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Integration of science in food chain

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OLD AND NEW CHALLENGES IN FRONT OF THE FOOD CHAIN

In our decades there is a never-seen flourishment of scientometrics, the different indicators of academic research are proliferating. Some years ago just a very narrow circle of specialist has applied such indicators as Hirsh index, the application of which had considerable debates[1], nowadays this is one of principal measures of scientific impact. At the same time, we have to ask ourselves, what is the ultimate goal of our work, is there yet a living and mutually productive interchange between science and the life? or the academic community is closing itself into his anecdotic ivory tower? or –citing the worsening financial conditions and hard bureaucratic constrains of development- tries to minimise its academic activity?

We deeply hope that the materials and results, presented at our conference will give a definite response to these questions, affirming and highlighting the commitment of food scientists to contribute to the resolution of global and local problems. Our conference is a tribute to the work and achievements of Professor József Farkas, whose activity has been focusing parallel on both aspects of academic life: on achievement of results of international significance and to contribution of upgrading of efficiency and competitiveness of food chains.

The challenges facing the global, European and Hungarian food chain in the 21st century are unprecedented in their scope, complexity and importance. During the last decades there have been considerable successes in decreasing number of undernutritioned persons, because their number decreased from 1.1 billion to 0.75, but even this is a very large number. The improvement has been achieved as a consequence of rapid economic development in Asia, but the economically depressed regions of the world are not capable to increase the level of food self-sufficiency and improve the food supply situation. The most important challenges before food supply chain seem to be as follows:

- increasing the level of food security in the world;
- increasing the food chain stability in crisis-hidden regions of the worlds;
- improvement of food safety, decreasing the food –borne diseases;
- improvement of food quality;
- contribution to the healthier life by production of functional foods;
- decreasing the environmental burden, caused by food production.

To address these challenges efficiently, the food chain must bring together the capabilities of agricultural production, food processing, logistics, trade and consumer education in a manner that optimally promotes the public interests. In this activity the food science is a key factor. The science and practice go hand in hand, always considering what states in the last scene Brecht's Galileo:

"*I maintain that the only purpose of science is to ease the hardship of human existence. If scientists… are content to amass knowledge for the sake of knowledge, then science can become crippled, and your new machines will represent nothing but new means of oppression*" [2].

[1]Kertész, J. (2009): A Hirsch-index második deriváltja, avagy új távlatok a tudományos minősítés előtt [Second derivate of Hirsch-index, or new horizons before scientific qualification, Magyar Tudomány, 170, 5. p. 602-603.
[2]Brecht, B. (1960): Plays, vol.I. (The Life of Galileo), Methuen, London, p.330 in Hungarian: Brecht, B: Galilei élete. Színművei. II. Budapest, Szépirodalmi Könyvkiadó p.233


Proceedings

EFFECT OF FREEZING ON A BAKERY PRODUCT

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SUMMARY

Freezing of bakery products is widely spread, as it allows manufacturer to plan the production according to consumer needs. However, frozen products after baking are thought to be not so good in quality as freshly baked products. Aim of our investigations was to compare freshly baked Kaiser buns with products which were pre-baked and frozen, or frozen without proofing. Organoleptic and rheological parameters were tested. The main question was, does freezing change the main characteristics of products to be detectable by consumers. Findings showed, that consumers preferred the products, which were frozen before proofing.

INTRODUCTION

Freezing technology is nowadays widely used also in bakeries. The reason for this is the achieved flexibility of the production times and the shift into the direction of "ready-baked" products. More and more supermarket used on-site baking facilities, to offer the freshness of the products to consumers, in a "controlled manner". The smell of the freshly baked bakery products are used also as "consumer magnets".

While the consumption of bakery products has not changed in the last years, the market share of frozen products is continuously growing.

The investment costs of the freezing technology is relative high, but the retailers of bakery products enjoy lower unit costs due to higher amounts of incoming products. The short shelf life of freshly baked bakery products became no hurdle for longer storage or further logistic ways in frozen state. (Werli, 2011)

At the beginning freezing was mainly done after baking, but with the developments of this technology the freezing became possible at different technological steps, such as before proofing (after kneading), and after proofing, before baking.

Because of the effect of the lower temperatures on the dough structure and yeast activity, adjustments on the recipe were needed. These adjustments include for example using less water, more yeast, different yeast strains, more emulsifier...etc. (www.pekforum.hu, 2013)
Classification of frozen bakery products can be according to:

- place of freezing in the production scheme (as mentioned above)
- treatments needed by baking off (in frozen state bakeable products, frozen products, which need to be proofed before baking, pre-proofed frozen products, which still need further proofing before baking, pre-baked or fully baked and then frozen products, which need to be baked a short time before serving) (Lajtos, 2013)

MATERIALS AND METHODS

Materials

Kaiser buns were produced on the same production line. Freezing was introduced at three stages of manufacturing: before proofing, after proofing and after baking. The recipe of the buns were similar: wheat flour (61-62%, BL55 type), yeast (4-5 % on flour base), sugar (1,5% on flour base), salt (1,5% on flour base), shortening (3% on flour base), skimmed milk powder (1,5% on flour base) and additives (containing enzymes) according to the recommendations for frozen products (0,4-3% on flour base).

Production of Kaiser Buns

After weighing flour has been sifted in the kneading bowl. Other ingredients are added to the flour directly. Kneading was done at low speed for 3 minutes, and at high speed for 6, 5 minutes. Resting time for the dough was 15-30 minutes. Forming and dividing products was done by hand. Proofing was done for 70 minutes at 35°C and 80% humidity. Baking was done at 245°C for 9 minutes (with the addition of 3, 5 l of steam).
In the case of so called green products (buns frozen before proofing), the buns were frozen in a freezing tower with -31 °C cold air for 58 minutes. After thawing products are proofed at 37°C for 70-75 minutes. Baking is done at 210°C for 11-12 minutes in a steamed oven.

In the production of pre-baked Kaiser Buns, the buns are baked in a Rotopass oven at 170°C for 15 minutes (with 3 l of steam). Products are cooled down at room temperatures before freezing at -30°C for 60-70 minutes in freezers. They will be transported in frozen state to the place of retail. The baking off is at 210°C for 4-5 minutes with the addition of 2 l of steam.

Methods

Texture measurements

The elasticity of the bread crumb was measured with a Stable Micro Systems TA-XT2i type texture analyzer according to the AACC 74-09 standard. 25 mm high samples were place below the measuring head of the analyzer. The probe moved with a speed of 10 mm/s until it reaches the sample, then at 0, 5 mm/s presses the sample to 40% of its original height. There is a 30 seconds of holding time (while the probe still presses the sample). During this time the measured resistance (Force against the probe) is decreasing. After 30 seconds, the probe will be raised, while the crumb follows its movement (force is still decreasing). When the sample is not following the probe, the measured force will be 0. The difference between the height measured at this moment and the original height refers to the non-reversible change in the crumb structure (relaxation).

7 samples were measured, 2 hours after baking (cooled at room temperature).
In Figure 1 the main parameters of the texture measurements are shown, which were later used in the evaluations. Force 1 is the total deformation (D), Force 2 is the plastic deformation (P), the difference between these two forces is the elasticity (R), and R/D quotients is used to describe relative elasticity.

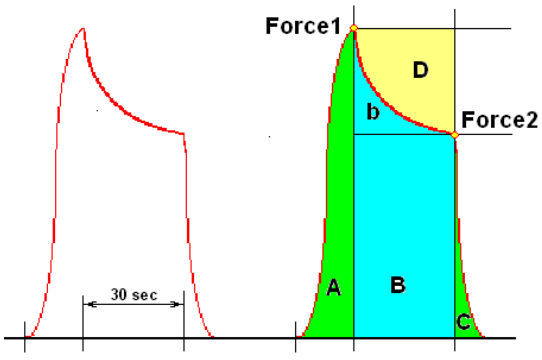

Figure 1: Typical curve of the texture measurements (Szalai, 2007)

Organoleptic evaluations

A list of 13 parameters (color, surface, shine, and roughness of surface, shape, smell, taste, crunchiness of crust, crumb porosity, crumb moistness, hardness, cohesiveness, crumb elasticity and chewiness) was set up before introducing the samples to the panel. Panelists had to judge these parameters on a non-structured linear scale. 12 panelists were taking part in the tests. ProfiSense software was used in the tests and their evaluation.

Results were compared with statistical tests (ANOVA).

RESULTS

Organoleptic evaluations

In the organoleptic tests no differences were significant in the color, the smell intensity, the crumb porosity, the cohesion (cohesiveness) and chewiness.

In the shine parameter panelists found the green products (buns frozen before proofing) as significant shinier, than the other products. It might be related to the higher amount of steam used by baking.

The roughness of the surface was significantly different (smoother) between the freshly baked sample and the other frozen products.

The final heights of products were similar between freshly baked and green products, being significant higher, then pre-baked products.

The crunchiness of crust was found to be the best in case of green products.

In the taste, both frozen products achieved significant better feedback, than the freshly baked product.

The moistness of the green product was rated as the highest. This might be due to the moisture, which the product picks up during proofing.

The hardness (of the first bite) was lowest in the freshly baked product and highest values were for the pre-baked product (significant differences).

Also the elasticity (the recovery of the sample after pushing together between fingers) was the best in the freshly baked product. Second came the green, and then partly baked product.

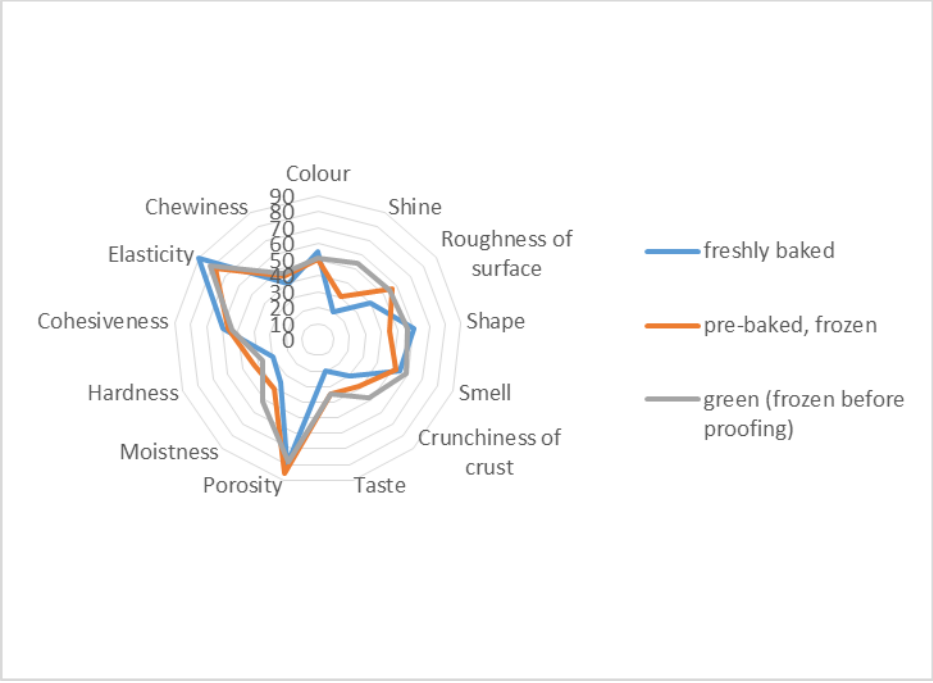

Figure 2. Sensory profiles of Kaiser buns

Results of the texture analyses

From the parameters shown in Figure 1 the results for Force 1 (hardness), Force 2 (plastic deformation), their addition (elastic deformation) and the quotient of elastic deformation and relaxation (Area D on Figure 1) were used in statistical analysis.
Figure 3 shows the measured values and their standardized error bars.

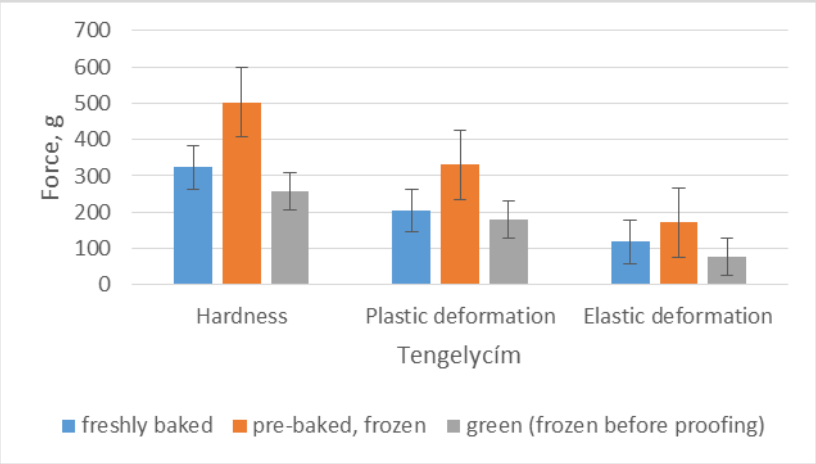

Figure 3 Parameters of texture analyses of Kaiser buns

In terms of hardness the texture analysis showed similar characteristics to the organoleptic test, as pre-baked product was significantly the hardest. Although differences between freshly baked and green products were calculated as significantly different (at 95% significance level), panelists could not detect it.
In general the best values in textural properties terms were achieved by the green product, next came the freshly baked product, and worst values got the pre-baked product.

CONCLUSIONS

Freezing of bakery products is not only a question of production management, but also a quality issue. Differences among freshly baked, pre-baked and green (frozen before proofing) were looked at in terms of organoleptic and textural characteristics.

The results measured with both human and mechanical test showed, that there is a clear difference between the qualities of the products in favor of the green product. It means, that it is not only a cost saving for the producer to freeze the products before proofing, and to leave the proofing and baking to the site of retail, but it also serves the quality requirements of costumers.

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EFFECT OF STATIC MIXER TO THE INITIAL FLUX OF RED- AND BLACK CURRANT JUICE USING MICROFILTRATION

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SUMMARY

The red currant (Ribes rubrum L.) and blackcurrant (Ribes nigrum L.) contain a lot of flavonoids, and have beneficial effects: protection against heart and cardiovascular diseases, stroke prevention, antioxidant protection, etc. The berries and the juices are used in many foods and drinks (e.g. as colouring agent). In our experiments the effect of using static mixer was examined during microfiltration. The initial flux was evaluated with static mixer and without it. For the measurements a ceramic tubular membrane was used with 800 nm pore size. Mild condition was used for the experiments: the temperature was 25 °C, the flow rate was changed between 50 – 250 L/h and the TMP was 2 – 4 bar. It was determined that applying static mixer increased the initial fluxes in case of both fruit juices with 25-45 %. Static mixer had no significant effect to the retention of dry matter content of the fruit juices.

INTRODUCTION

Flavonoids and related plant compounds in fruits and vegetables are of particular importance as they have been found to possess antioxidant and free radical scavenging activity. The berries of red- and blackcurrant are rich in polyphenolic compounds and especially in anthocyanins, demonstrating antioxidant activity. Antioxidant activities of plant products contribute multiple health benefits (e.g. anti-cancer and anti-oxidant activities) (Yamagata, 2015 and Kruger, 2014).

During the processing technology it is very important to preserve these valuable components in red- and blackcurrant juice. For the clarification of the fruit juices microfiltration was used (Bánvölgyi, 2009). This process is considered to be clean technology as it has the advantages of large-scale continuous separation without phase change, avoiding the use of organic solvents. It can achieved on low temperature, so the valuable compounds can be preserve (Strathmann, 2006). Our aim was to evaluate the effect of static mixer to the initial permeate fluxes of the fruit juices.

Since a sufficiently high value of the permeate flux can assure lower investment costs and the operation at lower energy consumption, the research work has been mostly focused on flux enhancement. Although static mixers reduce hold-up in the feed channel, static turbulence promoters increase wall shear rates and may produce secondary flows and instabilities, and the significant flux enhancements compared to a conventional cross-flow process have been reported (Krstic, 2007).

MATERIALS AND METHODS

The red- and blackcurrant juice was provided by the Fitomark Ltd. (Tolcsva). The juices were enzyme treated (Pektopol PT 400), conserved with sorbate and stored in fridge till the experiments.

For the microfiltration a ceramic tubular membrane (Pall, 800 nm pore size) was used with 0.005 m^2 active area. The flow sheet of the equipment can be seen in Figure 1. The experiments were carried out with static mixer (turbulence promoter) and without it. The temperature was kept on constant value (22 °C). The total soluble solid (TSS) content was

measured by an Atago PAL-α digital refractometer. The initial permeate flux was determined at different operating parameters.

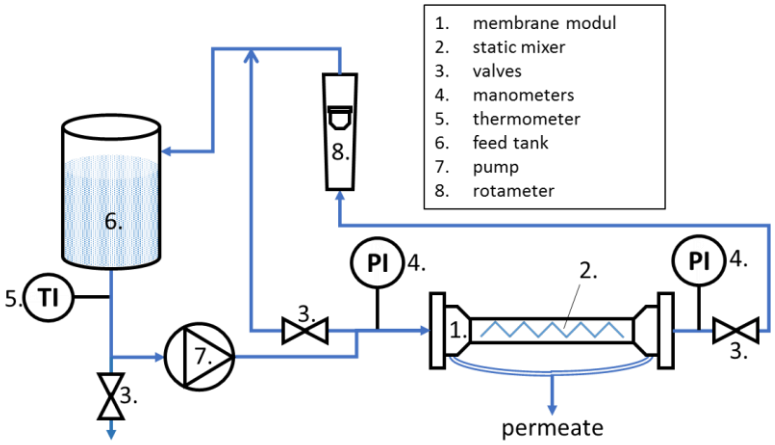


Figure 1.: The flowsheet of the equipment

To analyze the effect of operating parameters on the initial permeate flux of fruit juices 2^P type full factorial design was used. There were three independent variables (two quantitative, one qualitative). Ranges of these factors can be seen in Table 1.

Table 1.: Variables, their ranges, base level (Z_i^0), variation interval (ΔZ_i) :

Effect	Factor	Range	Z_i^0	ΔZ_i
TMP	Z_1	2 - 4 bar	3 bar	1 bar
Qrec	Z_2	50 – 250 L/h	150 L/h	100 L/h
Static Mixer	Z_3	SM or NSM	-	-

After we calculate the base level and variation interval, variables can be transformed, and we will get the equation of estimated regression planar:

$$Y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_{12} x_1 x_2 + \beta_{13} x_1 x_3 + \beta_{23} x_2 x_3 + \beta_{123} x_1 x_2 x_3$$

where, Y- initial permeate flux ($Lm^{-2}h^{-1}$), x_i- transformed factors, β_0- constant, β_i- coefficient of effect.

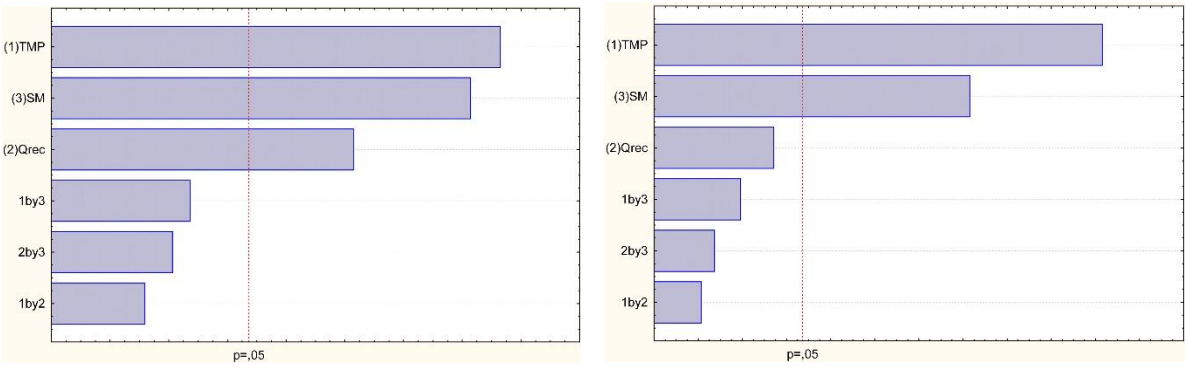
The model is adequate, if there is no significant difference between constant of linear model (β_0) and measured values in origin of 2^P type full factorial design. This means we had to measure the initial permeate flux in middle of the parameter range too (3 replicates).

RESULTS AND DISCUSSION

The TSS content was also measured by refractometer next to the initial fluxes. In all cases the TSS concentration was almost the same in the retentate and permeate. The applied microfiltration membrane is suitable for clarification of the red- and blackcurrant juice, it does not retain the valuable compounds.

Using the 2^P type full factorial design, we created the model for the initial permeate flux of red- and black currant juices in function of 3 different factors. The Pareto chart of standardized effects was calculated for confidence interval of 95%. From Pareto charts (Figure 2.) it was determined that for the initial fluxes of red currant juice TMP, Qrec and SM have also significant effect. For the initial permeate flux of black currant juice only TMP and

SM have significant effect. Third biggest value is effect of Qrec. In all cases the three main variable with the biggest effect were had respected for the model equations neglected the interactions.



a) b)

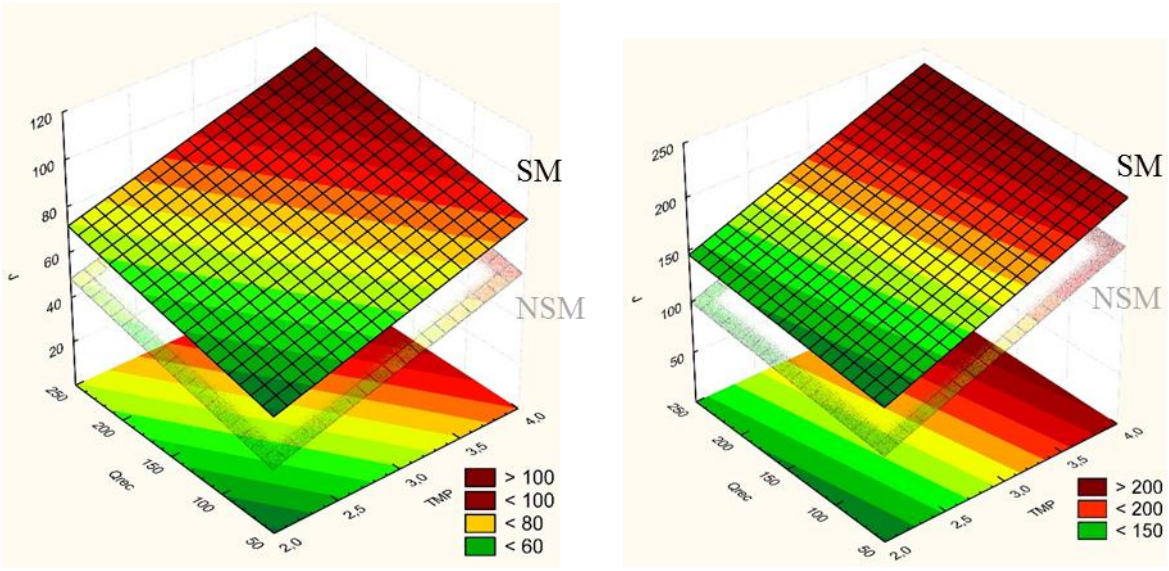
Figure 2.: Pareto charts of standardized effects (a) red currant juice (b) blackcurrant juice

The final equations for the initial permeate fluxes in the analyzed range:

red currant: $J_{rc} = 66.29 + 16.53 \cdot \left(\frac{TMP - 3}{1} \right) + 10.33 \cdot \left(\frac{Qrec - 150}{100} \right) + 11.54 \cdot SM$

blackcurrant: $J_{bc} = 159.28 + 45.14 \cdot \left(\frac{TMP - 3}{1} \right) + 8.42 \cdot \left(\frac{Qrec - 150}{100} \right) + 22.94 \cdot SM$

where TMP is 2 – 4 bar, Qrec is 50 – 250 L/h, SM is 1(SM) or -1(NSM)



a) b)

Figure 3.: Effect of operating parameters to the initial permeate flux of (a) red currant juice and (b) blackcurrant juice

The effect of TMP, Qrec and SM to the initial permeate fluxes of the fruit juices can be seen in Figure 3. In both cases using SM the initial permeate fluxes increased 20-40 %. Raising the TMP and Qrec the permeate fluxes was increased.

Beside permeate flux, one of the most important parameter from an economical point of view is the specific energy consumption (Espec) defined as the power dissipated per unit volume of permeate (Vatai, 2009). The hydraulic dissipated power is directly related to the

pressure drop along the membrane module (Δp) and the specific energy consumption can be calculated as:

$$E = \frac{Q \cdot \Delta p}{J_p \cdot A}$$

where J_p is the permeate flux and A is the membrane surface area.

Table 2. shows the energy consumptions. Diff Espec shows the ratio of specific energy consumption with SM and without it. It can be determined SM can be used economical at low Qrec where Diff Espec < 100%. At higher Qrec the energy costs increased appreciably.

Table 2.: The values of specific energy consumptions

		red currant			blackcurrant		
TMP (bar)	Qrec (L/h)	Espec-SM (kWh/m^3)	Espec-NSM (kWh/m^3)	Diff Espec	Espec-SM (kWh/m^3)	Espec-NSM (kWh/m^3)	Diff Espec
4	250	4,39	3,55	124 %	2,03	1,47	138 %
4	50	0,17	0,23	73 %	0,06	0,08	75 %
2	250	8,18	7,07	116 %	3,51	2,55	138 %
2	50	0,31	0,38	81 %	0,11	0,16	69 %
3	150	1,35	1,39	97 %	0,61	0,56	108 %
3	150	1,45	1,66	87 %	0,59	0,65	91 %
3	150	1,67	1,70	98 %	0,76	0,68	112 %

CONCLUSIONS

As a result of our experiments, we can tell that microfiltration is an effective clarification process, where different solid materials (seed, skin and vine residue) can be removed from fruit juices. At all operating parameters used in the experiments it was determined that the applied membrane with 800 nm pore size does not retain the valuable compounds. The TMP and SM (static mixer) have significant effect to the initial permeate fluxes of red- and blackcurrant juices. Since the effect of the Qrec is minimal, it should be kept on lower values (50-150 L/h) to ensure energy efficiency of the process.

The microfiltration process was modeled on the base of the experimental data. The model equations could be used for calculation the initial permeate fluxes of red- and blackcurrant juice in the named range of the operating parameters.

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COMPARISON OF CHEMICAL CHARACTERISTICS AND TECHNOLOGICAL PARAMETERS IN DURUM WHEAT BASED BREADS ENRICHED WITH HEMP SEED FLOUR

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SUMMARY

The effect of hemp seed flour addition on bread nutrition and physico-chemical properties were monitored in this study. The hemp seed flour concentration was set at 30%; in order to observe the water soluble total phenolic content (TPC) and the antioxidant capacity (FRAP) of the dough during the bread making process. The flour-mixtures to the dough were prepared by mixing Triticum durum L. wheat and hemp seed flour (Cannabis sativa L.) in several ratios.

INTRODUCTION

Nowadays, consumers from nutrition aspect are looking for consciousness. It is a fact, that parallels with the basic grains like wheat, corn or rye other cereals and pseudocereals appear on the market. The nutritional characteristics of these plants are strongly contribute to improve the quality of flour made from the basic grains. Preparing flour mixtures could help to get high standard quality bakery products.

Bread is a food produced using simple ingredients such as wheat flour, salt, yeast and water, but despite this, is one of the most consumed cereal products in many countries and a food at the basis of the diet of many people around the world (Alessandra Danza et al., 2014).In fact, nowadays consumers prefer to eat healthier foods in order to prevent non-communicable diseases (Hathorn et al.,2008). We prepared the bread with the aim of analysing the chemical characteristics, since there is a difference between the T. aestivum wheat and the durum wheat flour, and with the benefits of hemp flour contributed to a healthier product. Durum wheat is known for its good taste, pleasant aroma, and high nutritional value. Moreover, bread made from durum wheat flour can be kept fresh for a longer time than bread made from soft wheat flour (Aleksandra Torbica et al., 2011).

MATERIALS AND METHOD

During the measurements we used commercially available hemp flour (Natura Cookta), and durum wheat flour by Júlia Mill Kiskufélegyháza. For the breads, different mixtures of durum flour and hemp flour were used. The exact composition of the 4 bread samples are shown in Table 1.

Table 1. Sample codes

Flour composition of doughs	Sample codes
100% Triticum durum	100D
90% T. durum – 10% Cannabis sativa L.	90D
80% T.durum – 20% C. sativa L.	80D
70% T.durum – 30% C. sativa L.	70D

Breadmaking process

For each of the bread samples 300 g flour, 9g yeast, 6g salt and 1,5 g sugar were used, and 146 ml water for creating the proper consistency. With increasing the amount of hemp flour the water absorption capacity was decreased. . After mixing all the ingredients 5 minutes of kneading followed using a kneading machine. The finished dough was ripen in a thermostat at 31 °C, then 400 g of dough was taken and been formatted with rounder movement. The shaped samples were put into floured bowl, and leavened at 31°C for 30 minutes. When the rising stage ended the dough was slammed and wetted, and baked at 220 °C in LAINOX oven for 25 minutes (MSZ 6369-8:1988).

Analyses of the bread samples

Three samples were taken during the process:

- after kneading
- after rising stage
- after baking.

Water content of samples was determined by Sartorius M 50 Aquatest instrument. Before extraction the samples were homogenized by a hammer mill grinder (KT 100) and the extracts were obtained (0.10 g ml–1 in water after centrifugation for 10 min at 4 oC at 10000 r.p.m.). Chemicals were purchased from Sigma-Aldrich Co. and REANAL Finechemical Co.

Water soluble polyphenol content was measured by colorimetric method with Folin &Ciocalteu's phenol reagent (SINGLETON & ROSSI, 1965) and the results were expressed in Gallic Acid Equivalent (GAE) value (mmol gallic acid per g dry weight of dough).

The water soluble antioxidant activity were determined using FRAP (ferric reducing antioxidant power) assay (Benzie & Strain, 1966).

Statistical analysis

Data of samples were calculated on the dry weight of dough. Three individual extracts were made in the case of all samples and three different determinations were made from the same extract with a standard deviation ±5%.

RESULTS AND DISCUCCION

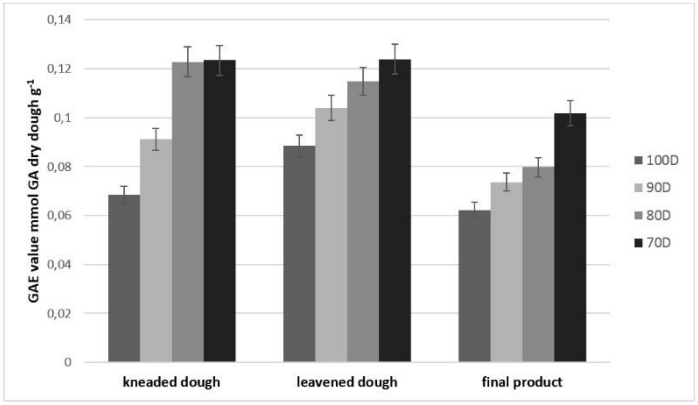

Figure 1. Determination of total phenolic content (TPC) during the bread making process

Water-soluble total phenolic content

Based on data shown in Figure 1, the water-soluble polyphenols content increased during the rising stage in the control samples, but in the final product the level of water-soluble polyphenols decreased below the measured value in the kneaded sample. The same trend was observed in the case of adding 10% hemp flour in all the three stages. The increased quantity of added hemp flour (20% and 30%) changed the experienced trend in the control samples, however in each sample of the final products contained the lowest water-soluble polyphenol content.

Figure 2 summarizes the change of polyphenol content in the final product, in the shell and in the crumb.

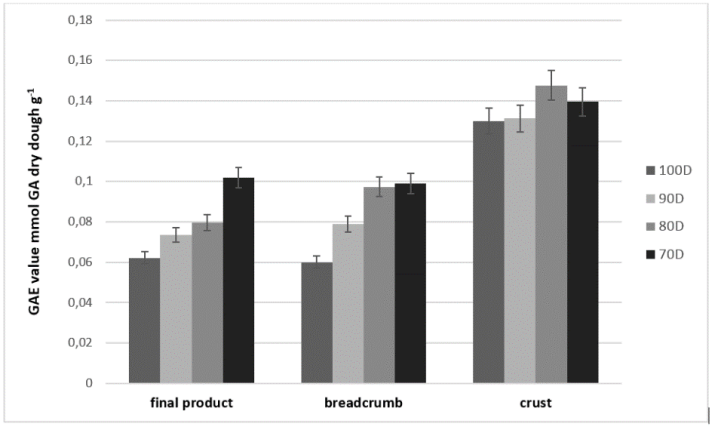

Figure 2. Determination of total phenolic content (TPC) of the final product, the breadcrumb and the crust samples.

We found it important to examine separately the crumb and the shell of the bread. We recorded significantly higher polyphenol level in the shell in each sample than in the crumb or in the final product. The high temperature caused the possibility of more water-soluble polyphenol compound formation, which was shown mainly in the crust.

Water-soluble antioxidant capacity

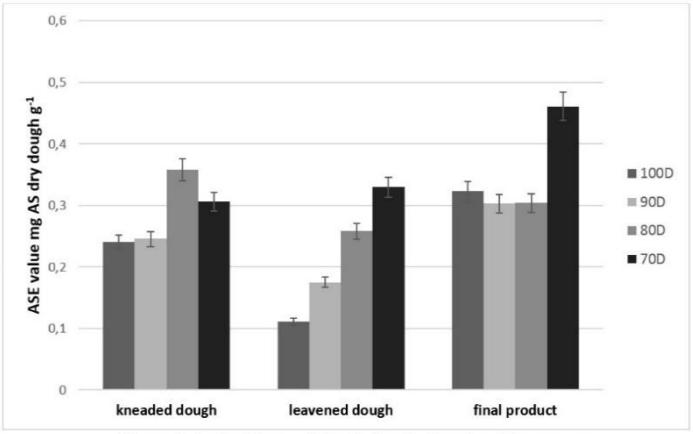

Figure 3. Antioxidant activity during the bread making process.

Figure 3 shows the water-soluble antioxidant capacity values of the samples from the different technological steps. Based on the data, the changes of the chemical parameters followed the same trend in case of the control sample, the 70D and the 80D samples: the initial value decreased during the rising stage, but increased in the following technological steps. The antioxidant capacity of sample 90D – which had the highest hemp seed flour content- shoed gradual increase during the bread making process.

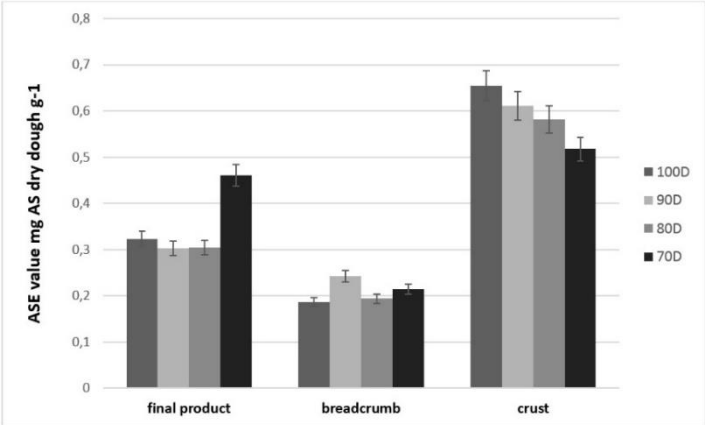

Figure 4. The results of the determination of antioxidant activity in the final product, in the breadcrumb and the crust.

As it is shown in Figure 4, the shell had the highest antioxidant capacity values in each of the samples, but in contrast to the polyphenol content the antioxidant capacity was significantly lower in the crumb.

CONCLUSIONS

Based on the result we can see that the polyphenol content and the antioxidant capacity can be increased in the final products when hemp flour was added, as the very complex microbiological and biochemical changes during the technological steps has a favourable effect on the nutritional values in the final products.

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METHODS FOR RISK PRIORITISATION TO SUPPORT FOOD CHAIN SAFETY DECISION MAKING

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SUMMARY

Tendencies of foodborne outbreaks show that the number of illnesses caused by Campylobacter spp. has been increasing recently in Hungary and in the European Union as well. The epidemiological status of Member States is diverse however according to the EFSA-ECDC zoonoses report campylobacteriosis was the most common zoonoses in the EU in 2013. As a solution, introduction of a microbiological criterion has been discussed and postponed by the European Commission, because of the limited information available on the epidemiology of Campylobacter spp. or on effective control measures. There are several methods that can be appropriate for ranking food chain safety hazards as well as for the estimation of the burden of food borne diseases. Although it is of great importance to find a proper methodology that is in line with the objectives. The aim of this work was to demonstrate different solutions applying food borne campylobacteriosis as an example.

INTRODUCTION

Regarding food chain safety controls and monitoring programs and thus risk based priority setting the role of risk assessment is emphasized. By the application of risk based priority setting resource allocation can be properly conducted as the probability of the hazards' presence in a certain food product and the effect of the hazard on human health. There are several qualitative and quantitative methods that can be applied but each of them has their own advantages and disadvantages. Although the applicable methodology depends always on the aim of the ranking whether hazards within one hazard group or between different hazard groups are being ranked (van der Fels-Klerx *et al.*, 2015).

Taking into consideration the interventions that can be applied against hazards occurring in the food chain, priority setting is also necessary, however in this case cost benefit analysis is the most suitable solution (Pitter *et al*., 2015).

Regarding recent tendencies of foodborne outbreaks it can be assumed that the number of illnesses caused by *Campylobacter spp*. has been increasing in Hungary and in the European Union as well. According to the EFSA-ECDC report on zoonoses monitoring activities carried out in 2013 (EFSA, 2015), campylobacteriosis was the most common zoonoses in the European Union. The annual number of confirmed human cases is above 200 000, but this is possibly only the tip of the iceberg. The actual number of gastrointestinal illnesses caused by *Campylobacter spp*. is estimated to be much higher (Sheerin *et al.*, 2014; Tam *et al.*, 2012; WHO, 2013). Furthermore, the number of campylobacteriosis has been exceeding the number of salmonellosis since 2009. In Hungary the number of human campylobacteriosis has been increasing recently as well.

Introduction of a microbiological criterion has been discussed as a solution by the European Commission as well common mandatory control programs on farms or microbiological criterion for products cannot be applied. Although EC is facilitating the Member States to start their own control programs depending on the epidemiological status of each MSs.

As foodborne diseases caused by *Campylobacter spp.* are of great importance, especially because of the severity and the frequency of them it is reasonable to find a proper method that can help for decision makers to distinguish between the available interventions.

MATERIALS AND METHODS

The aim of this study was to provide an overview on the available methods supporting decision making applying foodborne campylobacteriosis as a practical example, especially the critical review of the decision tool provided by the European Commission. While conducting this evaluation on the applicability of the decision tool methods related to Health Technology Assessment techniques have been used.

RESULTS AND DISCUSSION

Objectives of a food chain control system can vary widely between the identification of hazards to be focused on, the setting of public health related objectives to be achieved, and also the selection of appropriate interventions against a specific foodborne hazard. Currently applied risk ranking methods in food safety decision making are not comprehensive enough for ranking hazards occurring in the food chain as well as for taking into consideration the available interventions. In case of ranking the available control measures against a specific hazard the adaption of Health Technology Assessment techniques (HTA) can be the proper solution.

Campylobacteriosis in the European Union

According to the latest EFSA-ECDC zoonoses report (EFSA, 2015), campylobacteriosis was the most common zoonoses in the European Union in 2013. The annual number of confirmed human cases is above 200 000. In the case of 20-30% of human campylobateriosis, fresh broiler meat is recognised as the main source of infection, furthermore in the case of 50-80%, the poultry play a role as reservoir (EFSA, 2011), and those cases are primarily the consequences of inappropriate kitchen practices such as inaccurate heat treatment or cross contamination (Swart and Havelaar, 2013).

In Hungary the number of human campylobacteriosis has been increasing recently, with more than 7000 confirmed cases in 2013. Although the number of cases in Hungary represents not more than the 3% of all confirmed cases in the EU, Hungary holds the 6th highest number of cases out of the EU 28.

In the European Union the number of campylobacteriosis is three times as high as the number of salmonellosis thus the issue is considered to be substantial by the European Commission (EC) as well. Introduction of a microbiological criterion has been discussed as a solution, however the epidemiological status of Member States is diverse and also limited information is available on the epidemiology of *Campylobacter spp.* as well as on effective control measures. Therefore the introduction of a mandatory control program on farms or the establishment of a microbiological criterion for products has been postponed by the EC yet. Although EC is facilitating the MSs to start their own control programs depending on the epidemiological status of each MSs.

Member States attempt to manage the problem differently, at different points of the food chain, in line with the structure of industry and the prevalence that varies from state to state remarkably (ICF GHK, 2012). Multiple intervention points can be identified through the food chain where measures can be taken, e.g. the reduction of the levels of *Campylobacter* contamination in flocks and in fresh broiler meat (WHO, 2013), however the role of

dissemination of information between the stakeholders, including consumers, is not negligible either.

Cost-benefit analysis of certain control measures

As a promotion of the MSs' efforts against the presence of *Campylobacter spp.* in the food chain, a cost-benefit analysis has been developed by the European Commission (ICF GHK, 2012). The study summarizes all the measures and interventions that can be applied for the reduction of the level of contamination in the food chain. Analysing the costs of different control measures, considering on farm activities and potential measures during or after slaughter can provide information on possible intervention points as well as proposals for strategic decisions. The model includes not only the costs of the intervention techniques but the estimation of the reduced disease burden in terms of Disability Adjusted Life Years (DALY).

CONCLUSIONS

The cost-benefit analysis developed by the Commission was proved to be suitable for ranking the available interventions, however more detailed analysis can be gained by the application of Quality Adjusted Life Years (QALY).

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DEVELOPMENT OF AN HPLC-ESI-MS/MS METHOD FOR THE DETERMINATION TROPANE ALKALOIDS IN CEREAL-BASED FOOD

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SUMMARY

Atropine and scopolamine are toxic compounds belonging to the group of tropane alkaloids that are secondary metabolites and occur in several plant families including the common weeds of Datura species. These plants often contaminate cereal batches, especially millet and buckwheat. In our study an HPLC-ESI-MS/MS method has been developed for the quantitative determination of atropine and scopolamine for the exposed cereal and cereal-based foods. The analytes were extracted using a modified QuEChERS procedure, HPLC separation was carried out on C_8-C_{18} RP columns, while the analytes were quantified with ESI-MS/MS. The developed method proved to be selective in several matrices with a linearity range of 0.2-25 ppb (R^2=0.9998), LOD value of 0.05 ppb, and LOQ value 0.2 ppb for both analytes. The recoveries for atropine and scopolamine after a simplified spiking procedure were in the range of 82-88% and 67-85%, respectively. Food samples of Hungarian origin were found to contain even extreme (exceeding 6700 ng/g) total contamination of these compounds, calling attention to real and actual food safety problems.

INTRODUCTION

Tropane alkaloids are secondary metabolites that naturally occur in several plants and they are highly toxic to humans. The most studied tropane alkaloids are scopolamine and atropine: atropine is the racemic mixture of (±)-hyoscyamine while scopolamine is an oxidized derivative of atropine. The tropane alkaloids' common structural element is the tropane skeleton; the (-)-hyoscyamine and (-)-scopolamine are esters of tropane-3α-ol with tropic acid (Lounasmaa and Tamminen, 1993). Their structure is shown in Figure 1. Jimsonweed (*Datura stramonium*), henbane (*Hyoscyamus niger*) and deadly nightshade (*Atropa belladonna*) are long known for their content of tropane alkaloids. They can contaminate other plants especially cereals used for food and feed purposes. The most common problem in Europe is the contamination with seeds from jimsonweed (Adamse and van Egmond, 2010). The most exposed cereal is the millet (*Panicum miliaceum*), as it can not be easily separated from the seeds of the jimsonweed with sheer physical methods.

The toxicological effects of atropine and scopolamine are the same: delirium, hallucination, change in heart rate, peripheral vasodilatation, coma, ruddied skin, dilation of the pupils, damage in the central nervous system, inhibition of micturition, and decrease of secretions (Bordás and Tompa, 2006). The EFSA CONTAM Panel established a group ArfD (Acute Reference Dose) for the sum of atropine and scopolamine at the level of 0.016 ng/g body weight (EFSA, 2013). The EC has just adopted a recommendation (2015/976) for the monitoring of the presence tropane alkaloids (particularly atropine and scopolamine) in food, i.e., in cereals and cereal-derived products. The LOQ value of the methods to be used for atropine and scopolamine quantification should preferably be lower than 2 ng/g for finished foods (e.g., breakfast cereals) and 1 ng/g for cereal-based foods for infants and young children. Meeting the requirements of the recommendation is possible exclusively with an LC-ESI-MS/MS method.

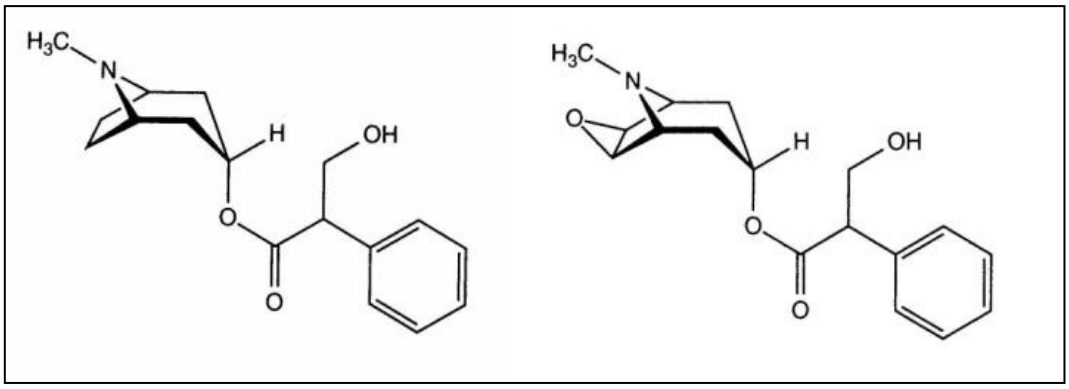

Figure 1. Structure of atropine (left) and scpolamine (right)

MATERIAL AND METHODS

Samples

The samples (husked millet, millet balls and millet flake) were obtained from local markets.

Chemicals

Atropine (≥99 %) and (-)-scopolamine hydrobromide (≥98 %) standards, ammonium formate (puriss p.a. for HPLC) and formic acid (~98% for MS) were supplied by Sigma-Aldrich (Schnellfdorf, Germany). Deionized water (18.2 MΩcm) was obtained from a Millipore purification system (Merck-Millipore; Darmstadt, Germany). Acetonitrile (Super Gradient Grade), $MgSO_4*H_2O$, methanol (HPLC Gradient Grade) and NaCl were supplied by VWR (Debrecen, Hungary).

Sample preparation

Two sample preparation methods were used in this work. The first was described by Vaclavik et al. (2014) and it uses a modified QuEChERS procedure. The other method was developed by Jakabová et al. (2012) and it is based on an ultrasonication-assisted methanolic extraction.

LC-ESI-MS/MS system

A triple quadrupole MS/MS device (Applied Biosystems 3200 QTRAP LC/MS/MS system) was used for the analyses. The mass spectrometer was coupled to an Agilent 1100 HPLC system (Agilent Technologies, Santa Clara, USA).

RESULTS AND DISCUSSION

LC-ESI-MS/MS method

All analytes were monitored in the positive ion mode with MRM methods. The precursor ion for atropin was set to m/z 290.2 for the transitions at 290/124, 290/93, and 290/77, showing the most intensive product ion at m/z 124. For scopolamine, the precursor ion was at m/z 304.2, while the transitions were set for 304/138, 304/156, and 304/103, with the most intensive product ion at m/z 138. Scopolamine and its fragment ions are shown as examples in Figure 2.

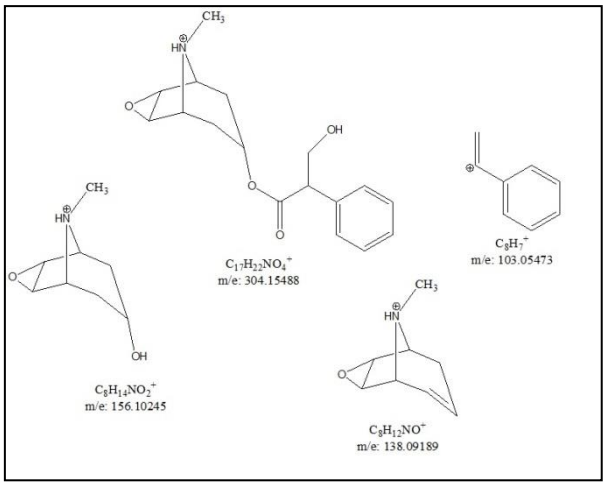

Figure 2. Structures and m/z values of scopolamine and its fragments

Optimization of HPLC parameters

The chromatographic process was built up through two main steps based on the study of Vaclavik et al. (2014). A C_8 RP column (15 cm x 4.6 mm x 5 µm) was used first to determine the two compunds' main elution behaviour. The mobile phase consisted of deionized water (eluent A) and methanol (eluent B) both containing 0.1 % formic acid and 5 mM ammonium formate. The final goal was to meet the usual requirements of routine laboratories to address a C_{18} narrowbore column (50 mm x 2.1 mm x 3.5 µm). The main parameters for the optimization process were as follows: chromatographic time, starting organic eluent concentration, signal-to-noise ratio, and peak width. The chromatographic development is presented in Figure 3: sample throughput has been doubled; the response factor has been increased more than 1.5-fold. From economic point of view it is important to highlight that methanol-water based eluent can be used at low (0.35 ml/min) flow rate.

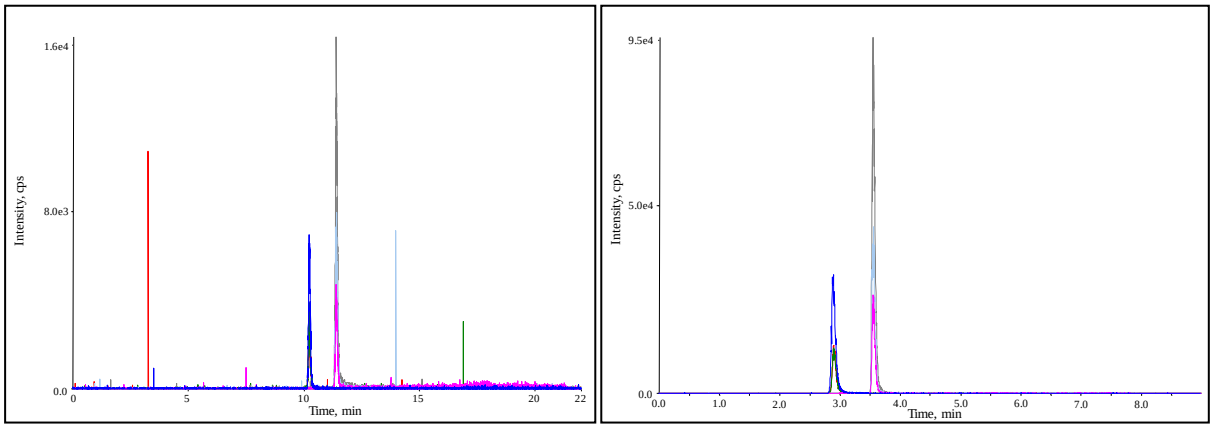

Figure 3. Chromatographic separation of tropane analytes on a C_8 column without optimization (left) and on C_{18} column after optimization (right). Elution order: scopolamine, atropine.

Merits of analytical performance and data from real world Hungarian samples

The selectivity of the method was assessed by analyzing blank samples of millet, millet balls, buckwheat, wheat and rye. No peaks were observed at the respective analyte retention times, i.e., the MRM spectra were interference-free. The linearity of the method was determined by measuring standard solutions at different concentrations. The linearity goes on

more than two orders of magnitude: the range of 0.2-25 ppb can be considered linear with high R^2 value. The limit of detection (LOD) and limit of quantification (LOQ) were calculated by injecting diluted samples and measuring the response at a signal-to-noise (S/N) ratio of ≥ 3 and ≥ 10 for the LOD and LOQ, respectively. The LOD value of our method is 0.05 ppb, the LOQ is 0.2 ppb for the injected extracts. Using diluted samples we could also observe that the matrix showed signal enhancement of the target analytes. Recovery studies were carried out with blank millet and millet ball samples at the spiking concentrations of 20 and 100 ppb for both matrix and both analytes. The recoveries for atropine and scopolamine were in the range of 82-88% and 67-85%, respectively.
An extract of the results are shown in Table 1. Taking into consideration the theoretically suggested future MRL (maximum residue limit) data for tropane alkaloids, i.e., 10-20 ng/g, the actual contamination of a huge number of Hungarian samples are shockingly high and calls for direct food safety interaction from Hungarian authorities to help producers to be prepared for the upcoming regulations.

Table 1. Atropine and scopolamine concentration in millet samples produced and/or purchased in Hungary

Sample	Atropine (ng/g)	Scopolamine (ng/g)
Millet, unhusked	< LOQ - 4255	< LOQ - 2516
Husked millet	< LOQ - 287	< LOQ - 235
Millet ball	< LOQ - 190	< LOQ - 272
Flavoured millet ball	< LOQ - 350	< LOQ - 332

CONCLUSIONS

The developed method shows better analytical performance for the quantification of tropane alkaloids than the methods actually available in the literature and it proved to be adequate to monitor the surprisingly highly contaminated millet samples of Hungarian origin. However, the fit-for-purpose characteristics of the method can only be judged after the publication of the final EU regulations defining both the MRL levels of atropine and scopolamine and the relevant food matrices to consider.

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MICROENCAPSULATION OF PROBIOTIC MATERIAL

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SUMMARY

At the Department of Food Technology of the University of Applied Sciences in Fulda, Germany, different methods for microencapsulation of probiotic material have been investigated. The aim of these studies was to develop a process to stabilize the probiotic bacteria for storage and to prevent them from the gastric conditions, so that a sufficient amount of the probiotics could reach their target location, the human intestine. Drying processes like spray drying and freeze drying have been tested as well as fluidized bed granulation with optional Wurster coating using different auxiliary materials.
In the performed studies the fluidized bed bottom spray granulation with an additional Wurster coating turned out to be an encouraging procedure to keep the probiotics in a stable form resistant against gastric conditions.

INTRODUCTION

Probiotic microorganisms play a major role in today´s nutrition. Various health benefits are verified on the consumption of different probiotics. To get a healthy effect on the consumer of probiotic food, the concentration of the viable microorganisms has to be in a sufficient range. Therefore it is a great challenge to bring the probiotics into a stable form, which guarantees, that the microorganisms reach their target location, the human intestine, in an adequate amount. This includes beside the production process itself and the storage period on the one hand the gastrointestinal passage of the probiotics on the other hand. So the aim of different studies, carried out at the Department of Food Technology of the University of Applied Sciences in Fulda, Germany, has been to develop a drying process with an additional coating process to prevent the probiotic microorganisms from the ambient conditions.

The studies have been performed on *Lactobacillus reuteri* DSM 20016. After cultivation of the *L. reuteri* the bacteria were dried in different drying processes and with different additives. Spray-drying processes were conducted with maltodextrin (MD), gummi arabicum (GA) or sweet whey powder as encapsulation material for the microorganisms. With two of these substrates (MD and GA) freeze drying processes were performed, too. In comparison to these straight drying processes for encapsulation of the *L. reuteri* a granulation process, operated in a fluidized bed granulator with bottom spray, was the topic of additional studies. Here, maltodextrin or sweet whey powder were used as encapsulation material. Furthermore the received granules were coated in a Wurster process with shellac to improve the resistance against the gastric conditions. Also in a fluidized bed granulation process, but in top spray mode, different additives for the protection of the bacteria against thermal stress during the granulation were investigated. The amount of viable cells in the obtained powders, granules and coated granules as well as in the original fermented slurry, specified in colony forming units per gram (CFU/g) or colony forming units per milliliter (CFU/mℓ), was evaluated by pour plate count method. Based on these bacterial counts the survival rates for *Lactobacillus reuteri* during the drying -, granulation - and coating processes and the storage periods could be determined. Additionally in this way the amount of viable cells during the simulated gastrointestinal passage and with it the survival and release rate under gastrointestinal conditions was evaluated.

RESULTS

(a) Drying Processes

In the freeze drying processes, operated with maltodextrin as well as gummi arabicum as encapsulation material, only low survival rates (between 5 and 19%) for the *Lactobacillus*

reuteri could be reached. The bacteria survival rate describes the relation between the amounts of viable cells in the bacteria slurry before the drying and in the rehydrated dry product; the concentration of the dry product in this suspension for determination of the viable cell numbers corresponds to the solid contend in the bacteria slurry before the drying [1]. This encapsulation technique for the microorganisms was not pursued furthermore.

Spray drying tests were performed with maltodextrin, gummi arabicum as well as with sweet whey powder as encapsulation material. The outlet temperature of the drying air was adjusted to 55°C. The survival rates of the *L. reuteri* after the spray drying process varied between about 1,5% for sweet whey powder as encapsulation material [2] and up to 90% for maltodextrin as supporting material [1]. Tests with higher outlet air temperatures were performed, too. In case of sweet whey powder as encapsulation material the increasing of the outlet air temperature up to 65% did not have any effect on the survival rate of the bacteria [2]. For maltodextrin the increasing outlet temperature up to 62°C caused a decreasing of the survival rate about 10% [1].

(b) Granulation Processes

As a further microencapsulation process the granulation in a fluidized bed was carried out. Therefor top spray technique as well as bottom spray mode were used. Thereby the bottom spray technique allowed a more stable process management. So longer process times, up to 1 hour, could be realized with bottom spray mode [3]. Top spray fluidized bed granulation was performed with sweet whey powder as encapsulation material at an inlet air temperature of 50°C. Hereby a relative viability for the *Lactobacillus reuteri* of about 17% could be reached. Trehalose or sorbitol, added to the bacteria slurry as heat protectant, could increase the bacteria survival up to about 43% [4]. The relative viability or bacteria survival rate SR quantifies the survival of the bacteria during the process [3, 4]:

$$SR = \frac{C_1 \cdot m_1}{C_0 \cdot V_0} \cdot 100 \qquad \text{Equation (1) [3]}$$

with:

SR [%]:	bacterial survival rate of the process
C_1 [CFU·g^{-1}]:	viable cell concentration of the product
m_1 [g]:	total weight of the product
C_0 [CFU mℓ$^{-1}$]:	viable cell concentration of the slurry
V_0 [mℓ]:	volume of the slurry

In contrast to the single drying processes a granulation process offers the opportunity to accumulate the bacteria in the dried product controlled by the process time. Therefore not only the bacteria survival rate but also the bacteria conversion rate is an important assessment criterion for the granulation process. The bacteria conversion rate CR describes the bacteria amount in the dried product in relation to the bacteria amount in the fermented slurry [3]:

$$CR = \frac{C_1}{C_0} \cdot 100 \qquad \text{Equation (2) [3]}$$

with:

CR [%]:	bacterial conversion rate of the process
C_1 [CFU g^{-1}]:	viable cell concentration of the product
C_0 [CFU mℓ$^{-1}$]:	viable cell concentration of the slurry

Fluidized bed granulation of the *L.reuteri* with maltodextrin as encapsulation material, operated with bottom spray technique, shows this accumulation effect (see figure 1 and 2) [3]. Although the bacteria survival rate decreased to about 60% for an atomizing pressure of 0,25 bar the conversion rate increased up to about 75% after 1 hour process time. The test series, diagrammed in fig. 1 and 2, were performed at inlet air temperature of 40°C due to a temperature sensitivity of the *L. reuteri* found in preliminary tests. In addition to the temperature sensitivity figure 1 and 2 also show a sensitivity of the bacteria against shear stress, expressed by the pressure [bar] of the atomizing air. In contrast to a survival rate of about 60% (conversion rate of about 75%) at an atomizing pressure of 0.25 bar an atomizing

pressure of 2 bar caused a decrease of the survival rate to about 25% (conversion rate of about 30%) [3].

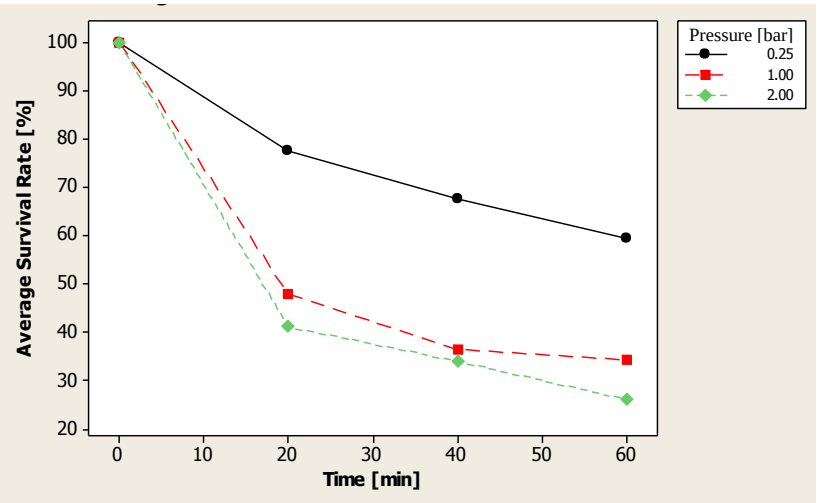


Figure 1 Scatter plot of the average bacterial survival rate as a function of the granulation time [3]

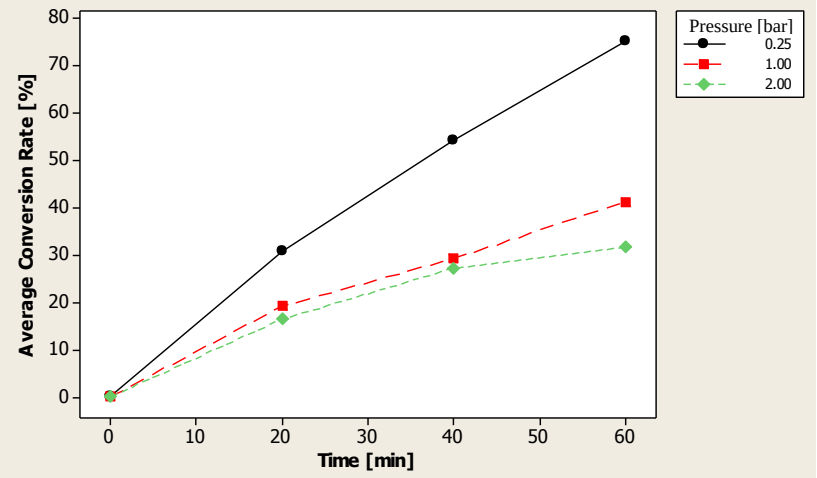
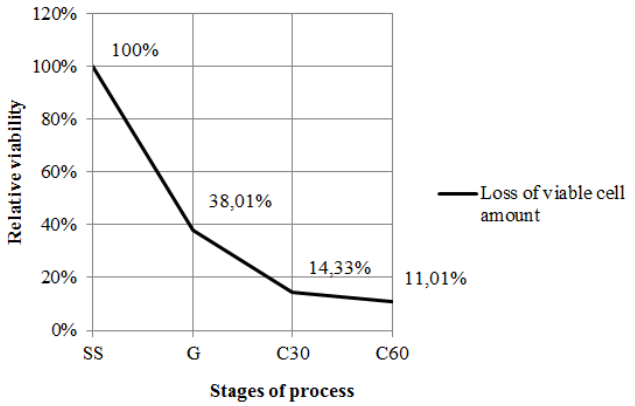


Figure 2 Scatter plot of the average bacterial conversion rate as a function of the granulation time [3]

Sweet whey powder as encapsulation material for the *Lactobacillus reuteri* in a bottom spray fluidized bed granulation required a higher a temperature for the inlet air and a higher pressure for the atomizing air. At an inlet air temperature of 60°C and an atomizing air pressure of 2 bar a stable granulation process could be generated. The relative viability of the *L. reuteri* was determined to about 38% after the granulation. An additional coating step in the fluidized bed with Shellac by using a Wurster device reduced the survival rate to about 11% after 60 minutes of coating, see figure 3 [5].



Process	Label	Time [min]	Colony forming unit [CFU/mL]
Bacteria strain preparation	SS	0	$1,96*10^8 \pm 3,62*10^7$
Granulation	G	60	$7,54*10^7 \pm 2,57*10^7$
Coating	C30	30	$2,94*10^7 \pm 1,05*10^7$
	C60	60	$2,21*10^7 \pm 8,62*10^6$

Figure 3 Relative viability of different stages during microencapsulation [5]

An artificial gastrointestinal test according to Picot and Lacroix [6] was performed to determine the survival respectively the release of the granulated and coated *L. reuteri* under simulated gastrointestinal conditions. The bacteria were exposed to simulated gastric juice (pH 1.9, containing pepsin) for 30 minutes. Afterwards the pH was adjusted to 7.5 and further digestive enzymes (bile salt and pancreatin) were added and the treatment was continued for additional 3.5 hours. The behavior of the *L. reuteri* microencapsulated with sweet whey powder in a bottom spray fluidized bed process and additionally coated with shellac is shown in figure 4. After an abrupt drop down after 30 minutes incubation time (according to the gastric conditions) the following intestinal conditions cause for the shellac coated microorganisms a steady rise of the bacteria survival/release rate up to 70% maximum with the incubation time [5].

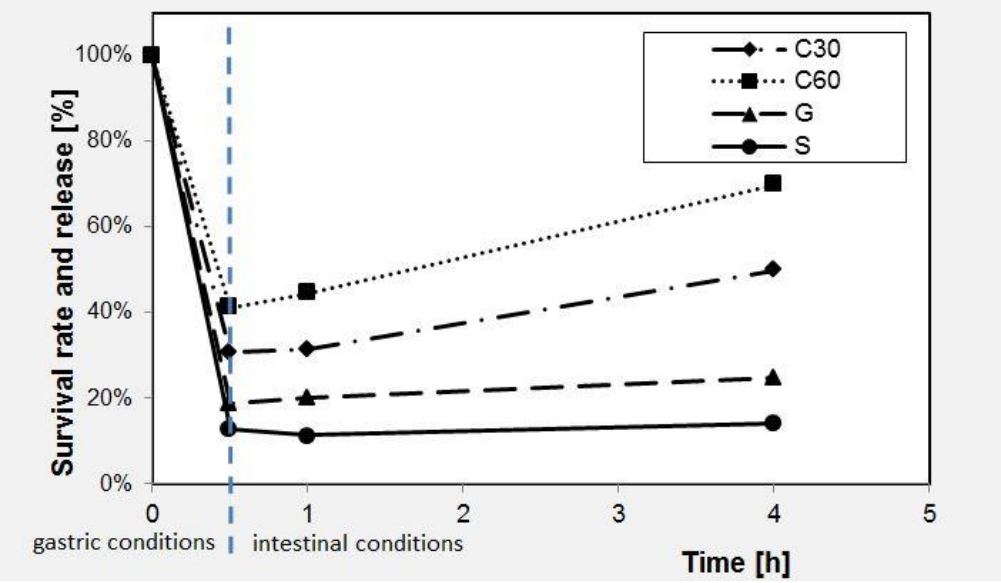


Figure 4 Survival and release rate of the *L. reuteri* granulated with sweet whey powder (G) and additionally coated with shellac for 30 minutes (C30) respectively 60 minutes (C60) in comparison to not granulated/coated material (S) during incubation in artificial gastrointestinal conditions [5]

CONCLUSIONS

The bottom spray fluidized bed granulation process turned out to be better than the single drying processes, because lower temperatures could be used and controlled by the process time more bacteria could be brought into the product. An additional coating with shellac can protect the *Lactobacillus reuteri* from the gastric conditions. Due to the sensitivity of the *L. reuteri* against heat stress and shear stress maltodextrin as encapsulation material seems to be more suitable than sweet whey powder. Maltodextrin allows a stable granulation process at lower temperatures and with a lower pressure of the atomizing air (representing the shear stress on the bacteria) than sweet whey powder.

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CHANGES OF PROTEINS IN RAW PORK AND BEEF MEAT CAUSED BY HIGH HYDROSTATIC PRESSURE TREATMENT

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SUMMARY

In the experiments we treated pork loin and beef sirloin between pressures of 100 and 600 MPa by 100 MPa steps for 5 minutes. We observed the color changes of samples and the changes of proteins.We examined the latter with isoelectric focusing and SDS polyacrylamide gel electroforesis. We experienced that the myoglobin behaved completely differently in case of different species. The results of isoelectric focusing showed that the myoglobin in beef resisted very well the high pressure treatment (it could retain the activity at a pressure of 600 MPa) and didn't suffer significant denaturation like in pork. Denaturation of myoglobin in pork was almost complete at 300 MPa or higher pressures and it lost its activity. The results of SDS-PAGE and the color measurement confirmed this finding. We concluded that the myofibrillar and sarcoplasmic proteins of beef didn't denaturate significantly and they were more pressure resistant than the proteins in pork.

INTRODUCTION

The increase of animal-based protein (ABP) consumption has started worldwide about 50 years ago. Composition of ABPs and role of meat within this category is different among countries (Sans et al.,2015). The consumption of raw beef and pig meat is significant all over the world. It is essential to ensure the microbiological stability and hygiene of meat to produce fine quality products. High hydrostatic pressure treatment (HHP) is a non-thermal process which reduces pathogens and also extends the shelf life of the product (Morales et al., 2008).

HHP treatment is a new alternative technology to thermal processing, because the retention of nutritional and organoleptic characteristics is better than in case of thermal processing. (Cheret et al., 2005). High hydrostatic pressure (HHP) treatment of foods can also modify protein structure and activity (Ko et al., 2003, Cheret et al., 2005) ,but the proteins react differently to the certain pressure values, moreover there are differences between some species. It is essential to the development of technological processes that we know the sensitivity and reaction of proteins of different species to the high hydrostatic pressure treatment.

MATERIAL AND METHODS

Pork loin and beef sirloin were bought in a local meat market. The laboratory of our department is equipped with a Resato FPU-100-2010 device, which was used during the treatment.

The samples were vacuum-packed and put in bags then treated between pressures 100 and 600 MPa by steps of 100 MPa for 5 minutes, which is used in the industry. After the tratment the color measurements were performed with a MINOLTA CR-400 trisrimulus colorimeter.

During the extraction of sarcoplasmic and myofibrillar proteins we have milled the control and treated samples by the help of a cutter. We have measured 1 g from the samples then added 2 ml NaCl solution to them and homogenized at 13500 rev/min for 3 minutes with an Ultra-Turrax T25 device. Samples were cooled by ice during homogenization. The resulted homogen suspension was centrifuged at 10000 rev/min with a Beckman J2-21 deice for 15 minutes then we filtered it. The supernatant contained the sarcoplasmic proteins. The

precipitate was washed with 0,05 M NaCl solution two times to extract myofibrillar proteins. The supernatant was removed then we added 2 ml of 0,7 M NaCl solution to the washed precipitate and homogenized for 1 minutes with Ultra-Turrax. After homogenization we centrifuged it for 15 minutes and the reduced supernatant was filtered. This filtered supernatant contained the myofibrillar proteins. We used a Bio-Rad device for the electrophoresis. Molecular weight of proteins was determined by gel electrophoresis (Bio-Rad mini Protein Tetra System, Bio-Rad, Image Lab 5.1 program). We used the method described by Laemmli (Laemmli, 1970) to the SDS- and native-PAGE. Concentrations of the running gel and stacking gel were 15% and 6%, respectively. Standard proteins; molecular weight 250-10 kDa (Precision Plus Protein Standards All Blue) were used in experiment.

During the isoelectric focusing we used 12x12 cm agarose gel. The pH range of added ampholytes was 5-8. The meat samples were homogenized and diluted with distilled water. The used anode solution was 1% sulfuric acid, and the catode solution was ethylene diamine. The used standard was horse myoglobin. The dye was made with pseudoperoxidase. During the focusing we changed the voltage parameters continuously.

RESULTS AND DISCUSSION

Color Measurement

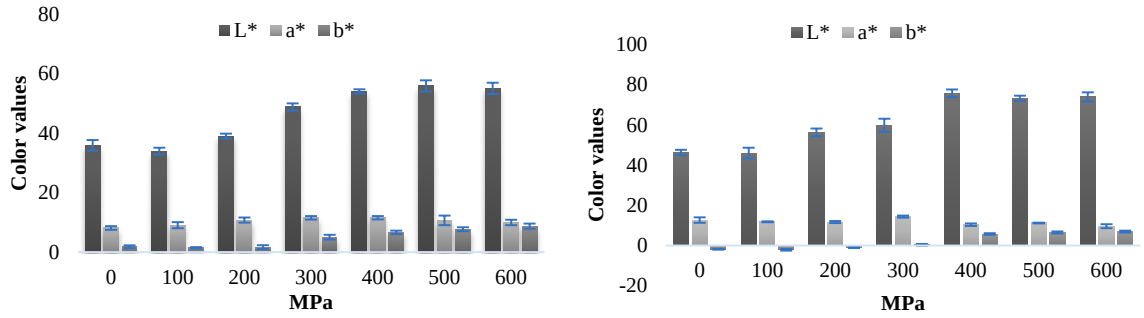

Fig 1. Results of beef sirloin and pork loin color measurement

In case of both meats, a ’jump’ was experienced in the L* values data caused by the treatment at 300 MPa, therefore this pressure can be regarded as a threshold.

The a* value of treated beef and pork meat did not change significantly. In the b* values of pork we experienced huge difference between the lower (100-300 MPa) and higher (400-600 MPa) pressures.

Isoelectric Focusing

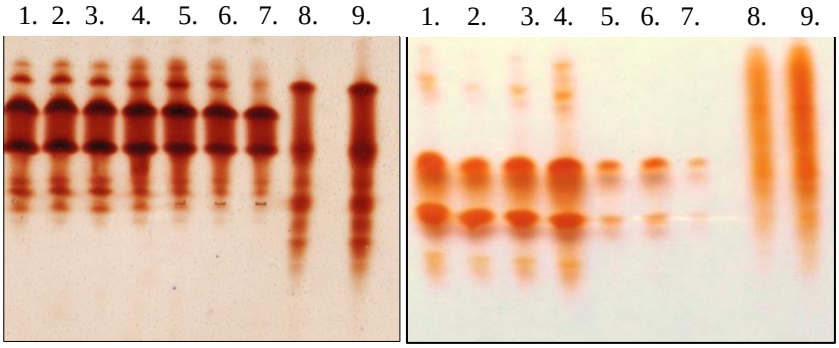

Fig 2. Results isoelectric focusing of beef sirloin and pork loin:
1. Control, 2. 100 MPa, 3. 200 MPa, 4. 300 MPa, 5. 400 MPa, 6. 500 MPa, 7. 600 MPa, 8-9. horse myoglobin standard

In the first picture we can see the results of isoelectric focusing of beef sirloin and the results of pork loin in the second one. The beef myoglobin treated by high hydrostatic pressure denatured only slightly, because its activity was lost, therefore the denatured myoglobin could not be stained by specific dye. However in the pictures we can see that the pork myoglobin has lost its activity at 300 MPa, but the beef myoglobin has been active at 500 MPa.

SDS-Polyacrylamide Gel Electrophoresis

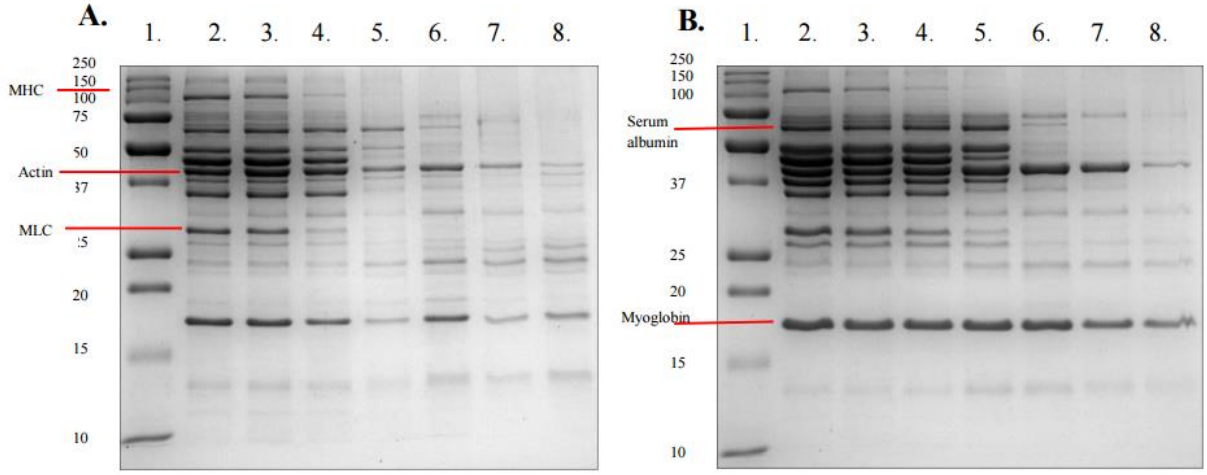

Fig. 3. Results of SDS-PAGE of beef sirlon
(A: myofibrillar proteins, B: sarcoplasmic proteins)
1. Standard, 2. 0 MPa, 3. 100 MPa, 4. 200 MPa, 5. 300 MPa, 6. 400 MPa, 7. 500 MPa, 8. 600MPa

The upper figures show the results of electrophoresis of the beef myofibrillar and sarcoplasmic proteins. The light and heavy chains of the myosin of myofibrillar proteins mostly denatured due to the pressure treatment, while in contrast, actin could be detected even after 600 MPa pressure treatment.

In case of low pressures (100 MPa and 200 MPa) the volume (intensity) of myofibrillar proteins (using SDS-PAGE) was similar to the control sample, but in case of higher pressures (300 MPa or higher) the volume of proteins decreased by more 50%, therefore 300 MPa is regarded as a threshold value. The lower figures show the volume intensity of the control sample and the one treated by 600 MPa.

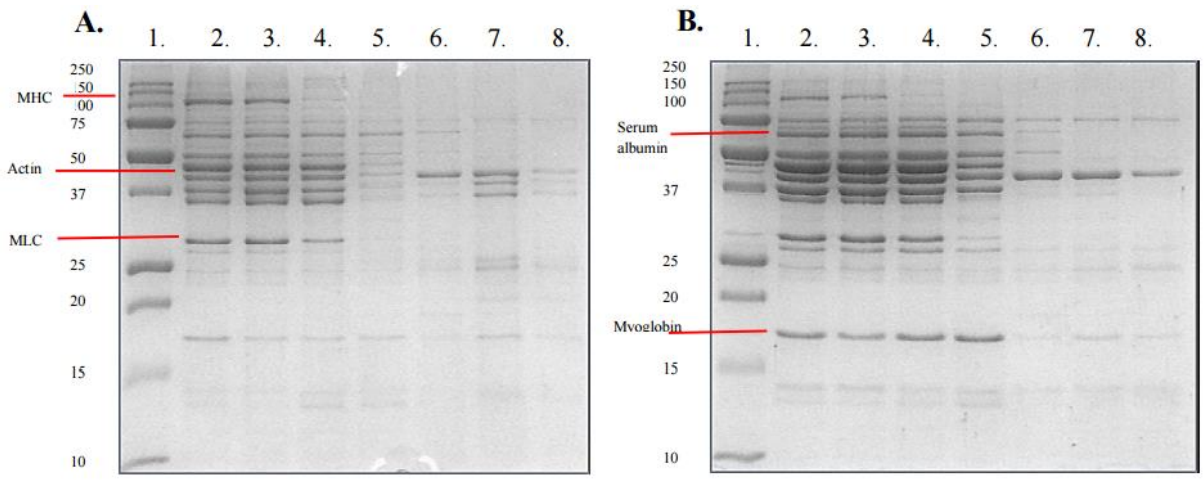

Fig. 4. Results of SDS-PAGE of pork loin
(A: myofibrillar proteins, B: sarcoplasmic proteins)
1. Standard, 2. 0 MPa, 3. 100 MPa, 4. 200 MPa, 5. 300 MPa, 6. 400 MPa, 7. 500 MPa, 8. 600MPa

The upper figures show the results of electrophoresis of the pork myofibrillar and sarcoplasmic proteins. Compared to beef meat the significant difference was (as indicated by isoelectric focusing, too) that the pork myoglobin denatured at above 300 MPa. The SDS gel electrophoresis illustrated that the reduction of the intensity was much higher in pork than in beef meat.

CONCLUSIONS

We conclude that the pork and beef have reacted to the pressure treatment differently. The technologically important myoglobin has lost its activity at 300 MPa pressure in case of pork, but the beef myglobin has been still active at 500 MPa or higher pressure. These results were confirmed also by the color measurement. We can say that the pork meat proteins are denatured and aggregated to a higher extent than beef proteins.

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VOLATILE CONSTITUENTS IN SEVERAL MUSHROOM SPECIES

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SUMMARY

Mushrooms are quite popular in Central and Eastern European cuisines as well. In Hungary, champignon is the most commonly consumed species, although a number of other mushrooms can be gathered. In this study, the fruiting bodies of four species (Agaricus bisporus, Boletus edulis, Cantharellus cibarius, Hericium erinaceus) were examined to review their aroma composition. Simultaneous distillation-extraction was applied to extract volatile compounds from mushrooms and the measurements were carried out with GC-MS. Although the fragrance of fungi is not so characteristic like in case of spices, several group of volatile compounds have been appeared in the mushrooms. The number of the identified components were between 61 and 100, with high ratio of eight-carbon volatiles, generally occurrent in fungi. Beyond common properties, individual attributes have been shown up as well – like outstanding ratio of benzene compounds in champignon, numerous N-containing volatiles in bolete, carotenoid degradation products in chanterelle or esters and fatty acids with high carbon number in lion's mane mushroom.

NTRODUCTION

Owing to their characteristic and delicious aroma, edible mushrooms have been used as foods and food flavorings in several dishes for centuries. Almost every species of mushroom has its own unique flavor (Hui, 2010). The volatile profile of mushrooms varies with species and varieties and can be affected by culture conditions as well (Venkateshwarlu et al., 1999). In this study, the volatile composition of mushroom species grown and collected in Hungary was examined. These species were *Agaricus bisporus*, *Boletus edulis*, *Cantharellus cibarius* and *Hericium erinaceus. Agaricus bisporus*, also known as champignon is the most common cultivated mushroom in the world. Therefore, a number of investigations have been dealt with its chemical composition, including its volatile constituents. *Boletus edulis* is also a very popular edible mushroom widespread in Europe. Due to its characteristic, marvelous-nutty flavor, 'king bolete' is consumed in raw, cooked and dried forms as well. Chanterelle (*Cantharellus cibarius*) is probably one of the most popular and especially favored wild-grown edible mushrooms in Europe. Its yellow pigmentation is developed by carotenoids, as in case of some plant species. This fungi has pleasant, specific fragrance reminiscent of fruits (mainly apricot) and also red pepper, which remains after dehydration as well. Its taste is spicily pungent in raw form, which disappears after food preparation. *Hericium erinaceus* (also called lion's mane) is a peculiar spectacle in oak, walnut and beech trees. Some parts of the world, this mushroom has long been used as medicines. Beyond its medicinal use, *H. erinaceus* is consumed as a peculiarity, owing to its special flavor reminiscent of seafood (Halpern, 2007). The publications about this particular mushroom mostly deal with its antioxidant properties and anticancer potential, its fragrance features are usually not under investigation.

In this study, four mushroom species were examined to reveal the main differences among their aroma properties. The results will be served as a base for our further investigations to identify the applied raw materials in several food products prepared from mushroom and truffle species.

MATERIALS AND METHODS

Samples

Fresh mushroom samples were provided from the Department of Vegetable and Mushroom Growing, Corvinus University of Budapest. *Agaricus bisporus* and *Hericium erinaceus* were cultivated, while *Boletus edulis* and *Cantharellus cibarius* were collected from their natural habitat. *A. bisporus* was cultivated on industrial straw-chicken manure based compost, while *H. erinaceus* on supplemented wood chips. The sample preparation was performed immediately after harvest.

Sample preparation

The extraction of the volatile compounds was accomplished by simultaneous distillation-extraction (SDE) in a modified Likens-Nickerson apparatus. Normal pentane of special purity was applied as an organic solvent. Three consecutive distillations were carried out, and the distillation time was one hour in every case. Subsequent to the LN-SDE, the extracts were frozen to remove the water traces and were concentrated.

Instrumental analysis

The analysis of the mushroom volatiles was performed on a GC-MS system (HP 5890/II – HP 5971A MSD). The instrument was equipped with a 60 m × 0.25 mm × 0.25 µm AT-WAX fused silica capillary column. Temperature program: initial 60 °C, and then an increase at a rate of 4 °C/min to a final temperature of 280 °C, held for 10 min; the injector temperature was 270 °C (in a splitless mode with a 100:1 split ratio). The detector was run in electron impact mode (70 eV) at 280 °C. Helium was used as a carrier gas (30 cm/s). Data were processed with the aid of the NBS49K, Wiley138, Wiley275, and NIST05 spectrum libraries.

RESULTS AND DISCUSSION

The numbers of the identified odorants in the mushroom species were among 61 and 100. The volatile fraction of *Boletus edulis* contained the fewest fragrance constituents, while *Cantharellus cibarius* included the most. The fragrances of the extracts were characteristic, strong mushroom-like in every case, although there were some differences among the odour impressions in diverse species. Among the identified scent constituents, there were some volatiles present in numerous mushroom species. These characteristic odorants in fungi are eight-carbon volatiles (alcohols, aldehydes and ketones) developing the peculiar mushroom flavor. The ratio of these specific C_8 compounds was quite different in various mushroom species (Figure 1.).

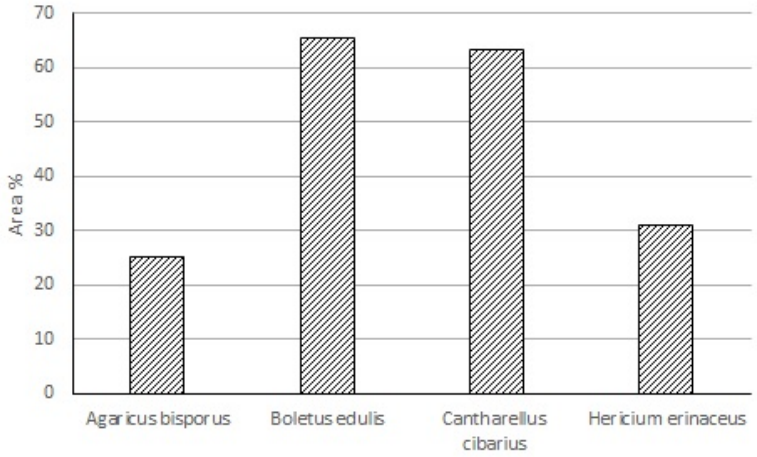

Figure 1.: The ratio of the C_8 compounds in the examined mushroom species

These volatiles are the degradation products of linoleic acid and arise from its oxidation and cleavage. Their share usually amounts to 44.3-97.6 % of the total volatile fraction in mushrooms (Combet et al., 2006; Venkateshwarlu et al., 1999). In *B. edulis*, *C. cibarius* and *H. erinaceus* 1-octen-3-ol (generally known as "mushroom alcohol") was the most intense peak on the chromatograms: its ratios from the total aroma were the highest in the distinctly fragrant *B. edulis* and *C. cibarius* (37.17 % and 46.58 %), respectively.

Beyond the common, characteristic "mushroom odorants" several other volatile compounds have been identified in the fungi. The percentage of these substances was the highest in case of *A. bisporus*. Benzyl alcohol, benzaldehyde and 3-octanone have been appeared with the most intense peaks on its chromatograms. The relative percentage of aroma constituents with benzene ring was outstanding in this fungi (32.73 %), similarly to the results of Venkateshwarlu et al. (1999). The precursor of the most intense benzene compounds benzaldehyde (11.45 %) and benzyl alcohol (14.60 %) is L-phenylalanine (Kawabe et al., 1994). This amino acid can be derived from the mushroom compost, which is a good source of organic nitrogen compounds. According to Halpern (2007), the flavor of some edible wild *Agaricus* varieties can be quite almondy. This fragrance property is presumably attributable to the presence of benzaldehyde, which compound possesses a characteristic odor similar to bitter almond (Burdock, 2010) and was present in these cultivated species as well. In case of *Boletus edulis*, the main differences compared to the other mushrooms were the greatest number of terpenes and nitrogen-containing compounds, and the total absence of acids. The main terpene constituent was 2-norcaranone, together with some fruity and spicy compounds like δ-cadinene, dl-limonene, viridiflorol, d-carvone, α-farnesene and α-copaene. The N-containing odorants are probably the volatile derivatives of high protein and free amino acid content of this fungi. In forming the fresh, fruity flavor of *Cantharellus cibarius*, lactones, terpenes and low-chain esters play an important role. The mentioned odorants in this mushroom are γ-octalactone, γ-nonalactone, δ-decalactone, β-ionone, farnesyl acetone, ethyl acetate and methyl octanoate. Except ethyl acetate, these volatiles were unique in this fungi. Some degradation products of carotenoids like β-ionone and dihydroactinidiolide were also present. Similarly to the fruits of some yellow and orange-coloured plant species, carotenoids are responsible for the yellow pigmentation of this mushroom as well. The volatile fraction of *Hericium erinaceus* can be described with increased number and ratio of esters and fatty acids. The number of these constituents and the ratios of both compound groups (6.90 % for esters and 9.81 % for acids) were the highest among the examined fungi. The members of both groups are volatiles with high carbon number and possess waxy, fatty odour. The really fragrant terpene components were present in low number and ratio in these fungi, and their

scent character was typically flowery. The mentioned terpenes were linalool, geraniol, dihydro methyl jasmonate and farnesol.

On the basis of our results, the examined mushroom species possessed unique fragrance compositions. Beside the common, specific C_8 "mushroom volatiles", the fungi could be characterized with other distinctive aroma components or compound groups. These odourants were benzene constituents in *A. bisporus*, terpenes and nitrogen-containing volatiles in *B. edulis*, lactones, terpenes and low-chain esters in *C. cibarius* and esters and fatty acids in *H. erinaceus*. Every mushroom contained individual scent constituents, specific only one species alone. The main differences in the ratio of the most important compound groups are represented in Figure 2.

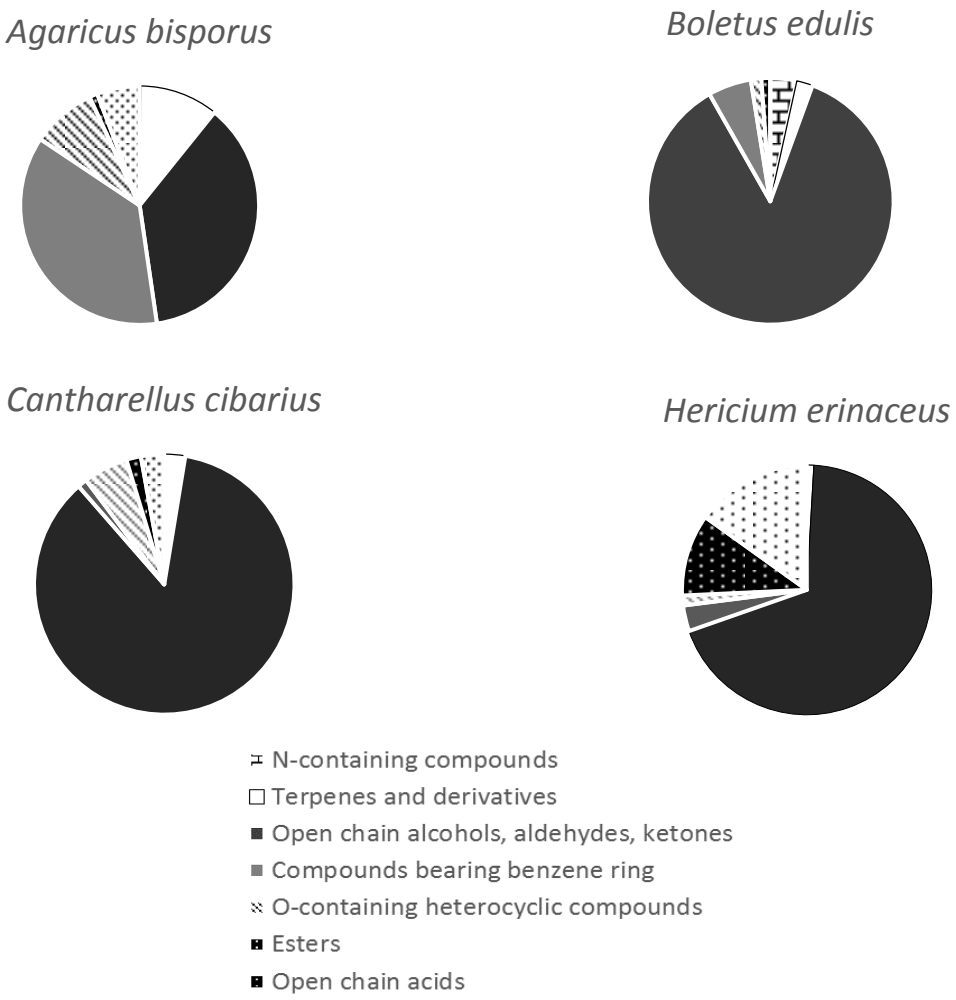

Figure 2.: The main volatile compound groups of the fungi

CONCLUSIONS

The characteristic fragrance composition of individual mushroom/plant species and varieties can be applied to identify the raw materials in food products. This identification has crucial importance in the examinations of food specialties. This method can namely help in revealing adulterations, when some valuable products (*e.g.* truffles or peculiar mushrooms) are attempted to replace with less expensive ones.

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EFFECT OF COMBINATIONS OF MINIMAL PROCESSING TECHNIQUES ON THE PROPERTIES OF PORK AND BEEF MEAT DETERMINED BY ELECTRONIC NOSE

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SUMMARY

At the development of modern food processes it is important to ensure the microbiological stability of the product with only the slightest damage in the quality parameters. This can be achieved by applying the principle of hurdle technology. The lethality of low temperature heat treatment could be enhanced by combining it with other physical treatments. High hydrostatic pressure technology seems to be promising for this purpose. Studies indicate that the electronic nose is suitable not only for monitoring the sensory properties of the products but also for the detection of microbiological changes during storage. Therefore the aim of our study was to examine -using the electronic nose- the preserved quality of the minced pork and beef meat right after the treatments and during storage (2 or 8C for 3 weeks) Samples were treated in combinations of low-temperature heat treatment (60°C 1 hour) and high hydrostatic pressure treatment (300 or 600 MPa, 5min).

INTRODUCTION

Food-quality and food-safety are the most important factors influencing consumer choices, and are also primary factors for food manufacturers and distributors in modern times. High hydrostatic pressure (HHP) technology, as a non-thermal preserving technique capable of inactivating or eliminating pathogenic and food spoilage microorganisms while retaining the valuable components of foods, has the potential to serve both purposes simultaneously. When using HHP treatment, an elevated pressure is applied in an instantaneous way throughout the product and subjects foods to 100-1000 MPa. Due to the instantaneously transmitted pressure, processing time and conditions are independent of the volume and shape of the treated sample (Gupta & Balasubramaniam, 2012). HHP processing can inactivate vegetative form of microorganisms efficiently unfortunately the spores and some types of the food enzymes show high pressure resistance.

Sous-vide cooking is a generally applied preservation technique in the catering industry to prepare ready to eat meals with enhanced shelf-life. The vacuum packaging can save the vast majority of the natural flavours and odours of the raw material. Furthermore the heat treatment at moderate temperature for long time has special effects on the characteristics of the raw materials derived from plant and livestock origin (Baldwin, 2012). This mild treatment is not suitable to inactivate all microbes in the food so the treated products have to be cooled down very fast right after the treatment and have to be kept at low temperature (below 3°C) to avoid the growth of the pathogens and their toxin production.

Beside the importance of novel food processing techniques, the monitoring of the olfactory properties of treated products plays also a main role. The electronic nose can be used on a very wide spread field of the product- and technology development e.g. food and drink, perfumery, household products, environmental monitoring, tobacco, smoke, health and pharmaceuticals, as well as quality control, process monitoring, shelf life and food industrial investigations (Hartyáni et al., 2013).

Since both preservation techniques have advantages and disadvantages their combination can provide longer shelf-life or less rigorous conditions in the cold chain. Therefore the aim of our study was to examine the preserved quality of the minced pork and beef meat right after the treatments and during storage (2°C or 8°C for 3 weeks) where the changes was monitored by electronic nose. Samples were treated in combinations of low-temperature heat treatment (60°C 1 hour) and high hydrostatic pressure treatment (300 or 600 MPa, 5min).

MATERIALS AND METHODS

Pork chop and beef round were purchased at a local market and was grinded using a Robot-Coupe grinder. 40-50g portion was measured into 90 µm PA/PE poaches than vacuum packaged using a Multivac C100 V.S. machine.

Meat samples were pressurized at 300 or 600 MPa for 5 minutes at room temperature in a RESATO FPU-100-2000 HHP unit (Resato International B.V., the Netherlands, **Figure 1**.). The pressure transmission fluid was glycol-oil mixture (Resato PG fluid, Roden, Holland). The pressure build-up rate was 100 MPa/min. Build-up and decompression times were not included in the treatment time. The rate of pressure build-up was so gentle that the increase of the temperature of the system (samples and pressure transmission fluid) was maximum 10°C. The sous-vide (LT-LT) treatment was carried out in a water bath (Labor Műszeripari Művek LP507/1). The thermostat was set at 60°C and the treatment lasted 1 hour. After heat treatments the samples were cooled in iced water.

Figure 1. Resato FPU 100-2000 high hydrostatic pressure food processor

Table 1. The applied treatment combinations

Treatment	1st treatment	2nd treatment
Sous- vide (SV)	60 °C, 1 h	
HHP 300	300 MPa, 5 min	
HHP 600	600 MPa, 5 min	
SV+ HHP 300	60 °C, 1 h	300 MPa, 5 min
SV+ HHP 600	60 °C, 1 h	600 MPa, 5 min
HHP 300+ SV	300 MPa, 5 min	60 °C, 1 h
HHP 600+ SV	600 MPa, 5 min	60 °C, 1 h

Heat- and HHP treatments were applied alone on the meat samples and they combinations were also performed in different order as **Table 1.** shows. After treatments the samples were put into a cooling cabinet (J 600-2, Thermotechnika Ker. Ltd., Hungary) then stored for 3 weeks at 2°C and 8°C. These temperatures were chosen to be below and to exceed the recommended <3°C storage parameter. Samples were analysed on day 0 and at the end of the storage period.

Head space analysis of meat samples was performed by an NST3320 type electronic nose (Applied Sensor A.G., Sweden, **Figure 2.**) with a built-in headspace auto sampler for 12 samples. The sample chamber contains 23 different sensors, software for collecting and processing the data of the specimen. Ambient air was used as reference gas for the sensors, which was filtered through a silica gel column and a combined moisture/hydrocarbon filter. The gas flow rate of the dynamic sampling was set to 50 ml/min. 3,5g amount of samples were filled into headspace vials (volume 30 ml). The vials were sealed with a Teflon-coated butyl septum and a screw cap. Three vials were filled per meat sample. One vial was measured three times so the results of 9 measurements were used for the statistical analysis.

Figure 2. NST 3320 electronic nose

The standby temperature, at which the samples are kept until their incubation phase starts, was set to 20 °C. The samples were equilibrated at 30 °C for 15 minutes. Then reference air was pumped over the sensor surfaces for 10 s (baseline) followed by the puree head-space for 30 s (sampling time) while the sensor signals were recorded. After sample analysis, the recovery phase of the sensors was set to 260 s including the flush time of the gas lines (60 s) with filtered air prior to the next sample injection to allow the re-establishment of the instrument baseline. The total cycle time per sample was 300 s.

The pattern recognition technique used in this work was canonical discrimination analysis (CDA). All statistical analyses were computed, created, and performed by SPSS 20.0 statistical software. Considering the relatively low number of samples, the classification models were validated by cross validation.

RESULTS AND DISCUSSION

Figure 3. shows the results of CDA of electronic nose measurement of samples of pork and beef meat.

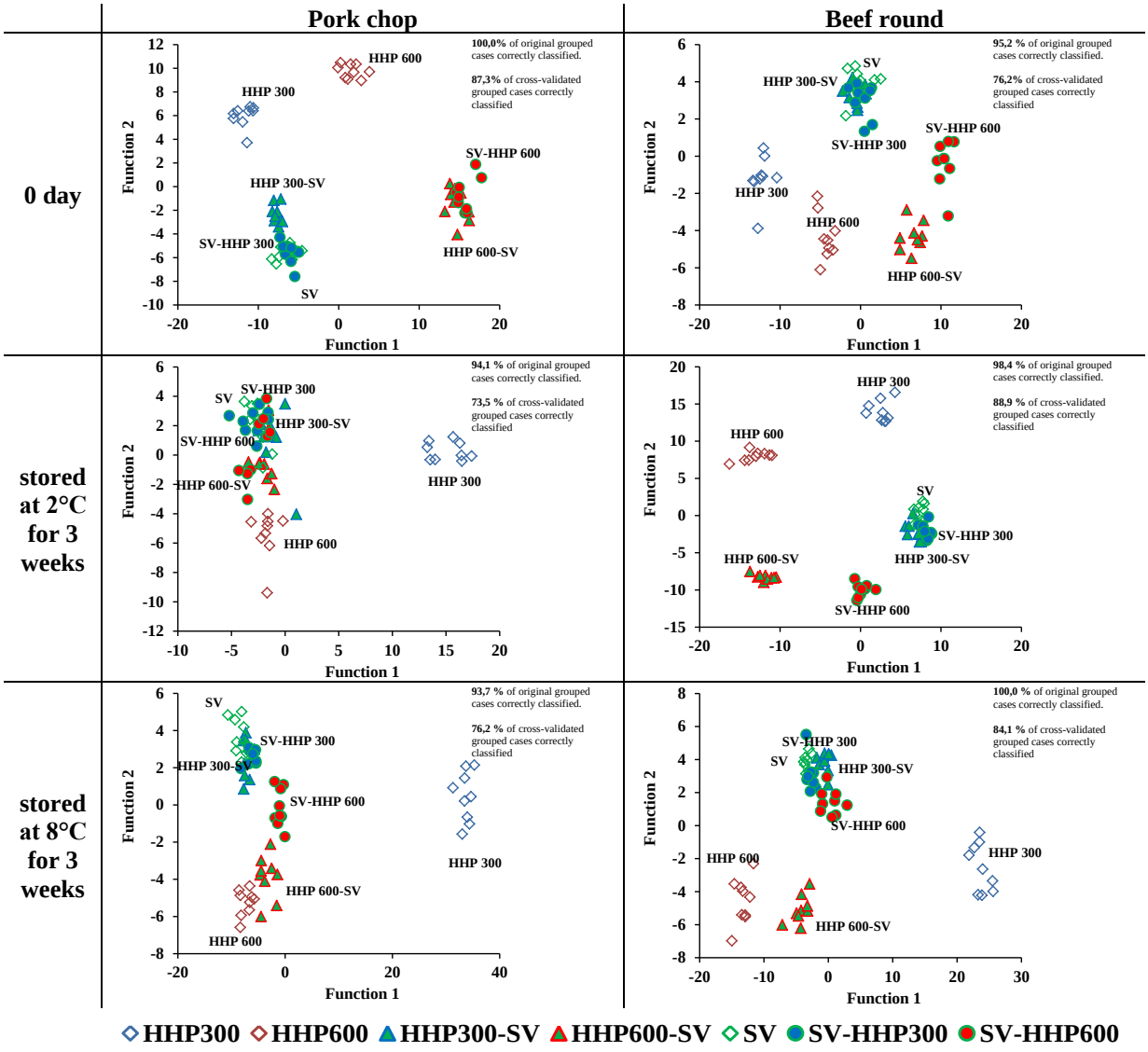

Figure 3. Results of canonical discriminant analyses based on the sensor responses of electronic nose in case of pork chop and beef round treated by different combinations of sous-vide treatment (at 60°C for 1 hour) and high hydrostatic pressure processing (at 300 or 600 MPa for 5 min) at the beginning and at the end of the storage period (at 2 or 8°C for 3 weeks

The electronic nose was able to differentiate each treatment efficiently. During the cross validation the rate of the correctly classified samples were ranged from 73.5 to 88.9. In all cases we got better classification ratio at beef loin samples which mean that pronounced changes were seen at this meat although the differences were not significant between the results of the two types of meat.

Right after the treatments the 7 groups clustered 3 major clusters in case of both meats. Based on the volatiles of the samples the treatments located close to each other on the discriminant space were more similar to each other than to the other treatments. The groups of SV, SV-HHP300 and HHP300-SV formed a clearly separated subgroup, SV-HHP600 and HHP600-SV formed another one although the two groups separated from each other at the beef meat. The HHP300 and HHP600 groups, where the pressure treatments were only applied, separated definitely from those groups where heat treatment was applied alone or in a combination although the separation was more significant at the beef loin since the separation can be done along the first discriminant direction while in case of pork chop the pressure-treated groups shifted away from the other groups along the second discriminant direction.

After the storage for 3 weeks at 2°C we could observe almost the same grouping at the beef loin as it could be seen at the 0 day samples. In case of the pork chop bigger changes in the location of the groups were noticed. The HHP300 treatment separated definitely from all other treatments. It is assumed that the microorganisms which survived this low intensity treatment produced off-flavour compounds. This our theory was confirmed by the results of 8°C storage since HHP300 groups were also shifted away in case of pork chop and beef round, respectively. This indicated that 300 MPa was not adequate to stabilize microbiological conditions over 3 weeks storage, and it showed that the low-temperature cold storage has significant role in the preservation of the quality. The eligibility of combined treatment could be concerned since while the pressure treatment at 300 MPa couldn't grant a stable quality during the storage at 8°C its combination with the sous-vide treatment was effective to ensure this requirement.

CONLUSIONS

Based on the results it can be concluded that the electronic nose was capable of distinguishing pork and beef samples treated by the combinations of sous-vide treatments and HHP processes so the changes can be monitored objectively during storage. The results of the experimental work have pointed out that better preservation of food quality can be achieved during the chilled storage by application of combined treatments.

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EFFECTS OF COMBINATIONS OF MINIMAL PROCESSING TECHNIQUES ON THE PROPERTIES OF SEASONED PORK MEAT DETERMINED BY ELECTRONIC NOSE

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SUMMARY
Studies showed that both the sous vide and the high hydrostatic pressure treatments have beneficial effect on the aromas of the treated products. In some cases stronger intensity could be observed after the treatments. Changes in the volatiles of minced pork and beef meat could be monitored well with the electronic nose. Our question was how the combined heat and high hydrostatic pressure treatments affected the characters of the spice added to the meat before treatments. Therefore, minced pork meat was seasoned with thyme or paprika powder and was heat treated at 60C for 1 hour or pressurized at 300 or 600MPa for 5 minutes or these treatments were applied in combinations. We investigated the changes right after the treatments and at the end of the storage period (at 8C for3 weeks)

INTRODUCTION
Food-quality and food-safety are the most important factors influencing consumer choices, and are also primary factors for food manufacturers and distributors in modern times. High hydrostatic pressure (HHP) technology, as a non-thermal preserving technique, applies 100-1000 MPa of pressure to inactivate pathogenic and food spoilage microorganisms while retaining the valuable components of foods. Sous-vide cooking is a applied preservation technique in the catering industry to prepare ready to eat meals with enhanced shelf-life. It is a method of cooking in which food is sealed in airtight plastic bags then placed in a water bath or in a temperature-controlled steam environment for longer than normal cooking times at an accurately regulated temperature much lower than normally used for cooking. After cooking if the meal is not consumed immediately it has to be cooled down as fast as possible and be kept under 3°C.

Both processing have a common advantage, namely, the food is treated in the packaging so the treated product cannot be contaminated from the environment unless the packaging is damaged. Application of the treatment in package has another benefit. The vacuum packaging can save the vast majority of the natural flavours and odours of the raw material. Studies showed that in some cases stronger intensity could be observed after the treatments. Changes in the volatiles of minced pork and beef meat could be monitored well with the electronic nose. For example, electronic nose was suitable to monitor the changes in beef meat samples which were treated by different kind of preservatives (lactic acid, sodium ascorbate, potassium lactate, potassium sorbate) and were stored under modified atmosphere at chilled storage to enhance the shelf-life of the raw meat. (Friedrich et al., 2008) This non-destructive, rapid method was also appropriate to compare the effects of different novel preservation techniques on food. Hartyáni applied high hydrostatic pressure and pulsed electric field processing to inactivate *Alicyclobacillus acidoterrestris* cells in apple and orange juice and by electronic nose she could monitor well how the surviving microbes produced off-flavour in the food matrix after treatments (Hartyáni 2012.) Dalmadi and his co-workers could observed the effects of HHP treatment and heat pasteurization on volatile composition of raspberry, strawberry and blackcurrant purees by electronic nose (Dalmadi et al., 2007). Influences of sous-vide and HHP treatments were also compared in case of grinded pork chop and beef round (Dalmadi et al, 2015) and pork patties (Kenesei et al., 2015).

In our study we aimed to use electronic nose to examine how the combined heat and high hydrostatic pressure treatments affected the characters of the spiced pork meat.

MATERIALS AND METHODS

Figure 1. Resato FPU 100-2000 high hydrostatic pressure food processor

Pork chop was purchased at a local market and was grinded using a Robot-Coupe grinder. One third of the meat was seasoned with dry paprika powder (4g paprika / 1000g meat) another third was seasoned with dry thyme (4g thyme / 1000g meat) and the rest of the meat was left without addition of any spice. 40-50g portions were measured into 90 µm PA/PE poaches than vacuum packaged using a Multivac C100 V.S. machine.

Meat samples were pressurized at 300 or 600 MPa for 5 minutes at room temperature in a RESATO FPU-100-2000 HHP unit (Resato International B.V., the Netherlands, **Figure 1**.). The pressure transmission fluid was glycol-oil mixture (Resato PG fluid, Roden, Holland). The pressure build-up rate was 100 MPa/min. Build-up and decompression times were not included in the treatment time. The rate of pressure build-up was so gentle that the increase of the temperature of the system (samples and pressure transmission fluid) was maximum 10°C. The sous-vide (LT-LT) treatment was carried out in a water bath (Labor Műszeripari Művek LP507/1). The thermostat was set at 60°C and the treatment lasted 1 hour. After heat treatments the samples were cooled in iced water.

Heat- and HHP treatments were applied alone on the meat samples and their combinations were also performed in different order as **Table 1.** shows. After treatments the samples were put into a cooling cabinet (J 600-2, Thermotechnika Ker. Ltd., Hungary) then stored for 3 weeks at 8°C. This temperature was chosen to exceed the recommended <3°C storage temperature so the efficiency of the combined treatment could be evaluated at this non-ideal storage condition. Samples were analysed on day 0 and at the end of the storage period.

Table 1. The applied treatment combinations

Treatment	1st treatment	2nd treatment
Sous- vide (SV)	60 °C, 1 h	
HHP 300	300 MPa, 5 min	
HHP 600	600 MPa, 5 min	
SV+ HHP 300	60 °C, 1 h	300 MPa, 5 min
SV+ HHP 600	60 °C, 1 h	600 MPa, 5 min
HHP 300+ SV	300 MPa, 5 min	60 °C, 1 h
HHP 600+ SV	600 MPa, 5 min	60 °C, 1 h

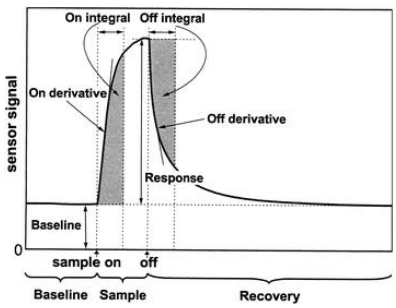

Figure 2. Sensor response of NST 3320 electronic nose

Head space analysis of meat samples was performed by an NST3320 type electronic nose (Applied Sensor A.G., Sweden, Dalmadi et al, 2015) with a built-in headspace auto sampler for 12 samples. The sample chamber contains 23 different sensors, software for collecting and processing the data of the specimen. Ambient air was used as reference gas for the sensors, which was filtered through a silica gel column and a combined moisture/hydrocarbon filter. The gas flow rate of the dynamic sampling was set to 50 ml/min. 3,5g amount of samples were filled into headspace vials (volume 30 ml). The vials were sealed with a Teflon-coated butyl septum and a screw cap. Three vials were filled per meat sample. One vial was measured three times so the results of 9 measurements were used for the statistical analysis.

The standby temperature, at which the samples are kept until their incubation phase starts, was set to 20 °C. The samples were equilibrated at 30 °C for 15 minutes. Then reference air was pumped over the sensor surfaces for 10 s (baseline) followed by the puree head-space for 30 s (sampling time) while the sensor signals were recorded (**Figure 2.**). After sample analysis, the recovery phase of the sensors was set to 260 s including the flush time of the gas lines (60 s) with filtered air prior to the next sample injection to allow the re-establishment of the instrument baseline. The total cycle time per sample was 300 s.

The pattern recognition technique used in this work was canonical discrimination analysis (CDA). All statistical analyses were computed, created, and performed by SPSS 20.0 statistical software. Considering the relatively low number of samples, the classification models were validated by cross validation.

RESULTS AND DISCUSSION

Figure 3. shows the results of CDA of electronic nose measurement of samples of unseasoned and seasoned pork meat. The electronic nose was able to differentiate each treatment efficiently. During the cross validation the rate of the correctly classified samples were ranged from 74.6 to 87.3. In all cases we got better classification ratio at 0 day samples which means that pronounced changes were seen at the beginning of the storage period although the differences were not significant.

Right after the treatment in all three cases the SV-HHP600 and HHP600—SV groups were separated from the other 5 groups and this separation happened along the first discriminant function which indicated that the combined treatment carried out at higher pressure level had the biggest effect on the volatiles detected by electronic nose. Another common characteristic of the unseasoned and seasoned samples that the HHP300 and HHP600 groups formed a subgroup on the CDA map and these 2 treatments shifted away from the other treatments along the second discriminant function. In all other cases the samples were heat treated alone or in a combination so they probably had cooked flavour character. Meanwhile it was interesting that SV-HHP600 and HHP600—SV treatments caused higher modification in the volatiles than HHP300 or HHP600 treatments did. This phenomenon was also observed at beef meat in our other study where unseasoned beef round were treated with the same treatments and the SV-HHP600 and HHP600—SV treatments had the biggest effect on the volatiles as well (Dalmadi et al. 2015). At the beef sample the HHP300 or HHP600 treatments were also clearly separated from the other treatment though they shifted away on the axis of the first discriminant function.

After the storage for 3 weeks at 8°C we could observe that in all three cases the HHP300 group located at the other part of the CDA map. It is assumed that the microorganisms which survived this low intensity treatment produced off-flavour compounds during storage. Pressure level of 300 MPa was not adequate to stabilize microbiological state for a 3 weeks storage at 8°C. This tendency was observed at beef samples too (Dalmadi et al. 2015). When the samples had been seasoned with paprika powder the samples, treated merely 600 MPa, were also shifted away together the HHP300 samples from the heat treated variants. Probably the paprika powder had contaminated by high number of microorganisms and the relatively high temperature of storage promoted their growth. The eligibility of combined treatment could be concerned since while the pressure treatment at 300 MPa couldn’t grant a stable quality during the storage at 8°C its combination with the sous-vide treatment was effective to ensure this requirement.

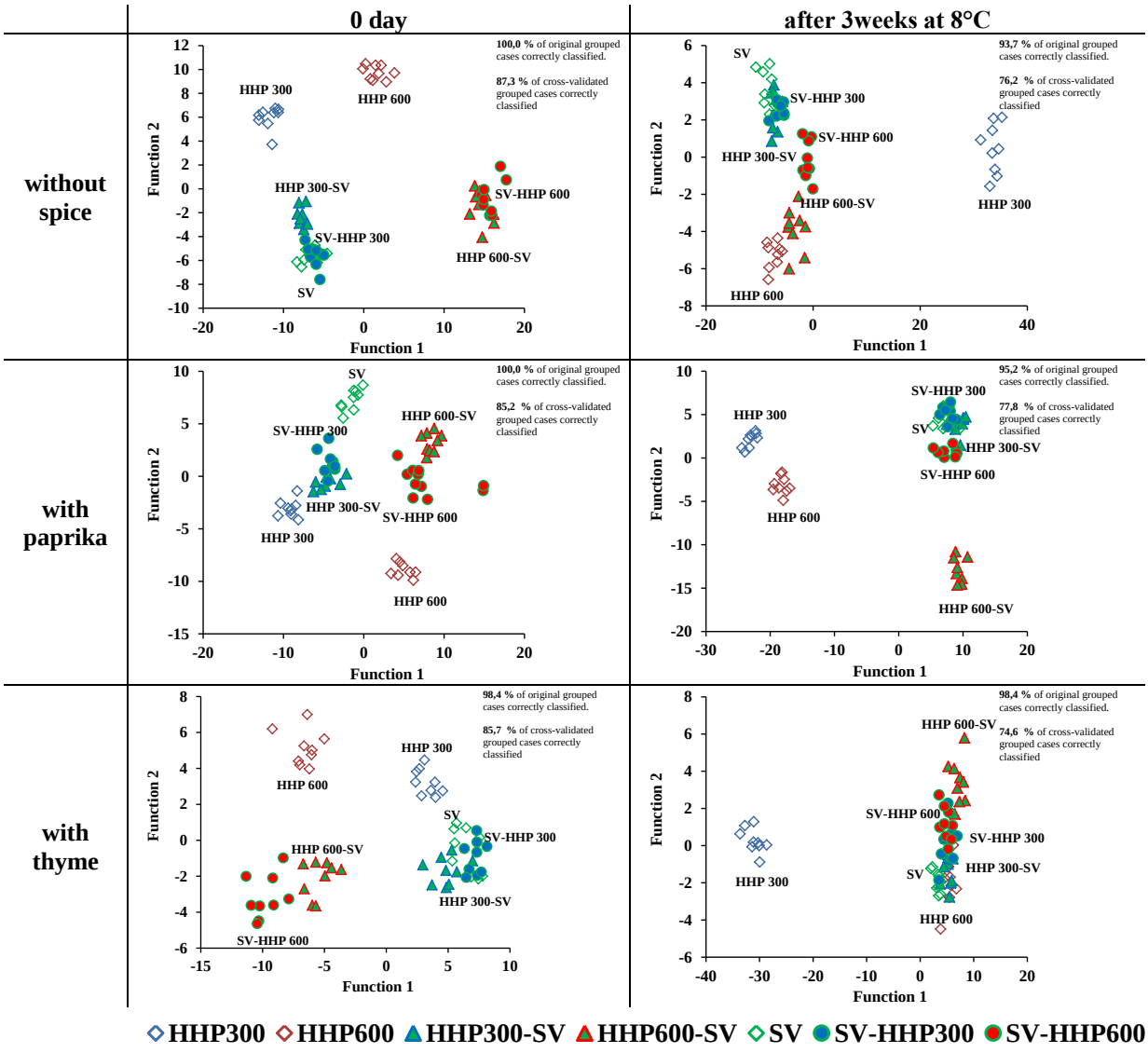

Figure 3. Results of canonical discriminant analyses based on the sensor responses of electronic nose in case of unseasoned and seasoned (with paprika powder or thyme) pork chop treated by different combinations of sous-vide treatment (at 60°C for 1 hour) and high hydrostatic pressure processing (at 300 or 600 MPa for 5 min) at the beginning and at the end of the storage period (at 8°C for 3 weeks)

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PRODUCTION OF LOW-PHOSPHATE FRANKFURTER WITH MICROBIAL TRANSGLUTAMINASE

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SUMMARY

The aim of this study was to show how microbial transglutaminase (mTG) can substitute the phosphate due to it is capability to improve texture through waterbindig. Frankfurters was made with 0.1%, 0.3%, 0.5% and 0.7% tetrasodium pyrophosphate and partly enzyme-treated with 0.2% commercial mTG enzyme preparation. The changes in colour and texture were analysed by pork meat batters and frankfurters with objective (extrusion, hardness, crunchiness) and subjective (sensory analysis) methods. Furthermore the quality of frankfurters were examined by measurement of water holding capacity (WHC). Our results show that mTG is capable to significantly improve hardness and crunchiness by frankfurtrs made with 0.1% phosphate addition. The cross-linking effect of mTG led to Our sensory evaluation suggests that mTG and phosphate should be applied in combination in order to have a recognisable more homogenous texture by the final product.

INTRODUCTION

Phosphates are generally applied for increasing water holding capacity (WHC) and cooking yield (Wang, 2009). However water-holding of phosphate is found to be limited at 0.4% concentration by emulsified sausages (Lü, 2000; Wang, 2009). Phosphate influences the hardness and chewability therefore the consumer acceptance of emulsified sausages as it increases the muscle protein extraction which leads to stronger gel matrix (Candogan, 2003). The most widespread functional phosphates are diphosphates (example: tetrasodium pyrophosphate, TSPP) as they act on actomyosin complex of meat protein at once and have high pH value (Nguyen, 2011). Moreover phosphates slow down the growth of some gram-positive bacteria (Bunková, 2008; Feiner, 2006). Recently there is a great attention focused on the reduction of salt and phosphate by meat products (Allais, 2010), which can be achieved by the crosslinking of protein molecules with calcium independent microbial transglutaminase (mTG, protein-glutamin γ-glutamyltransferase, E.C. 2.3.2.13). Due to the intra- and inter-molecular covalent bonds catalysed by mTG, the formed myofibrillar protein polimers can restore the texture of pale soft exudative (PSE) pork meat (Milkowski, 1999). Our aim was to analyse the simultaneous effect of 0.1-0.7% TSPP and 0.2% mTG. Furthermore we studied how phosphate could be replaced by mTG to produce a low-phosphate frankfurter with adequate texture.

MATERIAL AND METHODS

Manufacturing of frankfurters

Pork meat batter was prepared as following: half portion (235 g) of minced pork meat (80% purity) was ground first with half portion (180 g) of ice cubes, then additives and seasonings were added in four 1-minute-long steps (1.step: 0.1-0.7% Na-soluprate (TTSP, Solvent Inc., Hungary), 2. step: 1. 8% salt (nitrite curing salt containing 0.4-0.5% NaNO2, Salinen Austria AG., Austria) 3. step: remaining half portion of minced pork meat, 4. step: 1.25% ground dried paprika (Kalocsai Fűszerpaprika Ltd., Hungary), 1.2% ground white pepper (importer: R-Coop 3 Inc., made in Indonesia), 160 g of pork back fat (5 mm particle size), 5. step: 0.2% mTG (Probind TX, nominal activity: 85-120 U/g, BDF Natural Ingredients, Girona, Spain). The meat batters were partly analyzed (for surface colour,

extrusion, adhesiveness) partly stuffed into 21 mm diameter cellulose casings (Kalle Hungária Ltd., Hungary) and hand-linked. Frankfurters were pre-dried (60 °C, 15 min), cooked (until 72 °C core temperature) and cooled in shower (until 10 °C core temperature) in CS350 EL type smoking machine (Korax Ltd., Hungary). After drainage frankfurters were cooled to 5 °C and stored in vacuum-package at the same temperature for further investigation. Commercial frankfurter with 47% pork meat content and 0.5% phosphate concentration was also analysed for comparison.

Surface colour

CIELAB values (lightness, L*; redness, a* and yellowness, b*) of pork meat batters and frankfurters were evaluated on a Minolta CHROMA METER CHR-400 tristimulus colour measuring system (Konica Minolta Sensing Inc., Japan). Ten replicates of the analysis were performed for each formulation.

Texture Profile Analysis (TPA)

Extrusion and adhesiveness by pork meat batter

Extrusion and adhesiveness was measured with the conical measuring head of TA. XTPlus (Stable Micro Systems, Great Britain). The cross-head pushed the 90° cone probe of spreadability rig with 2 mm/sec speed into the sampling holder. The meat batter samples were tempered to 12 °C and the measuring time was 90 sec. Three replicates of each samples were evaluated using the official software of the instrument called Texture Exponent 32.

Hardness and crunchiness by frankfurters

Hardness was also measured with TA. XTPlus (Stable Micro Systems, Great Britain). Ten frankfurter cores (diam. = 21 mm, height = 12 mm) were axially compressed to 70% of their original height. Force–time deformation curves were derived from a 500N load cell applied at a crosshead speed of 2mm/s. The samples were tempered to 12 °C and the measuring time was 2 min. Crunchiness was measured with 2 mm cylinder probe with the above mentioned crosshead speed till 25 mm penetration. Frankfurters were cooked 1 min in boiling water just before measurement. Samples were fixed to the base with sample holder and 5 cm surface was analyzed through casing. The averages are results of at least 3 replicates.

Water holding capacity (WHC)

Water holding capacity (WHC) was measured according to Grau-Hamm method on pork meat batter.

Sensory analysis

After storage overnight the samples were evaluated with a trained panel (10 members) with difference test focusing on texture. Texture attributes as homogeneity of cut surface, springiness and porosity were ranked on a -5-0-5 score scale compared to reference sample, which was anchored at the exact middle of the scale (0 point).

RESULTS AND DISCUSSION

Main characteristics of low-phosphate pork meat batters

The enzyme treatment led to recognisable colour change at all phosphate concentrations (see Total Colour Difference, ΔE values in Table 1**)**. The extrusion force shows how easy is the stuffing of the meat batter.

The texture of meat batters made with industrially applied phosphate addition (0.3%) could not be enhanced by mTG, however WHC had 30% higher values because of the built protein network.

Table 1.: Technofunctional properties of pork meat batters depending on phosphate level

Phosphate level (%)	**ΔE**	**Extrusion** enhanced by mTG (%)	**Adhesiveness** enhanced by mTG (%)	**WHC** enhanced by mTG (%)
0.1	2.25	-2	2	20
0.3	1.29	1	1	30
0.5	3.35	79	88	0
0.7	0.74	20	25	0

Main characteristics of low-phosphate frankfurters

The enzyme treatment led to recognisable colour difference by all frankfurters, which were due to the more reddish and yellowish colour caused by mTG (data not shown). The mTG enzyme led to more hardness (11-16%) independent from actual phosphate concentration of the samples (Table 2.). We suggest the enzyme-treatment by 0.1-0.3% phosphate addition as mTG had in that interval the most significant effect on springiness and crunchiness of frankfurters. The enzyme treated 0.1% phosphate frankfurter had -3% hardness, 15% more springiness and 8% more crunchiness compared to commercial frankfurter with 0.5% phosphate level (data not shown).

Table 2.: Technofunctional properties of frankfurters depending on phosphate level

Phosphate level (%)	**ΔE**	**Hardness** enhanced by mTG (%)	**Springiness** enhanced by mTG (%)	**Crunchiness** enhanced by mTG (%)
0.1	2.08	13	4	19
0.3	2.43	11	2	69
0.5	1.35	16	7	42
0.7	3.26	16	11	16
Commercial (0.5)	1.58	23	11	37

Sensory analysis of low-phosphate frankfurters

The sensory evaluation shows the effect of enzyme treatment compared to the control at the different phosphate levels (Figure 1.). Porosity refers to the recognised air bubbles in the cooked meat batter. This could be eliminated with the help of mTG if at least 0.3% phosphate was added. Springiness was observed by touching the frankfurter cores between 2 fingers. The mTG could enhance springiness independent from phosphate levels. Among texture properties homogeneity was also enhanced by mTG, however the differences were noted rather at lower (0.1-0.3%) phosphate levels.

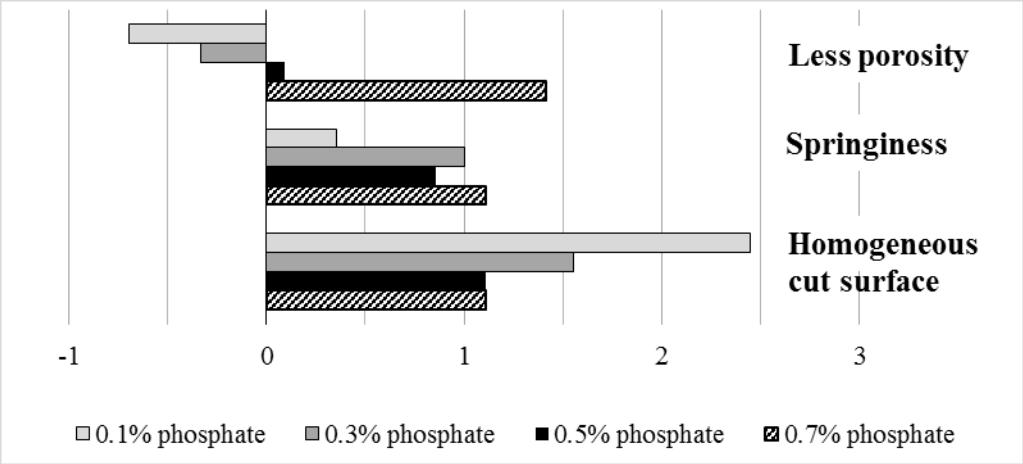

Figure 1.: Difference in sensorial parameters of frankfurters due to enzyme treatment

CONCLUSIONS

The results proved that 0.2% mTG may increase the water holding capacity with 20% by low-phosphate (0.1-0.3%) frankfurters and it also contributes to more homogeneous cut surface. According to our comparison with commercial product, low-phosphate (0.1%) enzyme-treated frankfurters have adequate hardness, and improved springiness and crunchiness, which is a promising sign for product development in the future.

ACKNOWLEDGEMENT: *We take this opportunity to express our profound gratitude for Krisztina Horti for optimization of texture measurements and Márta Csukáné-Nemes for her technical support. We thank for BDF Natural Ingredients to provide the commercial mTG preparation used for this study.*

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EFFECT OF TRANSGLUTAMINASE ON TEXTURE OF SEMI-HARD CHEESE DURING RIPENING

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SUMMARY

The purpose of our study was to investigate how microbial transglutaminase (mTG) can enhance cheese yield depending on fat level of cheese milk. Therefore trappist like semi-hard chesse samples were produced from 2.8%; 3.5%; 5% cows' milk. The effect of ripening was evaluated with Texture Profile Analysis (TPA) and sensory evaluation (scoring test, 10 trained panelists). The highest cheese yield improvement caused by mTG was 30% by 2.8% cows' milk. Springiness and cohesiveness values were significantly higher by enzyme-treated semi-hard cheese samples at lower milk fat levels. The decreasing in cohesiveness due to ripening was slowed down by mTG treatment as cross-binding led to more stable structure. Sensory evaluation showed that the enzyme-treatment led to higher scores by cheese samples made from 3.5% and 5% cow's milk.

INTRODUCTION

The ripening state of semi-hard cheese was mainly followed by imaging of the development of eye-growth (Huc, 2014a; Soodam, 2014). This technique has even more promising results if it is coupled with CO2 release measurement (Huc, 2014b). However the most evident and simple method seems to be the follow-up of texture modification during cheese ripening. However to our best knowledge microbial transglutaminase (mTG, protein-glutamin γ-glutamyltransferase, E.C. 2.3.2.13), affecting texture properties such as hardness of semi-hard cheese, was only analysed at selected ripening states: after 1 week (Cozzolino, 2003), 4 weeks (Aaltonen, 2014; Di Pierro, 2010), 24 weeks (Aaltonen, 2014), but not monitored during the whole ripening period. Therefore our main objective was to follow changes of hardness due to mTG during 4-10 weeks of ripening. Aims were extended with investigation how the fat level influences the texture modifying properties of mTG

MATERIAL AND METHODS

Manufacturing and ripening process of semi-hard cheese

Semi-hard cheese samples were made from 2.8 % fat pasteurized cows' milk and 30% fat whipping cream (Dabas Tej Ltd., Dabas, Hungary) was used to set the milk fat level to 2,8%, 3,5% and 5% fat. The milk was then heated up to 30 °C in a 10 L Armfield FT-20A type cheese-vat (Bridge House, Great Britain) and 0.025% (w/v) $CaCl_2$ (Sigma Aldrich Ltd., Budapest, Hungary) was added. A stirrer (14 rpm) was used for proper mixing during the whole process, except during curdling. In case of enzyme treated samples, 0.01% (w/v) mTG (Probind CH, nominal activity: 58 U/g, BDF Natural Ingredients, Girona, Spain) was applied. A freeze-dried concentrated lactic starter culture consisting of *Lactococcus lactis subsp. lactis*

Lactococcus lactis subsp. cremoris, *Lactococcus lactis subsp. lactis biovar. diacetylactis*, *Streptococcus thermophilus* was used as cheese culture (MA 4001, LYO 5DCU, Danisco, Epernon, France). After 15 min incubation 0.05 v/v% chymosin (Présure Simple Brun, Danisco, Melle, France) was added. Stirring was stopped 5 min after chymosin was added. After cutting, curd was heated until 39 °C to help whey separation. As a last step of curdling, whey was separated. The curd was pressed 24 h with a force of 20 kg per kg cheese and then cured in 20% (w/v) NaCl solution for one day.

The desiccation lasted 1 day, after which cheese was put in Flexo-vacuum PS650 five-layers vacuum package (oxygen permeability: 100 cm^3 m^{-2} 24 h^{-1}, steam permeability: 6 g m^{-2} 24^{-1}) and ripened at 13 °C for 10 weeks.

Determination of main characteristics of semi-hard cheese samples

The pH was measured once, after 4 weeks ripening, with a Testo 206 portable pH meter (Testo AG., Limburg an der Lahn, Germany). Dry matter content was measured both in cheese (after 4 weeks) and whey (on the day of production) with KERN MLS-N Moisture Analyser (Kern & Sohn GmbH, Balingen, Germany) using 105 °C as the drying temperature. The fat content of cheese samples were determined by Lindner method (Seidel, 1993) after 4 weeks. The protein content of cheese whey was determined by Lowry method (Lowry, 1951).

Texture Profile Analysis (TPA)

The samples were measured after 4, 6, 8 and 10 weeks of ripening. Each cheese was cut into horizontal halves, and 10 samples of 12 mm in height were taken from the center towards the rind, with a core sampler 21 mm in diameter. The prepared cheese core samples were tempered at 12 °C before TPA analysis. TPA analysis was performed with TA.XTPlus (Stable Micro Systems, Godalming, UK) Texture Analyser used with 500 N load cell. The cheese core samples were axially compressed two times to 70 % of their original height with 20 mm cylinder probe at a crosshead speed of 2 mm/s. Force–time deformation curves were evaluated with Texture Exponent 32 as the given software of TA.XTPlus and the springiness parameter was selected for analysis.

Sensory analysis

Sensory evaluation with 20 points Scoring Test was applied to evaluate the samples according their appearance (3), inner colour (3), odour (3), taste (5), texture (3), cheese eyes (3) using a comprehensive vocabulary of these descriptors (see in MSZ 12280-87). Sensory sessions were organised for at least 10 trained panellists.

RESULTS AND DISCUSSION

Main characteristics of semi-hard cheese samples

Our result highlights the effect of mTG on cheese yield, which is reduced by higher milk fat level (see Table 1). Due to the incorporation of whey proteins into the product the dry matter content of whey decreased which led to values by the final product. However mTG incorporated less fat into the cheese than expected. The pH of cheeses were lower than ideal (pH 5.2, Hui, 2004) but did not change significantly during 4-10 weeks of ripening (data not shown). Sensory analysis revealed that enzyme-treatment.

Table 1.: Cheese yield and chemical characteristics of semi-hard cheese samples

Cheese type	**Yield** (L milk/kg cheese)	**pH**	**Dry matter** (%)	**Fat content** (%)	**Protein of cheese whey** (%, w/v)	**Sensory analysis** (max. 20 points)
control_2.8%	9.52	4.89±0.08	58.00±0.37	20.44±0.71	1.03±0.01	17
enzyme_2.8%	7.35	4.97±0.05	62.81±0.52	19.23±1.15	0.85±0.01	15
control_3.5%	7.51	4.89±0.04	58.12±0.68	24.50±0.92	0.71±0.01	15
enzyme_3.5%	7.37	4.97±0.09	61.62±0.29	21.48±1.26	0.58±0.02	16
control_5%	6.94	4.97±0.11	61.59±0.45	30.02±1.04	0.70±0.02	12
enzyme_5%	6.88	4.96±0.04	64.14±0.17	28.16±0.49	0.59±0.01	18

Texture Profile Analysis of semi-hard cheese during ripening

The effect of enzyme treatment and fat content of cheese milk was also analysed during ripening with the help of TPA method (70 % compression) focusing on the springiness and cohesiveness of cheese (Fig. 1., Fig. 2.). According to our research results mTG significantly affected the springiness by lower fat levels (2.8% and 3.5%) independent from ripening time.

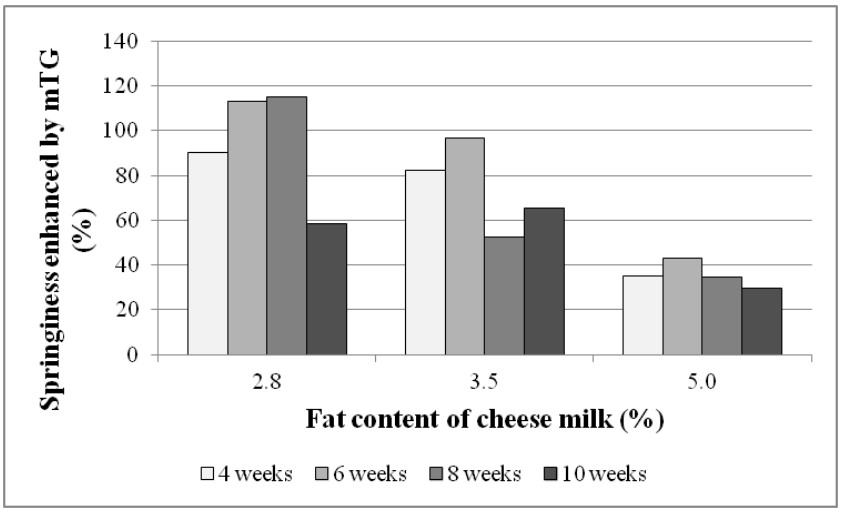

Figure 1.: Effect of fat content of cheese milk on the springiness development caused by mTG

The cohesiveness represents the inner binding force of the studied material. During ripening cheese tend to soften and crumble. The enhanced cohesiveness of samples suggests that the cross-binding effect of mTG leads to structure stabilization by 2.8% and 3.5% fat levels (data not shown).

Sensory analysis

The sensory evaluation showed that 2.8% fat is not sufficient for mTG to enhance textural properties, which probably influenced the panelists in the overall judging (Fig. 2.). We assume that 3.5% fat content is needed for mTG to enhance odour, taste and cheese-eyes development. However the enzyme-treatment could even improve the inner colour and taste of the semi-hard cheese samples when manufactured from 5% cows' milk

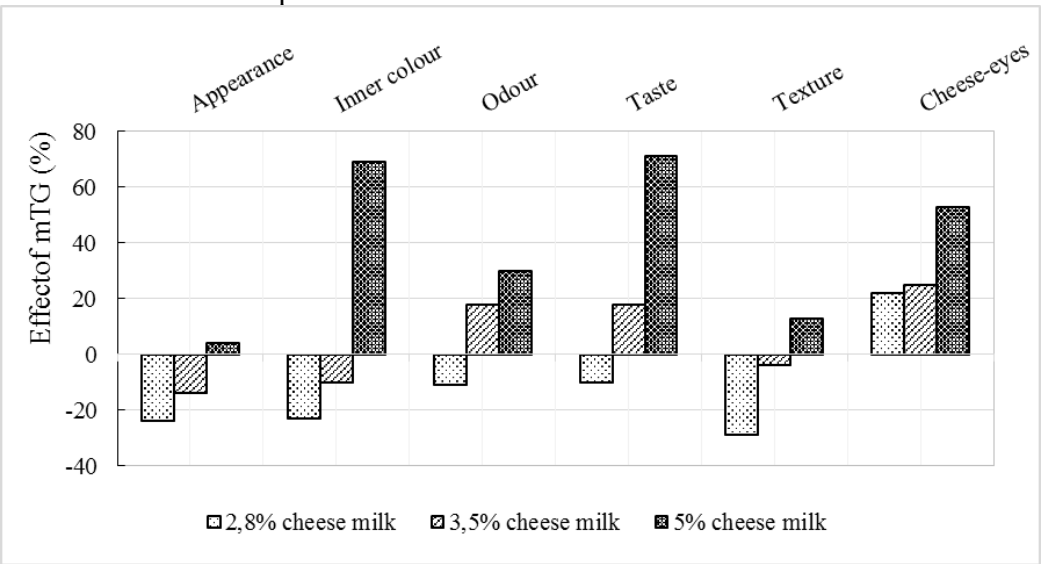

Figure 2.: Changes of sensorial attributes depending on fat content of cheese milk

CONCLUSIONS

The study proved that the cross-binding effect of mTG leads to higher cheese yield independent of milk fat level, which is due to whey incorporation. The enzyme-treated cheese samples has lower fat levels, but have higher springiness and cohesiveness throughout 4-10 weeks ripening time. Sensory analysis revealed that the enzyme-treatment improved nearly all sensory attributes if semi-hard cheese was produced from 3.5% or 5% cows' milk.

ACKNOWLEDGEMENT We thank for BDF Ingredients to provide the commercial mTG preparation used for this study.

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EFFECT OF BACTERIAL ISOLATES FROM FOOD PRODUCING ENVIRONMENT ON FOOD-BORNE PATHOGENIC BACTERIA (FOCUSING ON *SALMONELLA* HARTFORD)

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SUMMARY
Bacterial isolates derived from food processing environment or food materials were screened for potential antagonistic activity against foodborne pathogenic bacteria (Salmonella Hartford, Listeria monocytogenes, Escherichia coli and Yersinia enterocolitica). In case of co-culturing of the 37 isolates with each pathogenic bacterium 16 isolates showed inhibitory effect on one or two pathogens. The antagonistic isolates were identified by miniaturized test and molecular sequencing. Cell-free supernatants of the 16 isolates did not show total growth inhibition of the pathogens, however in some cases decline in the growth could be detected. One of the best inhibitory effects was detected in case of isolate CP-P-5 (Pseudomonas lundensis) on Salmonella Hartford. CP-P-5 was co-cultured with the pathogen in TS broth and plated onto CASO and Salmonella selective Harlequin media after different time intervals. The results showed that inhibitory effect of CP-P-5 isolate could only be detected after 24 hours of incubation when cell concentration of the antagonist was 10^3 times more(1:1000 rate) than Salmonella Hartford.

INTRODUCTION
Consumption of fresh or minimally processed (MP) foods is an increasing trend nowadays. These foods are often contaminated with food-borne pathogens like *Salmonella* spp., *Escherichia coli* or *Listeria monocytogenes* (Leverentz et al., 2006). For ensuring the safety of fresh or MP products certain techniques like heating, freezing, drying, salting, fermentation and smoking cannot be applied because these methods may contribute to changes in the products' sensory properties (Alegre et al., 2012). Preservation with chemicals is a possible method for enhancing the safety of food products, nevertheless consumers reject the application of these compounds in foods more frequently. Biocontrol can be an alternate technique to inhibit the growth of food-borne pathogens and ensure food safety without affecting nutritional values of the products.

The best sources of biocontrol microorganisms can be their natural environments: the food processing surroundings or the food itself (Leverentz et al., 2006). In several cases the native microbiota on fresh products (fruits or vegetables) contains potential antagonistic microbes against certain food-borne pathogens (Alegre et al., 2013, Trias et al., 2008). The presence of the antagonistic microbes does not mean inhibition in every case because of several environmental factors which can influence the interactions between microbial cells (Cray et al., 2015). To ensure the most effective inhibition of food-borne pathogenic bacteria with antagonists several tests should be done under different environmental conditions for determining the optimal inhibitory parameters.

The aims of this study were the isolation of bacteria from different food processing environments and determination of their inhibitory effects on different food-borne pathogens (*Escherichia coli* O157, *Listeria monocytogenes*, *Salmonella* Hartford and *Yersinia enterocolitica*).

MATERIALS AND METHODS

Isolation and characterization of bacteria

Bacterial isolates were collected from different foods and food processing environments.

Cultivation of the isolates was performed on tryptone-soy (TS) agar (Biokar Diagnostics, Beauvais, France).

For characterization colony morphological (applying WL nutrient agar), physiological (determination of optimal pH and temperature for growth) and biochemical tests (OF and KOH probes) were done.

Inhibition assays

Inhibition assays were carried out by co-culturing the pathogens and the test strains on TSA plates. Ten µl suspensions (approx. 10^6 cells) of the isolates were dropped onto the agar's surface inoculated massively by pathogenic bacteria. The plates were incubated on different temperatures (5, 10, 15, 20, 25, 30, 37 and 42°C) to be able to determine the optimal temperature for inhibition.

Inhibitory effect of 1-, 3- and 6-day-old cell-free supernatants generated from cultures of the isolates in TS broth was tested by Multiskan Ascent (Thermo Electron Corporation) for 24 hours at 25 °C and 595 nm. Inhibitory assays using cell-free supernatants of static and shake flask TSB and PGY cultures were carried out by Multiskan in case of strains which were able to inhibit the growth of *S.* Hartford in previous studies.

Typing and identification of the isolates

For identification of antagonistic bacterial isolates API 20E, API 20NE (Biomérieux) and Crystal GP tests (BBL) were applied. Molecular typing was done by using M13 and OPA 18 primers, while direct sequencing of partial 16S rDNA was used for molecular identification.

Protease and heat treatments of cell-free supernatants, and detection of siderophore production

Inhibitory effect of cell-free supernatants generated after 24 hours incubation of isolate CP-P-5 was tested against *S.* Hartford after protease treatment by proteinase K, protease from *Streptomyces griseus*, trypsin and α-chymotrypsin from bovine pancreas at 37 °C for 90 min. Heat treatment of the supernatant was performed at 95 °C for 5 and 30 min, or 121 °C for 15 min. The growth of *S.* Hartford in the presence of protease- or heat-treated cell-free supernatants was determined by Multiskan as described above.

The siderophore production of strain CP-P-5 was determined from cell-free supernatant by measuring the absorbance at 405 nm as described by Manninen and Mattila-Sandholm (1994).

Determination of inhibitory effect of selected isolate in culture broth by co-culturing

Co-culturing studies using TS broth was carried out in static cultures. Flasks containing TSB were inoculated with cells of *S.* Hartford and CP-P-5 in volume ratios of 1:1, 1:10, 1:100 and 1:1000, respectively.

The flasks were incubated for six days at 25 °C under static conditions. Samples were taken after one, two, three and six days of incubation, and cell counts were determined by spread plate method using CASO and *Salmonella* selective Harlequin™ agar (Lab M Limited, Lancashire, United Kingdom) plates.

RESULTS AND DISCUSSION

Altogether 44 bacteria were isolated: 20 from an abattoir, six from vegetable processing environment, and 18 from an egg processing plant. The isolates were grouped into 37 clusters by colony morphological characteristics based on shape, colour and acid production on WL nutrient agar plates. For further analyses one isolate was selected from each group. Out of the tested 37 bacterium isolates, 16 could inhibit at least one of the tested pathogens, and the most effective inhibition was detected between 20 and 30 °C. All antagonistic isolates proved to be mesophilic, neutrophilic, and based on the results of oxidation-fermentation (OF) tests ten could be characterized as fermentative carbohydrate utilizers. Using the KOH test eight isolates were clustered into Gram-negative, while eight into Gram-positive groups of bacteria.

Molecular typing with OPE18 primer was not successful as in many cases amplicons were not generated. However, results of typing by M13 primer showed that all 16 isolates were clonally diverse strains. Identification of the isolates was done by miniaturized identification tests and sequencing of 16S rDNA genes as well (Table 1). As it can be seen significant differences were observed in case of results of the two different methods. Identification of *Pseudomonas* isolates could only be accepted at genus level, while all the other isolates were misidentified by miniaturized tests, which emphasize the necessity of molecular identification of non-clinical isolates.

Table 1. Results of miniaturized identification and sequence analysis of antagonistic isolates

Source	Isolation Code	KOH	Results of miniaturised identification	Results of sequence analysis of 16S rDNA gene (similarity percentage)
Vegetable processing environme	6/2 Z	GP	*Lactococcus lactis ssp. cremoris*	*Bacillus toyonensis* (100%)
	C2Z	GP	*Enterococcus avium*	*Bacillus weihenstephanensis* (99.91%)
Abattoir	CP-P-2	GN	*Pseudomonas fluorescens*	*Pseudomonas azotoformans* (99.7%)
	CP-P-5	GN	*Pseudomonas putida*	*Pseudomonas lundensis* (99.9%)
	CP-P-8	GN	*Sphingomonas paucimobilis*	*Paenibacillus pabuli* (99.9%)
	CP-S-8	GN	*Pseudomonas fluorescens*	*Pseudomonas granadensis* (100%)
Egg processing environme nt	CSE-B-2	GN	*Acinetobacter baumanii/ calcoaceticus*	*Pseudomonas rhizosphaerae* (99.05%)
	CE-B-1	GP	*Corynebacterium renale*	*Macrococcus caseolyticus* (99.8%)
	CE-PT-1	GP	*Staphylococcus kloosii*	*Rothia endophytica* (100%)
	CE-EJ-2	GN	*Pseudomonas putida*	*Pseudomonas lundensis* (99.9%)
	CE-EJ-3	GN	*Pseudomonas fluorescens*	*Pseudomonas extremaustralis* (99.81%)
	CE-EJ-4	GN	*Pseudomonas fluorescens*	*Pseudomonas azotoformans* (99.59%)
	CSE-T-1	GP	*Lactococcus lactis ssp. cremoris*	*Staphylococcus vitulinus* (100%)
	CSE-T-3	GP	*Staphylococcus haemolyticus*	*Macrococcus caseolyticus* (99.8%)
	CSE-T-4	GP	*Helcococcus kunzii*	*Bacillus pumilus* (100%)
	CE-E-1	GP	*Staphylococcus haemolyticus*	*Macrococcus caseolyticus* (99.79%)

Cell-free supernatants of the 16 isolates did not have total inhibitory effect on growth of the pathogens as it can be seen in Table 2, however, in some cases decline in the growth could be detected (data are not shown). The most sensitive pathogenic bacterium proved to be *Y. enterocolitica*, as nine out of the 10 tested isolates had negative effect on its growth.

However, *L. monocytogenes* and *S.* Hartford could only be inhibited by two isolates. The best inhibitory effect was detected in case of isolates CP-P-5 (*Pseudomonas ludensis*) and CE-EJ-4 (*Pseudomonas azotoformans*).

Both isolates were able to inhibit *E. coli* O157, while CP-P-5 had inhibitory effect on *Y. enterocolitica*, and CE-EJ-4 inhibited the growth of *L. monocytogenes* as well.

In case of isolates CP-P-5 and CE-EJ-2 (both are *P. lundensis* strains) inhibitory effect on *S.* Hartford was not detected when the cells were cultivated in PGY broth and under shaken conditions. Furthermore, inhibition could only be observed after 24 hours of incubation; later this effect ceased, which refers to the possible primary metabolite characteristic of the inhibitory substance(s).

Table 2. Inhibitory effect of cell-free supernatants on growth of the pathogens

Code of the isolate	Growth inhibition				Code of the isolate	Growth inhibition			
	Lm.	*Ec.*	*Ye.*	SH.		*Lm.*	*Ec.*	*Ye.*	SH.
6/2 Z	(✓)	nd.	nd.	nd.	CE-PT-1	nd.	nd.	(✓)	nd.
C2Z	nd.	nd.	(✓)	nd.	CE-EJ-2	nd.	X	nd.	(✓)
CP-P-2	X	nd.	nd.	nd.	CE-EJ-3	X	(✓)	nd.	nd.
CP-P-5	nd.	(✓)	X	(✓)	CE-EJ-4	(✓)	(✓)	nd.	nd.
CP-P-8	nd.	nd.	(✓)	nd.	CSE-T-1	nd.	nd.	(✓)	nd.
CP-S-8	nd.	(✓)	nd.	nd.	CSE-T-3	nd.	nd.	(✓)	nd.
CSE-B-2	nd.	nd.	(✓)	nd.	CSE-T-4	X	X	(✓)	nd.
CE-B-1	nd.	nd.	(✓)	nd.	CE-E-1	nd.	nd.	(✓)	nd.

(✓): partial inhibition **X**: no inhibition **nd**.: not determined as inhibitions in case of co-culturing on CASO plates were not detected

Inhibitory effect of cell-free supernatant of CP-P-5 was not affected by proteolytic enzyme treatments, however it showed heat-sensitivity. Moreover, CP-P-5 did not produce siderophore(s). In co-culturing study of CP-P-5 and *Salmonella* Hartford using TS broth the best inhibition was observed after 24 hours of incubation, and the most effective inhibition was detected when the ratio of the antagonistic strain was 1000 times higher than that of the pathogen.

CONCLUSION

Antagonistic bacteria of food-borne pathogens were found in food processing environments or in/on the food itself. However, our results showed that not only extracellular (mainly primer) metabolites are responsible for the inhibition, but competition of the cells as well. Interestingly, strains inhibited the growth of human pathogenic *Salmonella* Hardford were identified as *Pseudomonas lundensis*, which species usually belongs to spoiling microbiota of food products, and had not been previously described as biocontrol agent.

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MINERAL ELEMENT COMPOSITION OF GRAINS

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SUMMARY

It is known for a long time that the composition, rate and application time of fertilization influences not only the amount of yield but the quality of winter wheat too. The advantageous effect of late nitrogen fertilization on quality has been widely evaluated thirty years ago but it has not been studied whether the modern wheat varieties show same quality improvement what the former ones showed. In a field experiment we have evaluated the effect of dose and application time on the quality of winter wheat. We examined the effects of these factors not only on the quality of final product but the changes of quality during maturing were also investigated. This presentation summarizes the results on the evaluation of mineral element composition of grains.

INTRODUCTION

It is a well-known fact that the different agro-technical processes are one of the most important influencing factors on the quality oriented crop production (use of fertilizers occupy prominent place among these processes). In this research the effects of the mineral fertilizers applied in different times and different quantities were evaluated on the element composition winter wheat varieties.

During the production of cereals the rate of applied nitrogen influences the protein content which results a strong connection with vitamins of the group of vitamin B (thiamine, riboflavin and nicotinic). It is a well-known fact that during winter wheat production late nitrogen fertilizer application increases the quantity of storage proteins (glutelin and prolamine). The altered protein composition changes the ratio of essential amino acids that and the late nitrogen dose reduces the quantity of lysine (HUNT 1927; NAHEIF, 2013) The proper quantity of applied nitrogen effects positively the indicators of different trace elements (Fe, Zn, Mn, Cu, Ni, Cd) except Na concentration (ZLATKO et al. 2013)

MATERIAL AND METHODS

Two winter wheat varieties had been selected for the experiments (Mv Suba from Martonvásár and GK Kalász from Szeged). The similar needs and growth intensity and the minimal difference in quality characteristics were the most important criteria during selection.

Three groups of fertilizer applications were performed in the experiment. The first could be considered as absolute control: the area had not been treated neither with basic fertilizer or top dressing. The other two treatment mean 90 and 150 kg ha^{-1} nitrogen fertilizer dose expressed in active agent. A third part of the fertilizer was applied as basic fertilizer and the remaining amount was applied as top dressing during the second year of vegetation period in one stock in the early spring, or divided to four parts and applied during early spring, tillering and in the beginning of stem elongation.

The fertilizer treatments evaluated in this paper are marked as follows:
C= absolute control
ES= early spring (1/3 basic fertilizer in autumn, 2/3 in early spring)
ES+T= early spring and tillering (1/3 basic fertilizer in autumn, 1/3 in early spring, 1/3 at tillering)
ES+SE= early spring and (beginning) of stem elongation (1/3 basic fertilizer in autumn, 1/3 in early spring, 1/3 at beginning of stem elongation)

The studied parameters were different chemical ones (falling number, protein content), rheological properties (valorigraphic and, farinographic test) and element content analysis. This paper summarizes the results of measurements of element composition. These analyses were performed by ICP-OES device after wet digestion of the samples.

The primary comparison of treatments was performed according to the dose of fertilizer than the secondary valuation according to the time of the application. The different species were marked with different colours in each diagram: blue colour means GK Kalász while green colour means Mv Suba. The marked doses mean the dose of active ingredient in every cases, thus during the experiment 90 kg ha^{-1} N and 150 kg ha^{-1} N active ingredient were applied.

RESULTS AND DISCUSSION

The increasing nitrogen fertilizer doses did not result significant change in the calcium contents of GK Kalász grain samples, but a moderate difference can be seen in the readings in the case of the lower fertilization dose: the early spring treatment in itself resulted a decrease in the Ca concentration, but when the spring dose was divided into two parts the more later application resulted more increase in the concentration. The higher fertilizer dose resulted decrease in Ca concentration, especially when it was applied as dingle treatment in early spring. In the case of Mv Suba the readings are much higher, but the effect of fertilization was also negligible on the calcium concentration and only in the case of the application at stem elongation was the value extremely low. (Figure 1.) Based on these results it was found that the timing of nitrogen application did not influence the calcium concentrations of evaluated varieties.

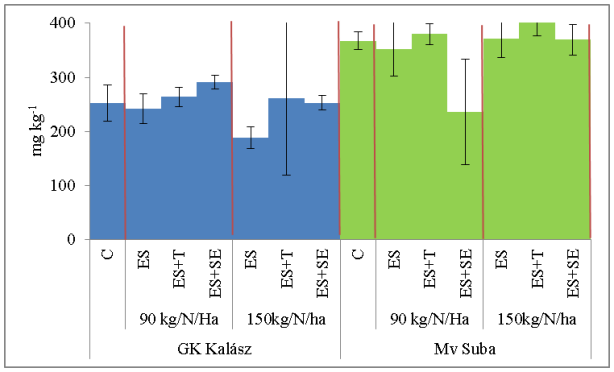

Figure 1.: Ca content of winter wheat grains

In the case of the magnesium content (Figure 2.) the lowest value was experienced at the 90 kg ha^{-1} N ES+SE treatment of Mv Suba but the 3rd treatment of the GK Kalász (90 kg ha^{-1} N ES, 150 kg ha^{-1} N ES+T and 150 kg ha^{-1} N ES+SE) also shows lower value compared to absolute control. The first variety with lower (90 kg/ha/N) dose shows continuous increase in magnesium content by the different application times. GK Kalász with the 150 kg ha^{-1} N dose shows lower values compared to the absolute control except for the ES application time.

The GK Kalász reached its lowest value with the 150 kg ha^{-1} N dose at ES+T time, this value is approximately 50% of the value of the 90 kg ha^{-1} N ES+SE which has the highest value. In the case of Mv Suba the 90 kg ha^{-1} N ES+SE dose resulted the lowest value and the absolute control differs minimally. In the case of 90 kg ha^{-1} N the ES and ES+T application times of Mv Suba show almost the same values while with the same dose in ES+SE time only equals with the values of the absolute control. In the case of higher dose it can be stated that the ES+T set up only reaches the 60 % of the value of 2 other set ups (ES and ES+SE) with the same dose.

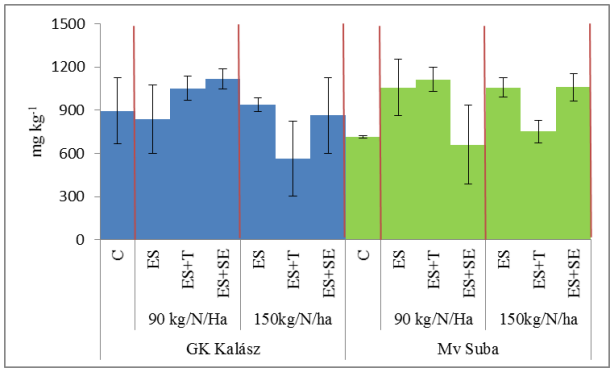

Figure 2.: Mg content of winter wheat grains

The 3. Figure presents the results of zinc concentrations. The fertilizer use effected positively the amount of this element and the increase of its amount can be achieved by sharing the fertilizer. In the case of GK Kalász with 90 kg ha^{-1} N dose the share of the fertilizer resulted positive effects by the increase of zinc content, in the case of Mv Suba the same dose (90 kg ha^{-1} N) showed inverse effect. The absolute control sample of the GK Kalász shows almost the same value as the setting of the 150 kg ha^{-1} N ES+SE. For this species the lowest value belonged to the 150 kg ha^{-1} N dose with ES+T application. The GK Kalász sample with the lowest value took up almost the same value as the Mv Suba absolute control's 90 kg ha^{-1} N ES+SE and 150 kg ha^{-1} N samples. The lower dose treatments of the second variety showed decreasing values with the share of the fertilizer, furthermore, for this variety the 90 kg ha^{-1} N dose at ES time resulted the highest value. The Mv Suba samples with 150 kg ha^{-1} N dose have not showed clear increase or decrease in the view of the date regarding to the continuity of applications. The highest reading was experienced in the case of single time fertilizer use while the second highest value was resulted by the last sharing application (ES+SE).

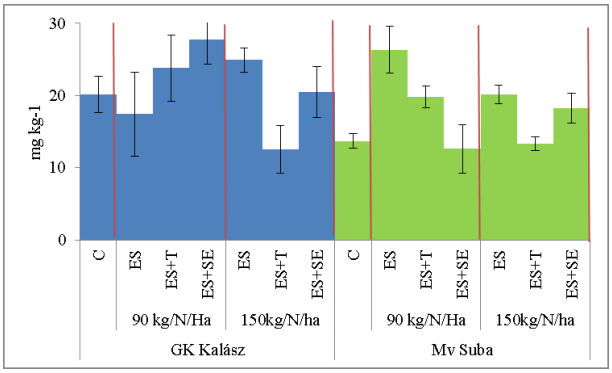

Figure 3.: Zn content of winter wheat grains

The 4th measured microelement was copper, which can be found in winter wheat in a low amount. The samples' copper amount was lower than mg kg$^{-1.}$

In the case of GK Kalász the samples treated with the lower dose (90 kg ha^{-1} N) reached similar values to the absolute control. Samples with the 150 kg ha^{-1} N set up the fertilizer division show positive tendency. With this dose the last application date reached the absolute control similar values as the 90 kg/ha/N samples. The lowest result was found in the case of ES treatment of 150 kg ha^{-1} N. In the case of Mv Suba compared to the absolute control only one treatment (90 kg ha^{-1} N) took up lower values. The sample with 90 kg ha^{-1} N dose and ES+T application time reached outstanding value. On the diagram the species highlighted with green with 150 kg ha^{-1} N dose settings had almost the same values and the values equal with the samples with the 90 kg ha^{-1} N ES+T settings.

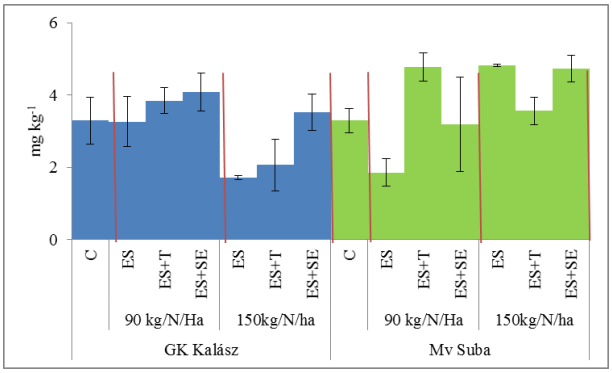

Figure 4.: Cu content of winter wheat experiment

CONCLUSIONS

We can achieve the genetic characteristics of the plant with proper agricultural engineering one of the methods is fertilizer application with proper amount and in the right time. Nowadays nitrogen has the largest share in fertilization. Nitrogen application in the proper phenological phase of plant can cause quantity and quality changes.

As a result of share the GK Kalász's 90 kg ha^{-1} N set ups shows continuous quantity increase in case of the calcium, magnesium, copper and zinc content. For this variety the higher (150 kg ha^{-1} N) dose did not meant positive effect in every case moreover some elements it showed continuous decreasing tendency (Mg, Zn).

The Mv Suba samples treated with 90 kg ha^{-1} N dose with ES+SE application had the lowest values (Ca, Mg, Zn content) in most of the cases. While from the 150 kg ha^{-1} N dose group (except for the Ca content) in every case the samples with ES+T application time took up the lowest values (Mg, Zn, Cu).

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COMBINED EFFECT OF NACL AND LOW TEMPERATURE ON ANTILISTERIAL BACTERIOCIN PRODUCTION OF *LACTOBACILLUS PLANTARUM*

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SUMMARY

Limited numbers of models are available for bacteriocin production and the food industry needs sufficiently complex and accurate models for growth of several pathogens and beneficial bacteria. The modified (0, 2, 4, 6% NaCl) MRS broth was inoculated with L. plantarum and incubated at 20, 25 and 30 °C. Changes in cell number and bacteriocin activity in the cell-free supernatant were recorded in every 3 hours for 48-60 h. Bacteriocin activity was determined by MicroTester apparatus. Listeria monocytogenes 1486/1 (from cheese) were used as target strains. A model was set up to describe the effects of different NaCl concentrations and temperature on microbial behavior.

INTRODUCTION

The occurrence of *Listeria monocytogenes* in fermented foods are quite frequent in the European Union (EFSA, 2015). Besides that, the fermented foods are containing bacteriocin producer lactic acid bacteria. *Lactobacillus plantarum* is a well-known lactic acid bacteria, which producing plantaricin (class II) bacteriocin. Numerous studies have been found about the antilisterial bacteriocin producer *Lb. plantarum* (Hata et al., 2010; Martinez et al., 2013; Todorov et al., 2010). Limited numbers of models are available in the literature on bacteriocin production under different environmental circumstances. The fermented food industry has an interest in reliable, stable starter culture. To provide this demand, the characterisation of lactic acid bacteria and the bacteriocin production have to be done. The aim of our study was to examine the combined effect of NaCl and low temperature on antilisterial bacteriocin production of *Lactobacillus plantarum* ST202Ch.

MATERIALS AND METHODS

Origins of bacteria and storage conditions

Bacteriocin producer *Lb. plantarum* ST202Ch strain (Todorov et al., 2010), previously isolated from fermented meat sausage and deposited in the culture collection of Escola Superior de Biotecnologia (ESB), were selected for this study.
L. monocytogenes from the culture collection of the LRCESB 1486/1, serogroup IIb, isolated from cheese was selected as target strain.
Lb. plantarum was cultured in de Man, Rogosa and Sharpe (MRS) broth (Biokar) at 37 °C for 18-22 hours; *Listeria monocytogenes* was grown in Tryptone Soy Broth (TSB; Biokar) at 37 °C for 18-22 hours. All strains were stored at -20 °C in appropriate culture media containing of 15% (v/v) glycerol. All bacterial strains were subcultured twice under appropriate conditions before use in experiments.

Examination the combined effect of NaCl and temperature

The modified (0, 2, 4, 6% NaCl) MRS broth was inoculated with *Lb. plantarum* and incubated at 20, 25 and 30 °C. Changes in cell number and bacteriocin activity in the cell-free supernatant were recorded in every 3 hours for 48-60 h. Bacteriocin activity was determined by MicroTester apparatus (Reichart et al., 2007). *Listeria monocytogenes* 1486/1 (from cheese) was used as target strain. Neutralized cell-free supernatants with antilisterial activity (hereafter referred as supernatants) was prepared as described by Van Reenen et al. (1998).

Determination of the bacteriocin activity by MicroTester apparatus

The MicroTester apparatus operates on the principle based on the detection of the change in redox-potential caused by microbial activity. The MicroTester apparatus is a computer-controlled 12-channel measuring system and the software was developd by Reichart et al. (2007). In the test cells (Fig. 5.1.) redox potential electrodes are dipped into the examined sample. The sample contained 0,5 ml of *Lb. plantarum* supernatants and 9,5 ml TSB broth inoculated with 1 % (v/v) *L. monocytogenes* 1486/1. The sample without supernatants referred as positive control. The value of bacteriocin activity is expressed in ΔTTD (TTD=time to detection) . ΔTTD is a virtual TTD which equal the the differences between the TTD of the sample and TTD of the positive control.

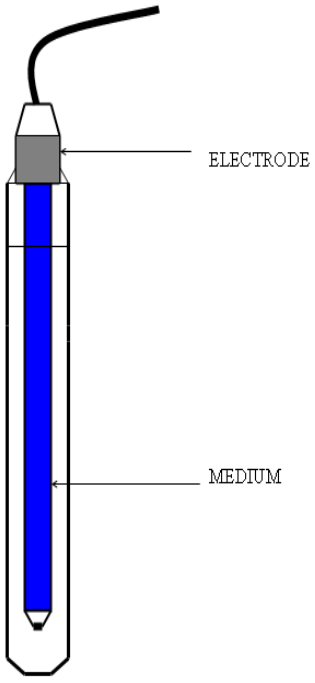

Figure 1. The test cell (microtest.co.hu)

RESULTS AND DISCUSSION

The results of antilisterial production at 20, 25, 30 °C in MRS broth containing 0, 2, 4, 6% NaCl are shown in Figs. 2 - 4. It was observed that the bacteriocin activity was influenced by the incubation temperature more than the concentration of NaCl.

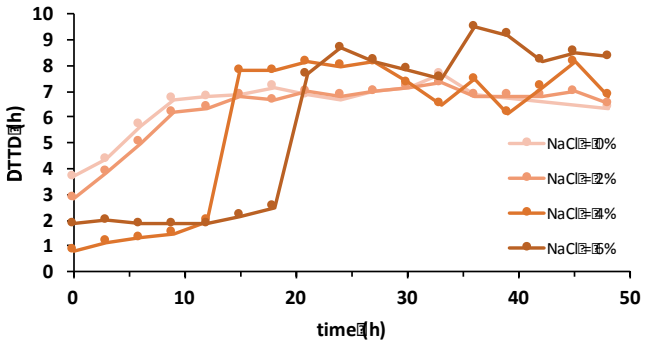

Figure 2. Antilisterial activity of neutralized cell-free supernatant produced by *Lb. plantarum* ST202Ch during growth in MRS broth at 30 °C using *L. monocytogenes* 1486/1 as indicator strain.

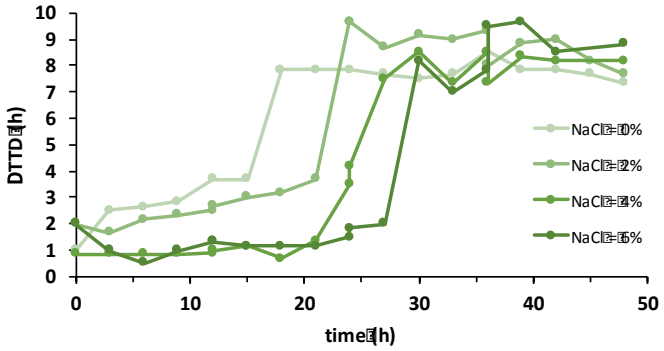

Figure 3. Antilisterial activity of neutralized cell-free supernatant produced by *Lb. plantarum* ST202Ch during growth in MRS broth at 25 °C using *L. monocytogenes* 1486/1 as indicator strain.

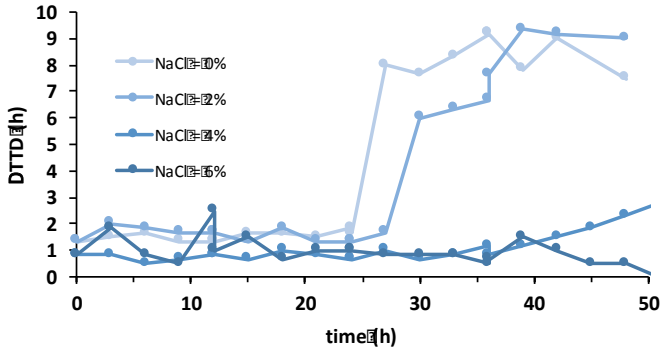

Figure 4. Antilisterial activity of neutralized cell-free supernatant produced by *Lb. plantarum* ST202Ch during growth in MRS broth at 20 °C using *L. monocytogenes* 1486/1 as indicator strain.

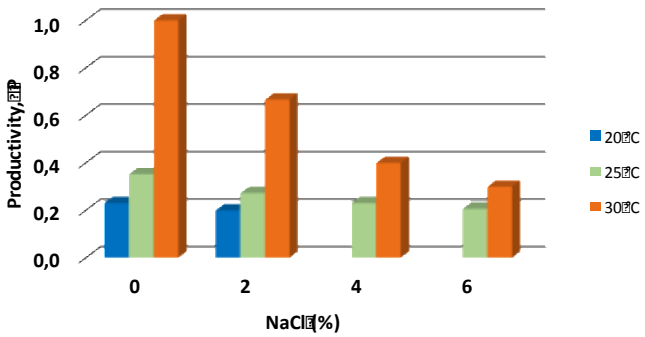

Figure 5 The productivity of antilisterial activity of neutralized cell-free supernatant produced by *Lb. plantarum* against *L. monocytogenes* 1486/1

The rate of antilisterial substance production of *Lb. plantarum* can be described as Productivity ('P'). The P is the time needed to achieve a given TTD (ΔTTD_c). ΔTTD_c is chosen to be in the linear phase of the antilisterial activity.
A model was set up to describe the effects of different NaCl concentrations and temperature on microbial behaviour. Lower temperature and higher NaCl concentration clearly affected the production of bacteriocin (Fig. 5).

ACKNOWLEDGEMENT Providing the *Lactobacillus plantarum* ST202Ch strain is highly acknowledged to Svetoslav D. Todorov PhD, Universidade Federal de Viçosa, Veterinary Department, Viçosa, Minas Gerais, Brazil.

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NON-DESTRUCTIVE IMPACT METHOD FOR FIRMNESS MEASUREMENT

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SUMMARY

Quick and reliable quality assessment of the fruits and vegetables is especially important. Monitoring of the maturation and ripening process, early detection of diseases, decision about harvest date and postharvest treatment need reliable, objective and – preferably – non-destructive quality testing methods. Our objective was to develop method and appropriate portable instrumentation to measure surface hardness – as quality measure – with a nondestructive method. The computer controlled instrument has an electromagnetically excited impactor fitted with a piezoelectric acceleration sensor. To ensure the uniform contact behavior (contact area) between the impactor and the tested produce of wide range of shape, spherical head was applied. Conclusively, Hertz contact theory is to be applied for evaluation of the impact signal. Instead of using empirical "hardness index" our objective was the physical interpretation of the contact phenomena and to characterize the process with the force vs. deformation relationship, similarly to the widely used texture analyzers, penetrometers.

INTRODUCTION

Research works of last years offer a wide range of novel methods for quality evaluation of products of horticulture, for example new, dynamic methods for non-destructive measurement of mechanical properties of fruits and vegetables. They are really useful tools for monitoring of the maturation and ripening process, early detection of diseases, decision about harvest date and postharvest treatment, etc., but with strong limitations in applicability area and/or physical interpretation of the measured parameters. The only dynamic method, with practically no limitations in application field, is the impact method, applying an instrumented impactor to hit the surface of the sample with low kinetic energy and analyzing the contact parameters. The different research groups apply different approaches either in measurement technic or in interpretation of the results. For interpretation of the impact signal, a number of parameters were used: the maximum of the impact signal, the time to reach the maximum of the signal, slope of the impact curve, area below the curve, combination of this parameters, etc. (García-Ramos et al., 2003). The common property of these approaches is, that the base of the selection of a parameter is the correlation between the given parameter and mechanical characteristics, instead of searching for a physical model behind the impact event. Our aim was to measure surface hardness – as quality measure – with a nondestructive method. Furthermore, our objective was to find the physical interpretation of the impact process, to describe the event with terms of mechanics (force, deformation, speed, energy, etc.). It is the base of development the instrumentation-independent description of the sample properties, offers the possibility of comparison of the results of different instruments.

MATERIALS AND METHODS

Impact measurement system

The base of the measurement system was an electromechanically excited impactor, fitted with a miniature, lightweight (0.7 g), ceramic shear ICP® accelerometer with sensitivity of 1.02 mV/(m/s²). A computer-controlled digital storage oscilloscope (Velleman, PCSGU250) was applied to measure the acceleration. The sensor was fixed at the end of an impactor arm. The impact movement of the arm was initiated by an electromagnetic coil. The impact speed can be controlled by the excitation voltage applied at the coil. Spherical impact tip (of 16 mm diameter) was applied to avoid the uncertain contact surface area and sharp edge effects between the sample and the impactor. It provides with uniform contact behavior (contact

area, depending on the displacement of the impact) between the impactor and the tested produce of wide range of shape (Hertz model).

Evaluation methods

Applying a calibrated acceleration sensor, kinetic approach can be applied in physical interpretation of the contact process. The speed of the sensor (v(t)) can be calculated integrating the measured acceleration values (a(t)) in time, and similarly, the displacement of the impactor (s(t)) can be determined, as the integrated value of the speed:

$$v(t) = \int_0^t a(t)dt + v(0) \quad (1)$$

$$s(t) = \int_0^t v(t)dt + s(0) \quad (2)$$

The boundary conditions (in this case the initial value of the impactor speed at the contact moment, v(0)) are crucial questions, as it is typical in differential equation based description of movements. In our approach we supposed, that the moment of the maximum deceleration (minimum acceleration), of maximum penetration and of zero speed belong to the very same time (t_{a_min}). In this case, Eq. (1) can be evaluated in multiple steps:

$$v'(0) = 0 \rightarrow v'(t) = \int_0^t a(t)dt \rightarrow v_0 = -v'(t_{a\_min}) \rightarrow v(t) = v'(t) + v_0 \quad (3)$$

The force 'F', characterizing the surface firmness, is proportional to the measured acceleration signal. The coefficient, determined by the inertial behavior of the (impactor arm+sensor+impactor tip) system, can be substituted by a constant "*effective mass*" (m*):

$$F(t) = m^* \cdot a(t) \quad (4)$$

This way, we have the possibility to characterize the mechanical properties of the sample surface with the *Force* vs. *Deformation* relationship. It ensures the possibility of comparison with the traditional, compression based methods and to characterize the samples with parameters of physical dimensions (N, N/mm, Pa, etc.). The advantage of applying spherical impactor is a uniform contact surface for – practically – any shape and in any position of the sample. However, the this contact surface is depending on the momentary penetration depth. Conclusively, the Hertz theory of the normal contact of elastic bodies is to be applied for evaluation of the *Force vs Displacement* relationship (Mohsenin, 1986):

$$F = \frac{4}{3}\frac{E}{1-\mu^2}\sqrt{R}d^{\frac{3}{2}} \quad (5)$$

where 'F' is the applied force, 'E': elastic modulus of the sample, 'μ': the Poisson's ratio, 'R': radius of the impactor tip and 'd' is the penetration depth. In this approach, the impactor tip is supposed to be rigid and much smaller, than the sample.

This way, a *Dynamic Coefficient of Elasticity* ("*DCE*") parameter can be introduced, including the Poisson number (which is generally not known exactly, but supposed to be 0.35-0.45 for fruits and vegetables), and connected strictly to the Young's modulus (*E*):

$$DCE = \frac{E}{1-\mu^2} = \frac{3}{4\sqrt{R}}\frac{F}{d^{\frac{3}{2}}} \quad (6)$$

The dimension of *DCE* is MPa, if the Force is given in N, and the penetration depth and impactor radius are given in mm.

Tested materials

For methodological investigations of the system, model materials (rubber, elastic foam etc.) were used to avoid the variability of the material properties. Applicability of the method and instrument for horticultural products (non-destructive nature, repeatability, etc.) was tested on different fruit and vegetable samples in wide size, shape, hardness and structure ranges (apple, nectarine, tomato, bell pepper, etc.). The samples were collected from a local market.

Metrological investigations

The *repeatability* of the system was characterized by repeating tests on the same sample, same location, under the same conditions (excitation voltage). In the same time, if the tested sample is a fruit or vegetable, the bruise, caused by the test, can be evaluated, simply with occurrence of a monotonous change in the result during the short-time repetitions. To characterize the *reproducibility*, the repeated impact tests were performed on different locations of the tested horticultural sample. In this case, of course, the variability is depending rather on the biological variability of the sample, than on the repeatability of the instrument.

RESULTS AND DISCUSSION

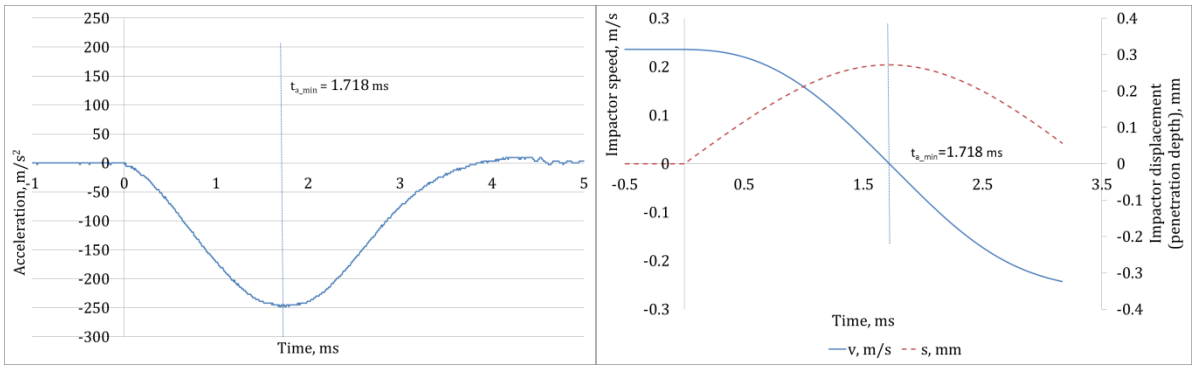
The impactor

A typical acceleration signal (a) and the impactor speed and displacement values (b), calculated according to the Eq. (3) are shown in Figure 1. (sample: nectarine). The impact parameters, used by different research groups (time to the first peak, amplitude, area below the curve, etc.) can be read from the figure (a), however, the applied approach of physical interpretation provides with more information about the process and the given sample (v0 , max. penetration depth, penetration work). Furthermore, the force, decelerating the impactor can be calculated according to (4). The m* effective mass can be determined by preliminary calibration of the given system.

Repeatability and reproducibility

The Coefficient of Variation (CV%) parameter of the repeated test characterizes the repeatability of the instrument and the method. It was typical for all model materials tested (rubber and elastic foam balls, etc.), resulting in CV% around 1-3% (it is in accordance with the literature). It shows a good/very-good repeatability of the measurement.

A difference in behavior can be experienced in case of fruit and vegetable samples. A small scale (virtual) hardening after the first impact is very typical most of the samples. The microscopic evaluation of possible bruise showed no destruction even after 10-20 repeated tests. It is emphasized by the penetration depth: it was between 0.1-0.3 mm during the investigations, which is much below the bioyield deformation of the samples.



(a) (b)

Figure 1. Typical acceleration signal (a) and the impactor speed and displacement values (b), calculated according to the Eq. (3) (sample: nectarine)

Conclusively, we supposed, that no destruction was caused by the impact test with the given instrumentation and setup. According to our approach, realignment in the intracellular water content can happen due to the impact, resulting in some changes in mechanical properties, increasing of the DCE parameter. The repeatability (of the instrument and the method) in this case can be characterized by the CV% of the tests from the 2nd impact. Results of some produces are summarized in Table 1.

Table 1. Mechanical characteristics of the tested cultivars (average of 20-20 samples)

Tested sample	Apple	Nectarine	Tomato	Bell pepper	Cucumber
Dynamic Coefficient of Elasticity, MPa	28.24	5.12	2.36	3.02	36.33
Coefficient of Variation, %	0.83	1.15	1.56	2.06	1.63

The reproducibility tests (repeated impacts on a sample on different locations, e.g. around the equator) resulted – of course – in much higher CV%-s (5 to 20 %, depending on the cultivar). It calls our attention to the necessity of the repeated test on a sample in order to get a representative characteristic of the mechanical stage despite of the high biological variability of the samples.

CONCLUSIONS

The developed instrumentation was found to be able to measure of surface mechanical properties of wide range of fruits and vegetables non-destructively, with very good repeatability. Due to its construction and non-destructive nature, it is suitable for in-vivo application as well, offering an especially useful tool for organic horticulture in maturation monitoring, treatment evaluation, defect detection as well. It is sensitive and accurate enough to detect and measure even the small scale changes during the maturation and postharvest handling of the samples. New mechanical characteristic (Dynamic Coefficient of Elasticity), was introduced, connected directly to the Young's modulus (E). The advantage of the physical interpretation of the process and determining mechanical parameters in physical dimensions instead of empirical indices is obvious (calibration transfer, comparability of different instrument, different setups, etc.).

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Zlatko Svecnjak, Marina Jelen, Marija Bujan, Dubrovka Vitali and Irena Vedrina Dragojevic, Trace element concentrations int he grain of wheat cultivars as affected by nitrogen fertilization, Agricultural and food science (2013) 22: 445-451

NATURAL FOOD PRESERVATIVE AGENTS FROM FRUITS (QUINCE, SEA BUCKTHORN AND SOUR CHERRY)

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SUMMARY

Due to the negative feelings of consumers for synthetic additives, it became necessary to investigate alternate opportunities, such as the use of bio-preservatives, which are suitable to large amount of synthetic preservatives. The aim of this study is to examine the antimicrobial effect of plant species, which, based on our previous results, significantly inhibit the growth of microorganisms. All the cultivars of the chosen plant species (sea buckthorn, sour cherry, quince) are rich in endogen compounds. Antioxidant status was determined by FRAP (Ferric Reducing Ability of Plasma) and TPC (Total Polyphenol Content) searching for correlation between antioxidant content and antimicrobial effect. Antimicrobial effect was tested on strains of three bacteria (E. coli, S. aureus, L. innocua). The degree of inhibition was measured by a rapid method: RABIT (Rapid Automated Bacterial Impedance Technique). We are expecting promising results, which can be applied by the food industry, satisfying the needs of conscious consumers by decreased synthetic preservative use.

INTRODUCTION

In recent years, there is a growing interest for the disciplines of food safety worldwide, and their relevance is growing continuously. Inhibiting the growth of microorganisms is a serious challenge for the food industry, as they are a threat to human health as well as to the economy and commerce. There are several kinds of preservatives and applications which are used by the food industry in order to lower the risks. However, the growing chemicalization lowers the confidence of consumers toward the food products. The majority of people have negative feelings for synthetic additives, and they consider their use to be unnecessary and unhealthy (Tarnavölgyi, 2000); as a result, more and more research aims to develop natural additives (Alzamora, 2003; Najeeb, 2014).

There are hundreds of natural compounds, which were proved to have antimicrobial effect on microorganisms, and there are several available methods, which are suitable to test their efficiency, and determine their antimicrobial spectrum (Davidson, 2001; Davidson et al, 2005; Michel et al, 2012; Mohácsiné & Kiskó, 2006, Papp et al, 2008).

In the present study the antimicrobial efficiency and antioxidant capacity of the fruits of three different fruit species (buckthorn, quince and sour cherry) were investigated. RABIT (Rapid Automated Bacterial Impedance Technique) was used to measure the degree of antimicrobial inhibition. This technique is based on the detection of admittance change caused by the growth of microbes (Fung, 2002). It is suitable for the determination of microorganisms (bacteria, yeasts and molds), which are present in food products, giving both qualitative and quantitative results, in an indirect, automated way (Mohácsiné & Kiskó, 2006).

MATERIAL AND METHODS

All the cultivars of the investigated fruits (buckthorn - *Hippophae rhamnoides* L., sour cherry - *Prunus cerasus* L. and quince - *Cydonia oblonga* Mill.) have Hungarian origin, and they were selected from several widespread Hungarian cultivars based on their high antioxidant capacity in our preliminary study. The following cultivars were involved in the study: ’Pető 1’ (buckthorn), ’Pipacs 1’ (sour cherry), and ’Konstantinápolyi’ (quince).

The fruits were equally prepared for the examinations. After grinding, the samples were lyophilized, and then 40 and 80 mg/ml solutions were prepared by water extraction. The samples were placed into an ultrasonic bath, followed by a filtration with filter wool. Because of the variance in the inhibitory strength between the fruit extracts, the data of the following solutions are shown in the present study: 40 mg/ml solution for quince extract; 80 mg/ml for sour cherry, and both 40 and 80 mg/ml for sea buckthorn (indicated as buckthorn [L], and buckthorn [H], respectively).

Antioxidant capacity was determined by the FRAP (ferric reducing ability of plasma) assay (Benzie & Strain 1996), and the results are expressed in ascorbic acid equivalent (mM AS/L). Furthermore, the TPC (total polyphenol content) of the samples was also determined based on the method of Singleton and Rossi (1965); results are expressed in gallic acid equivalent (mM GS/L).

Antimicrobial effects were measured with a RABIT system in the Corvinus University of Budapest, Dept. of Microbiology and Biotechnology. Two ml broth was dosed into the RABIT test cells containing sterile electrodes, followed by 700 µl bacteria suspension and 300 µl fruit extract. Samples containing only broth and bacteria suspension were used as positive control, while the negative control contained broth alone. The measurement period was 24 hours; admittance was measured automatedly in every six minutes. Incubation temperature was set to 37 °C.

The following bacteria strains were used to measure antimicrobial inhibition: *Escherichia coli (6739)*, *Staphylococcus aureus (ATCC 6538)*, and *Listeria innocua (CCM 4030)*. These bacteria strains are maintained by the Dept. of Microbiology and Biotechnology (Corvinus University of Budapest). The data of the RABIT system was assessed by calculating the area under the curve of admittance. Integral calculus was made by the R-project 3.2.1. software, while statistical analysis has also been made.

RESULTS AND DISCUSSION

Characterizing the antioxidant state of fruits, average values of FRAP and TPC are shown in Figure 1.

The results showed significant differences between the species ($p_{FRAP}=1.15*10^{-8}$, $p_{TPC}=2.06*10^{-9}$). FRAP values had a range from 2.1 to 8.4 mM AS/100g. The ascorbic acid equivalent of sour cherry is approximately one third of the ascorbic acid equivalent of quince, and less than the quarter of the FRAP of buckthorn.

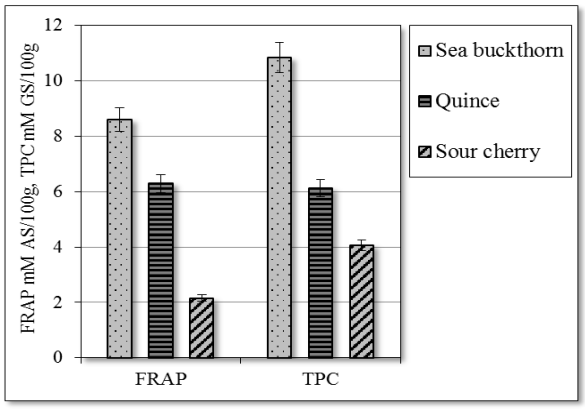

Figure 1: The avarage results of antioxidant state (data shown in mM AS/100g for FRAP, and mM GA/100g for TPC)

The total polyphenol content of the samples range from 4.1 to 11.2 mM GS/100g, the value of buckthorn is outstanding, being three times higher than of the sour cherry.

The admittance curves created by the impedimetric RABIT system are showing the admittance change caused by the metabolic processes of bacterial growth, and have a resemblance to the standard growth curve of bacteria. The initial period of the curve (lag phase) is indicating the adaptation of the bacteria to the particular environmental factors. This is followed by a rapidly rising phase (exponential phase), where the maximum intensity of microbial growth is reached.

The RABIT assay showed that *L. innocua* had a low growing rate in the broth, or it has produced very low amount of metabolites which affect the admittance of the solution. In the case of this strain, further methodical development is necessary to get reliable data with the RABIT assay. In contrast to this, in the case of *E. coli* and *S. aureus*, the antimicrobial effect of the fruits was well measurable. The admittance curves of these are shown in Figure 2. and Figure 3.

On the left side, the bacterial growth in the 40 mg/ml solutions of both quince and buckthorn is shown by the admittance curve; while on the right side of the Figures (2. and 3.) the samples of the 80 mg/ml sour cherry and 80 mg/ml buckthorn solutions can be seen. The fruit extracts were typically slowing the growth of *E. coli*, lowering the number of bacterial cells, except in the case of the 80 mg/ml buckthorn solution, where the beginning of the growth was also delayed in addition to the reduction of cell numbers. In the case of *S. aureus* the initial (lag) phase of the growth became longer, with the exception of the quince samples, where more likely the growing rate was lowered by the added fruit extract.

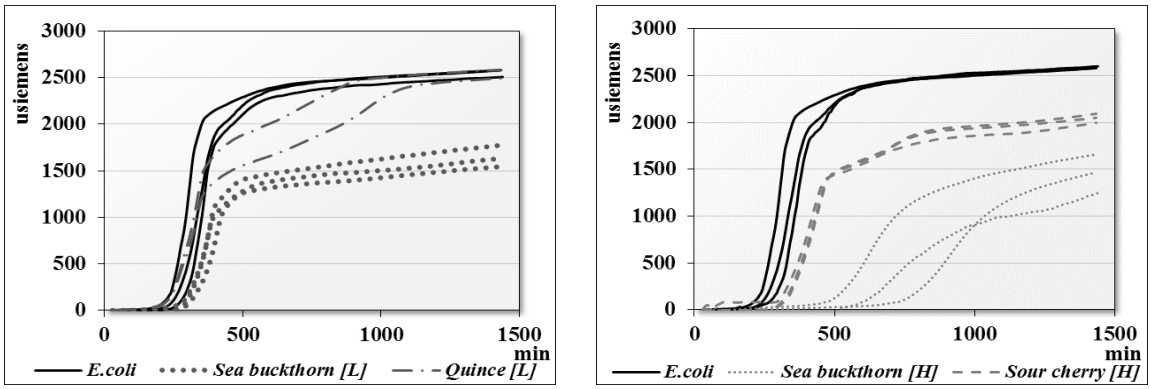

Figure 2: The admittance curves of antimicrobial inhibition effect of the samples on E. coli,, 40 mg/ml extracts: '[L]', 80 mg/ml extract: '[H]'

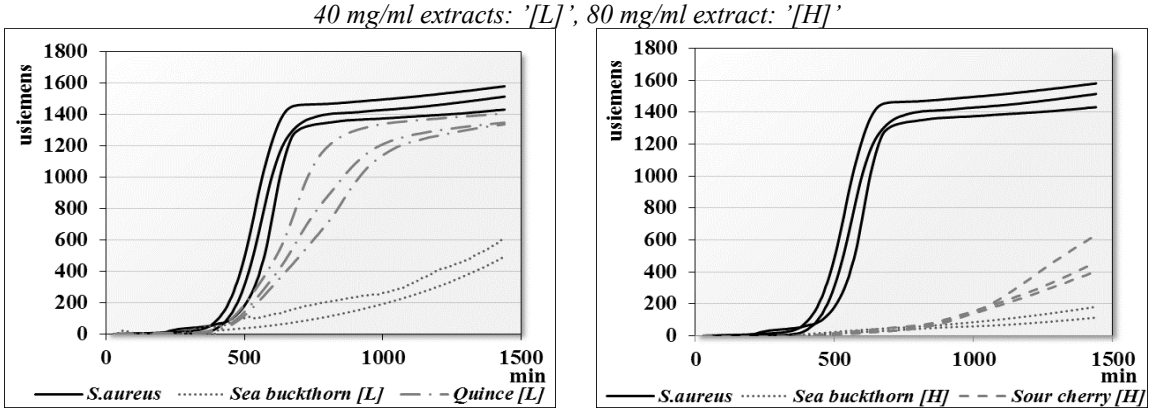

Figure 3: The admittance curves of antimicrobial inhibition effect of the samples on S. aureus, 40 mg/ml extracts: '[L]' 80 mg/ml extract: '[H]'

Average values of the areas under the admittance curves are shown on Figure 4. By adding any more fruit, the area under the admittance curve was decreasing significantly. The size of the area under the admittance curve, and the number of bacteria cells are directly proportional.

The strongest inhibition was shown in the case of buckthorn samples; adding sour cherry samples resulted in the weakest inhibitory effect.

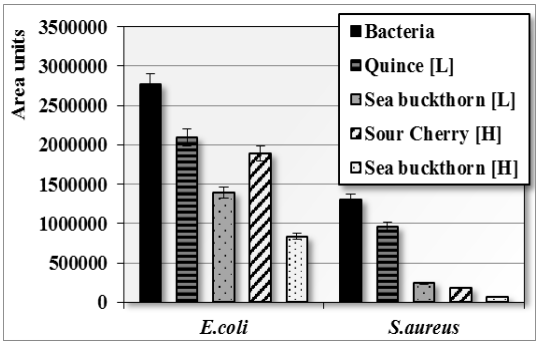

Figure 4: Avarage values of the areas under the admittance curves, 40 mg/ml extracts: '[L]' 80 mg/ml extract: '[H]'

CONCLUSIONS

However we investigated three different bacteria strains, difficulties with the detection were only experienced in the case of the *L. innocua* strain, which is the result of the instability of the bacteria. An efficient method can be developed by choosing more suitable broth, and using longer measuring period. In the case of the two other bacteria, it was possible to follow process of the antimicrobial effect with the RABIT assay.

Against the investigated bacteria strains, evidence were gained about the antimicrobial efficiency of buckthorn, quince, and sour cherry, which can be suggested for further testing as natural preservatives in the food industry. Due to the outstanding antioxidant content of buckthorn, it had the greatest antimicrobial effect on the investigated bacteria strains. By using natural antimicrobial materials, the concentrations of preservatives and shelf life extenders of food products could be lowered, which were a huge step on the field of healthy food production, satisfying the needs of the conscious consumers.

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APPLICATION OF MATRIX-ASSISTED LASER DESORPTION IONIZATION-TIME OF FLIGHT MASS SPECTROMETRY FOR DETECTION OF SALMONELLA SPP.

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SUMMARY

Salmonella is a zoonotic food-borne pathogen that is the major causative agent of gastrointestinal outbreaks. The classical international standard methods of Salmonella detection are laborious and might take 5 to 7 days depending on the realization of biochemical and serological confirmations. For these reasons there is an increasing need for such alternative methodologies which allow fast and reliable analysis and identification. This study was performed to evaluate the potential place of matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry in the system of Salmonella detection. Furthermore, the possible application of MALDI-TOF MS for pre-screening of the epidemiologically important Salmonella enterica subsp. enterica serovars was investigated. Based on our former results MALDI-TOF MS is showed to be a simple and accurate tool for a more reliable and even faster identification and confirmation of Salmonella spp. By analysing mass spectra in detail, potential serovar-identifying biomarker ions were found. These ions represent an opportunity for pre-screening Salmonella serotypes successfully.

INTRODUCTION

Whole-cell matrix-assisted laser desorption ionization time-of-flight mass spectrometry, based on intact protein profiling, revolutionized microbiology as a new alternative technique for rapid identification of bacteria and fungi. The acronym MALDI-TOF denotes a special mass spectrometer in which a photoionization source is combined with a time-of-flight mass analyser. MALDI is considered as a soft ionisation method as macromolecules are ionized without significant degradation by embedding them within crystals of small organic compounds, called matrices which absorb radiation from the laser and transfer energy to the analytes (Pavlovic et al.,2013). Identification of microorganisms is based on genetically coded proteins which generate characteristic protein profiles that can be used as "fingerprints".

Salmonella is a zoonotic food-borne pathogen that still remained the most frequently detected causative agent of gastrointestinal outbreaks. According to the latest scientific report of EFSA and ECDC (2015), a total of 82.694 confirmed salmonellosis cases were reported in 2013, from which 59 had a fatal outcome, but it has to be noted that these data only show us the tip of the iceberg and many cases remain unrecorded.

Traditional culture-based international standard methods for *Salmonella* spp. detection requires 5-7 days depending on the realization of biochemical and serological confirmations. Automated PCR methods and immunoassays are also widely used for the detection of *Salmonella spp.*, providing negative results within 1-2 days. However, culture methods and confirmations must be performed in the case of supposedly positive or inhibitory samples.

Differentiation of *Salmonella* serovars by serotyping plays an important role in public health monitoring of infections as it helps to recognize outbreaks and to investigate the sources and possible contamination routes. At present more than 2500 *Salmonella* serovars have been described based on their antigenic variability but only a few of them represent the majority of isolates. Generally, *Salmonella enterica subsp. enterica* (subsp. I.) is responsible for more than 99% of human salmonellosis cases (Grimont and Weill, 2007). Some studies indicated that polymorphisms in highly expressed genes can result amino acid variations in proteins, which can be observed by MALDI-TOF MS allowing distinguishing *Salmonella* subspecies or even serovars (Dieckmann et al., 2008; Dieckmann and Malorny, 2011).

This study was performed to evaluate the potential place of MALDI-TOF MS in the system of routine *Salmonella spp.* detection. Furthermore, the possible application for pre-screening of epidemiologically important *Salmonella enterica* subsp. *enterica* serovars was also investigated. Because of the fact that comparison below the species level requires highly informative mass spectra with quality and reproducibility, we extended the analysis for experimental factors, e.g. growing conditions, sample preparation.

MATERIAL AND METHODS

Samples, bacterial strains, isolates and culture conditions

Altogether, more than 200 cultures, prepared on selective and non-selective growth media, were analysed for *Salmonella* spp. presence/absence by MALDI-TOF MS. Standard MSZ EN ISO 6579:2006 was used as the reference method. Additionally, a total of 45 *Salmonella enterica* subsp. *enterica* serovar strains were used with the purpose of studying the medium-dependence of mass spectra, and for the analysis of serovar-identifying biomarker ions. Reference strains and several isolates were obtained from the collection of WESSLING Hungary Ltd. Serotyping results according to the reference White-Kauffmann-Le Minor scheme and further isolates were kindly provided by Food Microbiological National Reference Laboratory of National Food Chain Safety Office. Bacteria were grown at 37°C for 24±1 h on selective XLD, *Harlequin*^{TM} *Salmonella ABC* and non-selective Plate Count, Columbia Blood and Mueller-Hinton agar.

Sample preparation for MALDI-TOF MS analysis

Two different sample preparation protocols were carried out according to Bruker Daltonics Inc. Samples were taken from individual colonies with sterile sampling tools. In the case of direct sample introduction, samples were directly applied onto the target plate and 1-1 µl 70v/v% formic acid was added. After evaporation of the solvent, 1 µl α-HCCA (10mg/ml) matrix solution was pipetted onto the sample and the spots were crystallized by air drying. In the case of the other protocol using ethanol extraction, a single colony was taken with an inoculation loop and the culture was suspended in 300 µl of deionised water in an Eppendorf tube. 900 µl of absolute ethanol was added to the suspension and mixed thoroughly. After centrifugation at 14500 rpm for 2 minutes, the supernatant was removed and the bacterial pellet was resuspended in 70% formic acid. Next the same amount of acetonitrile was added, which was followed by a centrifugation for 2 minutes at 14500 rpm. Subsequently, 1 µl of the supernatant was transferred onto the target plate, then after drying it was overlaid with 1 µl of the matrix solution and dried again.

MALDI-TOF MS parameters and data evaluation

Mass spectra were acquired with Bruker Microflex LT MALDI-TOF mass spectrometer operating in positive linear mode, with molecular mass range of 2.0-25 kDa. Identification of *Salmonella spp.* was carried out by *MALDI Biotyper Realtime Classification software (RTC)*. At least 200 shots gave satisfactory spectra with good signal-to-noise ratio. Calibration had to be performed before sample measurement with the usage of *Bruker Bacterial Test Standard (BTS)*. With the aim of analysing mass spectra of serovars in detail, 640 shots were performed and mass data files were transferred to *flexAnalysis software*. Mass spectra were processed with baseline correction, smoothing, peak finding and internally calibrated using a set of ribosomal *Salmonella spp.* genus-specific biomarker ions.

RESULTS AND DISCUSSION

Identification results of *Salmonella spp.*

MALDI-TOF MS identification technique was linked into several phases of *Salmonella spp*. detection process consisting of the classical standard method MSZ EN ISO 6579:2006 complemented by validated PCR based methods or automated immunoassays (e.g. VIDAS) which is shown in Figure 1.

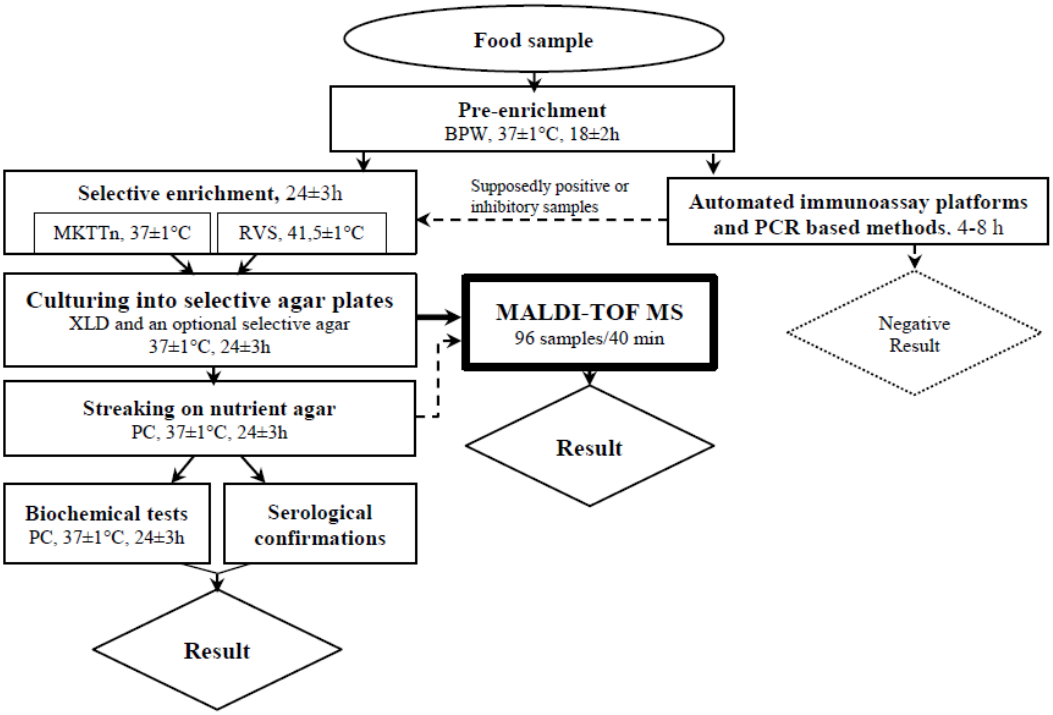

Figure 1.: The place of MALDI-TOF MS in the system of *Salmonella* spp. detection due to standard MSZ EN ISO 6579:2006 complemented by validated PCR based methods and automated immunoassays

In order to evaluate the applicability of MALDI-TOF MS for detection of *Salmonella spp.* presence/absence, more than 200 cultures were analysed in parallel with the reference standard method MSZ EN ISO 6579:2006. Method validation was performed according to standard ISO DIS 16140-2:2013, which gave the following convincing parameters, summarized in Table 1.

Table 1.: Validation results of the analysis of *Salmonella spp.* presence/absence in accordance with the instructions of standard ISO DIS 16140-2:2013

Relative accuracy (AC)	100 %
Relative specificity (SP)	100 %
Relative sensitivity (SE)	100 %
False positive ratio (FP)	0 %
False negative ratio (FN)	0 %

According to the results of this study MALDI-TOF MS method fits well into the system of *Salmonella spp.* detection and it has the ability to identify samples reliably even by analysing cultures prepared on selective media, meaning that this technique can give results within 4 days. Furthermore, MALDI-TOF MS identifies microbes based upon their proteomic similarities which make detection of microbes scientifically sound and exact. The above discussed results proved that this method could substitute biochemical tests which are laborious and give inconclusive or even contradicting results in some cases.

Analysis of biomarker ions below the subspecies level

By analyzing different *Salmonella enterica* subsp. *enterica* serovars using MALDI-TOF mass spectrometry, several potentially serovar-identifying biomarker ions were found but it has to be noted that many of them were present in more than one serovar. Thus, it is recommended to define combinations of these biomarkers for prescreening. For example absence of m/z 6009 combined with the presence of m/z 6036 were uniquely found in the analysed strain of *Salmonella* Enteritidis (Fig. 2/a), which is in accordance with literature. Some of the observed mass shifts corresponding to amino acid variations are shown in Figure 2. By evaluating the effect of some experimental parameters, culturing on non-selective growth media and sample preparation using ethanolic extraction resulted mass spectra with satisfactory properties for comparison.

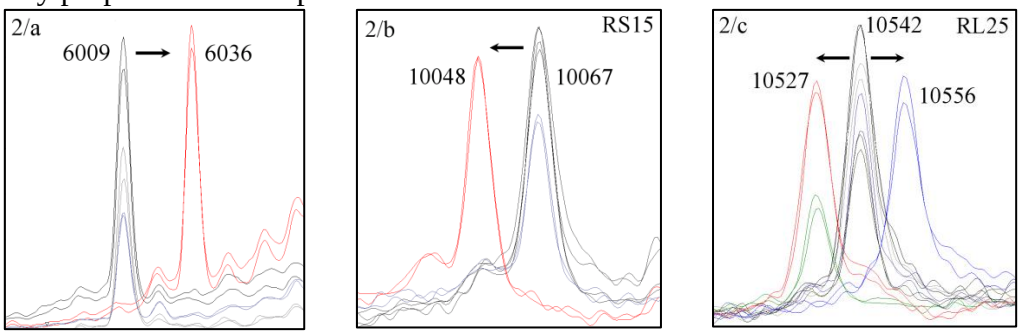

Figure 2.: Characteristic mass shifts in different *Salmonella enterica subsp. enterica* serovars

CONCLUSIONS

In summary MALDI-TOF MS is showed to be a simple and exact tool for a more reliable and even faster identification and confirmation of *Salmonella* spp. which was easily implemented into routine analysis because of its high throughput and relatively low-cost. Identification on the genus level was not influenced by selective growth media and did not require special time-consuming sample preparation. In these terms it means that with the usage of MALDI-TOF technique, streaking of typical or suspect colonies on nutrient agar and the classical biochemical confirmations could be replaced, thus the analysis time of *Salmonella* spp. could be reduced by at least 24 hours or even 48 hours. Comparison below subspecies level requires ethanol extraction protocol preferably which provides more informative mass spectra with better reproducibility and bacterial culturing must be carried out on non-selective agar media. To improve results in the future, it is important to collect additional mass spectra of the same strains and use additional strains of *Salmonella* serovars.

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CHANGES IN POLYPHENOLS, ANTHOCYANINS AND COLOUR INTESITY OF KADARKA, KÉKFRANKOS AND CABERNET FRANC WINES DURING WOODEN BARREL STORAGE

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SUMMARY
Phenolics compounds are one of the most important compounds of red wines, mainly because they responsible for oxidation with change of colour of red wines and the formation of wine character. The anthocyanin and polyphenol content in red wines depends not only on the original composition in Vitis vinifera grapes, but also on the enological techniques applied. Monomeric anthocyanins, like malvidin contribute the main part of colour in the young red wines. During wooden barrel storage the contents of the monomeric anthocyanins in red wines decline constantly, whereas red wines can still maintain an essentially red color thanks to the catechin and leucoantocyanin. This study summerized the change of monomeric anthocyanins, leucoanthocyanin, catechin and other polyphenols, like trans-resveratrol in Kadarka, Kékfrankos and Cabernet Franc after 2 years of aging.

INTRODUCTION

Phenolic components of wines are the most important group of compounds from oenological point of view, as those play an extraordinary role in the development of red wine characteristics, at the same time are responsible for processes involving browning. However, the concentration of polyphenol compounds is different in the wines, which is mainly determined by the original quantity of these compounds in the raw material, in the grape. The other important factor is wine-making technology - the period and the temperature of fermentation on the skin that also significantly influences the concentration of polyphenols in the wine. In addition to the importance of wine-making, the role of wine storage and ageing is also extraordinary. In case of a red wine the given wine making technology is playing an extremely important role in the volume of polyphenol that is present in the wine, because these compounds - quercetin, catechin, epicatechin, proantocianidine, resveratrol - are concentrated in the grape skin structure. The longer is the fermentation process on the skin, the more is the quantity of polyphenol compound released from the skin. (Creasy and Coffee, 1988.) In freshly harvested grape skin there can be even 50-100 micrograms of resveratrol. Resveratrol content of wines in the case of red wines is 0.1-14 mg / l, in the case of white wines, 0.04-3.5 mg / l. (Mark et al., 2005.)

LITERATURE REVIEW

Several studies prove that cultured and moderate red wine consumption may be physiologically beneficial, and its positive impact on health and the development of organoleptic properties are due to the fine composition of wines. Three large poliphenol groups can be distinguished: flavonoid phenols, non-flavoid phenols and tannins. (Peri and Pompei, 1971.) Catechin, anthocyanin and leukoantocianin monomers belong to flavonoids of which procyanidins are built. These possess superior antioxidant activity, responsible for the organoleptic changes in the wine and also for the predisposition to browning. (Singleton and Esau, 1969.) The sense of bitter, tart taste and structure are determined by catechins leucoantocyanins. (Kennedy, Saucier and Glories, 2006.) Flavanols as well as cinnamic acid and its derivatives play an important role in the stabilization of anthocyanins during the colour

development of the new wines. (Boulton, 2011.) Resveratrol belongs to the group of non-flavonoid phenols, which belongs to the family of stilbens, and has an important function in terms of health effects as well. On one hand it has a positive effect on health - protects against cardiovascular diseases and shown to be effective in some cancer lesions. (Guerrero RF et al., 2009.) Gautam's researches have proven that trans-resveratrol has an important cell inhibiting and killing function in the case of leukemia cells. (Gautam et al., 2000.) - On the other hand it protects the grape against pathogen fungal infections. (Seigneur et al., 1990.)

OBJECTIVE

The objective of the research is to determine the practical aspects with scientific underpinning, chemical follow-up of wine ageing phases, monitoring observation and quantification of the changes in polyphenol concentration. The total polyphenol content of three red wines are examined. These are different varieties, but from the same wine region. The wines are aged in standard and barrique oak barrels in normal operating conditions. During the two-year ageing period, polyphenol changes are closely monitored with biannual sampling, regardless of the vintage.

MATERIAL AND METHOD

Three characteristic wine varieties of Szekszárd wine region have been tested: Kadarka, Kékfrankos, Cabernet Franc. The wines received no other treatment than a racking and a basic sulphuring. The items were racked twice and were treated with for 35 mg / l free sulfur concentration. The harvest dates were different in case of the three varieties, cabernet franc harwest was at the beginning of October, Kadarka in mid-October and the Kékfrankos took place in late October. In case of all three varieties healthy raw materials were processed. Alcoholic fermentation happened within controlled conditions during a 8-14 days period on a temperature of 18-24℃ using Uvaferm BDX yeast. Following the alcoholic fermentation the wines were racked into wooden and barrique casks, the control samples were in stainless steel tanks. Samples were taken in every six months, the last, the third one was at the end of April 2015. The basic analysis of wines and the polyphenol content were examined. Application of spectrophotometric methodologies: total polyphenol content was determined with the use of Folin-Ciocalteau reagent, calibrated on gallic acid (Kallay and Török, 1999.); theamount of leucoantocyanins was defined on spectrophotometer after heating it with the 40:60 mixture of hydrochloric acid and butanol containing iron (II) sulfate based on Flanzy (1970) method; anthocyanin content was measured with spectrophotometer at 550 nm, after a dilution with 96% ethanol that contained HCI in 2% v / v concentration also based on the modified Flanzy (1970), method; catechin content was measured in the wine that had been diluted with alcohol reacted with sulfuric acid reacted with vanillin, on 500 nm with the use of spectrophotometer. (Rebelein, 1965.) Application of High Performance Liquid Chromatography (HPLC) methodology: cis-resveratrol and trans-resveratrol concentrations of wines were determined by direct injection, appling HPLC method (Kallay and Török, 1997.)

RESULTS AND DISCUSSION

The results support the fact, that trans-resveratrol shows a clear increase after the third sampling. (Figure 1.) (Source: own result) Trans-resveratrol, that is one of the two geometric isomers of resveratrol, is present in the grape skin in the form of glucoside called piceide. This piceide transformed into trans-resveratrol during the alcoholic fermentation in the way, that glycosidic bond was decomposed by β-glucosidase enzyme. (Kallay and Nyitrai-Sardy, 2007.)

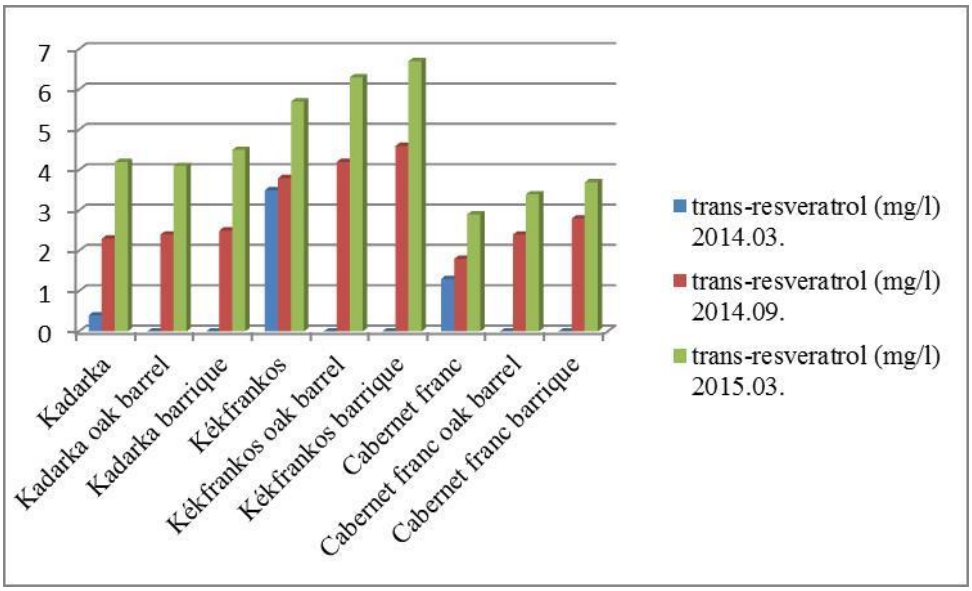

Figure 1.: The increase of trans-resveratrol in Kadarka, Kékfrankos and Cabernet Franc during wooden barrel storage

CONLCUSION

In case of the studied wines varieties our primary goal was to perform field trials. Our previous test results clearly show, that different wine varieties have different starting polyphenol concentration and also the changes of the initial tannin phenols are developing in different ways as far as wine varieties and ageing methods are concerned. (Table 1.) Based on the first chart it can be concluded that the growth of total polyphenol concentration was experienced during the oak ageing of these three varieties, Kadarka, Kékfrankos and Cabernet franc. As it was observed, the amount of trans-resveratrol, that has physiologically important role, showed growth in volume in case of Kadarka, Kékfrankos and Cabernet Franc varieties during the oak barrel aging and more specifically during barrique ageing.

Table 1.: Baseline concentrations of polyphenols

Wine	Total polyphenol (mg/l)	mono. ant.(mg/l)	leuco ant. (mg/l)	catechin (mg/l)	polim (%)	color int.	color tone	cis-resver. (mg/l)	trans-resver. (mg/l)
KAD	1567	234,0	1354	1021	44,00	5,30	0,96	0,4	0,4
KFK	2355	473,0	2022	1825	38,00	6,82	0,67	1,00	3,5
CAF	2683	380,0	2474	2675	31,00	3,58	0,61	0,2	1,3

Source: own result

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THE EFFECT OF ESSENTIAL OILS ON FOODBORNE PATHOGENIC AND SPOILAGE BACTERIA OCCURING ON THE SURFACE OF FRESH VEGETABLES

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SUMMARY
In appropriately low concentration the mixture of different essential oils of plant origin may represent an alternative solution for preservatives applied in vegetable protection. Based on results of organoleptic analysis the utilisation of essential oils is acceptable for consumers. Some of the chemical components of the essential oils decrease cell viability, changing the cell membrane permeability, resulting in changes of the cellular structure and perturbing the normal cellular functions. During the experiments we study the antimicrobial activity of some selected essential oils on pathogenic and spoilage bacteria isolated from the surface of fresh vegetables. Based on the results we find that individual essential oils and mixture compositions (due to synergic effect) could be good candidates for the preservation of fresh vegetables.

INTRODUCTION
Fresh vegetables may be contaminated with pathogenic and/or spoilage bacteria during their growth, harvesting, transportation and processing. The main bacterial species isolated from fresh vegetables are: *Aeromonas hydrophila*, *Bacillus cereus*, *Campylobacter jejuni*, *Clostridium botulinum*, *C. perfringens*, *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella enterica* serotypes, *Shigella sonnei*, *Yersinia enterocolitica*, *Staphylococcus aureus*, *Vibrio cholerae* (György, 2014). Due to their high moisture content (~80-90%) vegetables are considered as highly perishable products and present a major problem that their surface may easily be contaminated by soil microorganisms. In spoilage of vegetables the primordial role have bacteria for example: *Pseudomonas*, *Flavobacterium*, *Acinetobacter*, *Alcaligenes*, *Xanthomonas*, *Chromobacterium*, *Enterobacter*, *Klebsiella*, *Serratia*, *Micrococcus*, *Lactobacillus*, *Corynebacterium*, as well as *Bacillus* strains (Deák, 2006). A variety of methods are used (physical, chemical, biological, and combined) to inactivate and/or to remove these pathogenic microbes, but the lack of a truly effective antimicrobial treatment may still present great concern from the point of view of food safety (Ramos, 2013; São José, 2014). The mixtures of different essential oils of plant origin at quite low concentration could be an alternative solution in case of vegetables to the conventional synthetic preservation agents. For example, the mixtures of the oregano and rosemary essential oils (obtained from *Origanum vulgare* L. and *Rosmarinus officinale* L., respectively) in low concentration (without individual inhibitory effect) was effective bacteriostatic and bactericide against pathogenic bacteria occuring on the surface of minimally processed fresh vegetables. Based on the results of organoleptic tests, the utilisation of these essential oils was acceptable for consumers (Alves, 2011). The combination of carvacrol and 1,8-cineole has bacteriostatic and bactericidal effect on *Listeria monocytogenes*, *Aeromonas hydrophila* and *Pseudomonas fluorescens* in mixed cultures. These essential oil components reduce the cell viability, by growing the cell membrane permeability and triggering cell structure modifications (Rimá de Oliveira, 2015). The mixtures of natural antimicrobial substances (carvacrol+thymol and carvacrol+thymol+eugenol) possess a strong inhibitory effect against bacteria occuring on the surface of fresh vegetables (Zheng, 2013).

To increase the shelf-life of minimally processed fresh-cut vegetables an alternative solution may be the application of edible films. Combining the coating material with natural

antimicrobial agents could represent a viable possibility for increasing the safety of the fresh-cut produce (Ciolacu, 2014).

The antimicrobial mechanism of essential oils

Essential oils (EO) are complex mixtures of plant secondary metabolites, composed mostly of terpenes, terpenoids, phenylpropanoids and other compounds with various structures (e.g. volatile sulphur and/or nitrogen-containing compounds). Due to the large structural variety of bioactive components, the molecular and cellular mechanism of the antimicrobial activity is multiple (Burt, 2004). Moreover, the activity depends on structural differences of the cell walls between the Gram-positive and Gram-negative bacteria (Nazzaro, 2013). The main effect on the bacterial cell is the membrane swelling and even membrane deterioration causing leakage and membrane selectivity disturbance, given by EOs pronounced lipophilicity (Burt, 2004; Saad, 2013). Terpenes, as single compounds, in general cause only the swelling of the membrane, but facilitate the action of terpenoids, that are membrane disruptors and cause cytoplasm coagulation (Nazzaro, 2013). The essential oil components inserting in lipid bilayer cause the distortion of the lipid-protein complex structures. The result is serious perturbation in trans-membrane ion transport (mainly for H^+ and K^+). The membrane potential changes, the ATP-level of the cell decreases drastically and energetic depletion occurs. The decrease of ATP-pool may be caused by reduction of ATP synthesis and/or increase ATP hydrolysis (Saad, 2013). In our opinion the intense ATP hydrolysis is probably the result of intensive ion pumps ATP-ase activity, which trying to maintain the homeostasis of the cell against the intensive uncontrolled ionic influx, similar to effect on cardiomyocytes of some toxines (András, 2011). A major inhibitory mechanism of the EO is the quorum sensing (QS) inhibition, as the QS phenomena has an important role in bacterial pathogenicity, antibiotic resistance and biofilm formation in several systemic and local infections (Szabó, 2010). Another important aspect is the potential of some EOs to reverse the antibiotic resistance of microbial strains (Becerril, 2012). In combination, the EOs often shows synergic antimicrobial effect at low concentration against various microbial strains (Lv, 2011). This synergism could be a main advantage of EOs as antimicrobial agents, as the multiple combination possibilities enhance the probability of increasing effectiveness against the phenomena of antibiotic resistance (Baby, 2009; Yap, 2013, 2014). The EOs may complete or, in some cases replace the synthetic products in food preservation and packaging, as they possess good efficiency and are environment-friendly (Adelakun, 2016). In contrast, the aroma intensity, even at low quantities, may cause organoleptic problems in foods (Hyldgaard, 2012), and restrains their applicability in specific modalities, as coatings and special packaging additive components.

MATERIAL AND METHODS

During our work we determined the antimicrobial activity of different essential oils (thyme, fennel, lemongrass, oregano, sage, juniper) with agar diffusion method on pathogenic (*Escherichia coli*, *Salmonella* sp., *Campylobacter jejuni*, *Bacillus cereus*, *Staphylococcus aureus*) as well as spoilage bacteria (*Pseudomonas* sp., *Bacillus subtilis*, *Bacillus* sp.) isolated on selecive culture media from the surface of various fresh vegetables (tomato, pepper, cucumber, lettuce, cabbage, radishes).

At the same time, in the case of some volatile oil combinations we determined the phenomena of synergism and antagonism: oregano+sage, oregano+lemongrass, oregano+thyme, juniper+fennel, sage+lemongrass, sage+fennel, sage+thyme, sage+oregano, lemongrass+thyme, lemongrass+fennel. In the case of the agar diffusion method, in the sterilized Petri dish 20 ml Nutrient agar medium is poured. After solidification the medium is inoculated on the surface with 0.1 ml suspension of microorganisms taken in study. In the

center of all of the inoculated mediums a 8 mm diameter hole is cut with the help of a sterile test-tube. In the hole 0.05 ml of essential oil is dropped. The incubation is realized at the temperature of 37 ºC, 24-48 hours. The dimension of the inhibitory area is measured. In case of the used essential oils the phenomena of synergism and antagonism can be determined within the agar diffusion method. In the holes made in the inoculated mediums, essential oils (0.025 ml) are added, obtained from two studied species of plants. After incubation the results are read and expressed in accordance with the size of the inhibition zone.

RESULTS

In our work we investigate the antibacterial activity of six essential oils against twenty bacterial strains isolated from the surface of different fresh vegetables. In 20 bacterial isolates, thyme essential oil shows inhibitory effect in 13 cases, followed by the oregano essential oil (inhibitory effects against 9 different bacterial isolates), then by sage and fennel (inhibitory for 8 bacterial isolates), as well as juniper (7 bacterial strains) (Table 1., Table 2., Table 3.). The lemon grass essential oil shows bacteriostatic effect only against *Salmonella* sp. isolated on salad. The largest inhibitory zones were observed in cases of thyme and juniper essential oils, the smallest for fennel seeds essential oil. For inhibition of spoilage microorganism the juniper essential oil was the most effective. In the majority of cases for the combination of essential oils antagonistic effect was observed, but for 3 combinations synergism was observed, as follows: junipera-thyme, oregano-thyme and sage-caraway. For example in the case of the *Escherichia coli* strain isolated from cucumber: the dimension of the inhibition zone for junipera essential oil is: 18.5±0.77, for thyme: 21.13±1.09, and for junipera-thyme essential oil combination is: 33.87±1.089.

Table 1. The effect of the thyme essential oil at the studied bacterial isolates I.

Studied bacteria (Source)	*E. coli* Cucumber1	*E. coli* Cucumber2	*E. coli Radishes*	*E. coli* Lettuce	*E. coli* Cabbage	*Salmonella* sp. Lettuce	*Salmonella* sp. Cucumber2
Dimension of inhibition zone in mm (average±SD)	21.13 ±1.09	18.28 ±1.36	18.9 ±2.94	19.07 ±4.19	16.75 ±2.53	25.97 ±0.59	11.41 ±0.74

Table 2. The effect of the thyme essential oil at the studied bacterial isolates II.

Studied bacteria (Source)	*Bacillus cereus* Radishes	*Bacillus cereus* Cucumber1	*Campylobacter jejuni* Cucumber2	*Bacillus subtilis* Cucumber2	*Bacillus* sp. Cucumber1	*Pseudomonas* sp. Cucumber2
Dimension of inhibition zone in mm (average±SD)	16.65 ±1.20	18.85 ± 0.81	27.77 ±0.92	18.37 ±1.07	21.89 ±3.82	21.11 ±3.08

Table 3. The effect of the oregano essential oil at the studied bacterial strains

Studied bacteria (Source)	*E. coli* Cucumber1	*E. coli* Lettuce	*Salmonella* sp. Lettuce	*Salmonella* sp. Cucumber2	*Bacillus cereus* Radishes	*Bacillus cereus* Cucumber1	*Bacillus* sp. Cucumber1	*Bacillus subtilis* Cucumber2	*Pseudomonas* sp. Cucumber2
Dimension of inhibition zone in mm (average±SD)	16.93 ±0.71	19.49 ±4.69	20.38 ±1.17	14.88 ±3.31	10.31 ±0.73	13.39 ±0.62	22.02 ±0.77	13.24 ±2.29	15.94 ±1.70

CONCLUSIONS

Among the studied essential oils the most effective was the thyme essential oil. The synergic effect was observed in case of the following essential oil combinations: junipera-thyme, oregano-thyme and sage-fennel. The sensitivity of the isolated bacterial strains against the different essential oils shows a great variability, differences were observed even among the sensitivity of the same strains isolated from different vegetables.

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VARIATION IN THE MINERAL CONTENT OF WINTER WHEAT IN HUNGARY

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SUMMARY

Nowadays, beside the food quality more and more important fields of interesting of consumers are the food safety and the wholesomeness of foods. This revalued the importance of compounds in small amounts; e.g. vitamins, toxins, mineral elements, aroma components and so on. The element composition of foods determines that they are healthy and safety or not. We have been regularly measured in the last decades the element content of winter wheat and in the meantime, this research has become an important task, especially due to finding of long-term effects. The aim of this study was to compare the concentrations of Ca, and Sr in the whole meal samples of various ages, as previously published data suggested that the mineral content of wheat declined to about half from 1942 to the present day. Based on the results of NPK fertilization increased the strontium contents of winter wheat grains. Over the past 170 years the strontium content of wheat grains has increased.

INTRODUCTION

In Hungary, winter wheat accounts for more than 30% of total energy intake, therefore the quality of winter wheat is especially important in case of these agricultural products. In previous years the technological quality parameters (such as gluten content, falling number and dough properties) were much more important. Today there is an increasing demand for the determination of macro- and micro elements. For a healthy nutrition the mineral content is very important to analyze and the new methods give us opportunity to determine more and more elements. Regarding the essential micronutrients wheat is one of the main sources in human diet (Kent, 1990). The mineral content of cereals depends on growing conditions and genetic properties. Mineral fertilization has significant effect on element concentration of wheat grain (Dikeman et al. 1982). Several researchers have been summarized the long-term effects of mineral fertilization, breeding, weather conditions, soil conditions on the mineral content of winter wheat in several articles.

Ekholm et al. (2007) found that trace element density in vegetable foods has decreased in Finland over the past three decades but only the K content was decreased significantly in cereals. All the changes in agricultural practice: new cultivars, different fertilizers and so on, have together caused the decrease in the trace element contents of vegetable food.

Burján and Győri (2013) showed that the different cropping sites of the Hungarian National Long-term Fertilization Trials, such as Iregszemcse, Karcag, Kompolt and Nagyhörcsök caused significant differences in the K, P, S, Mg, Ca, Fe, Mn, Zn, Cu, Sr element ($P<0.01$) and protein contents ($P<0.05$) of the winter wheat whole grain. Kirchmann et al. (2009) reviewed that significantly declining Pb and Cd concentrations in wheat grain could be explained by lower atmospheric deposition. A doubling of Mo concentrations in grain since 1975 resulted in Cu/Mo ratios often below one, which may cause molybdenosis in ruminants. The increase in Mo concentrations in crops correlated with the decline in atmospheric deposition. Concentrations of Cu and Fe declined in NPK-fertilized wheat as compared to unfertilized or manure-treated wheat. According to Fan et al. (2008) research the concentrations of zinc, iron, copper and magnesium remained stable between 1845 and the mid 1960s, but since then have decreased significantly, which coincided with the introduction of semi-dwarf, high-yielding cultivars. Győri (2015) has published the Cu, Zn and Mn contents in the whole winter wheat grains to have decreased but the calcium content increased, over the past 170 years. Bálint et al. (2001) and Skrbic and Cupic, (2005) communicated data from micro elements content of different wheat varieties in the region.

MATERIALS AND METHODS

Samples were collected from various locations in Hungary on fertilization; irrigation and crop variety testing are conducted. These included the experimental nurseries of the University of Debrecen, experimental stations involved in Hungarian National Long-term Fertilization Trials (OMTK) and the field variety trials of the National Institute for Agricultural Quality Control (OMMI), all of which have different types of soil and climatic conditions. The number of samples analyzed between 1974 and 2006 was close the ten thousand. Three samples (from 1909, 1919 and 1936) were found in the OMMI archives and one in the Hungarian Agricultural Museum (Pannonhalma Collection).

Between 1974 and 1988 the element contents of the samples were measured using an atomic absorption spectrophotometer, while from 1988 inductively coupled plasma optical emission spectrometers (ICP-OES and ICP-MS) were used, after digestion with HNO3-H2O2 solution (Kovács et al. 1996; 1998). The data were analyzed using the SPSS for Windows 22 software package.

RESULTS AND DISCUSSION

In the present study, the contents of two mineral elements were analyzed: Ca and Sr. Ammonium-nitrate and superphosphate as Ca-rich fertilisers contain also high amounts of Sr. That is why the uptake of this element and the Sr contents of the part of the crop that is meant to be used as food has to be regularly tested since high Sr contents carry risks. Figure 1 shows the results of sample analyses from National Long Term Field Experiment), which was set more than forty years ago. The data show that the Sr contents in wheat increased at all production locations and the most significant increases were found in Keszthely, Karcag and Putnok. Other findings from the experiments in Debrecen, where the Sr contents data are illustrated comparing to Ca contents and shows a linear correlation (Figure 2). We found that 600 mg/kg and 400 mg/kg Ca contents are accompanied by 4 mg/kg and 3 mg/kg Sr contents, respectively.

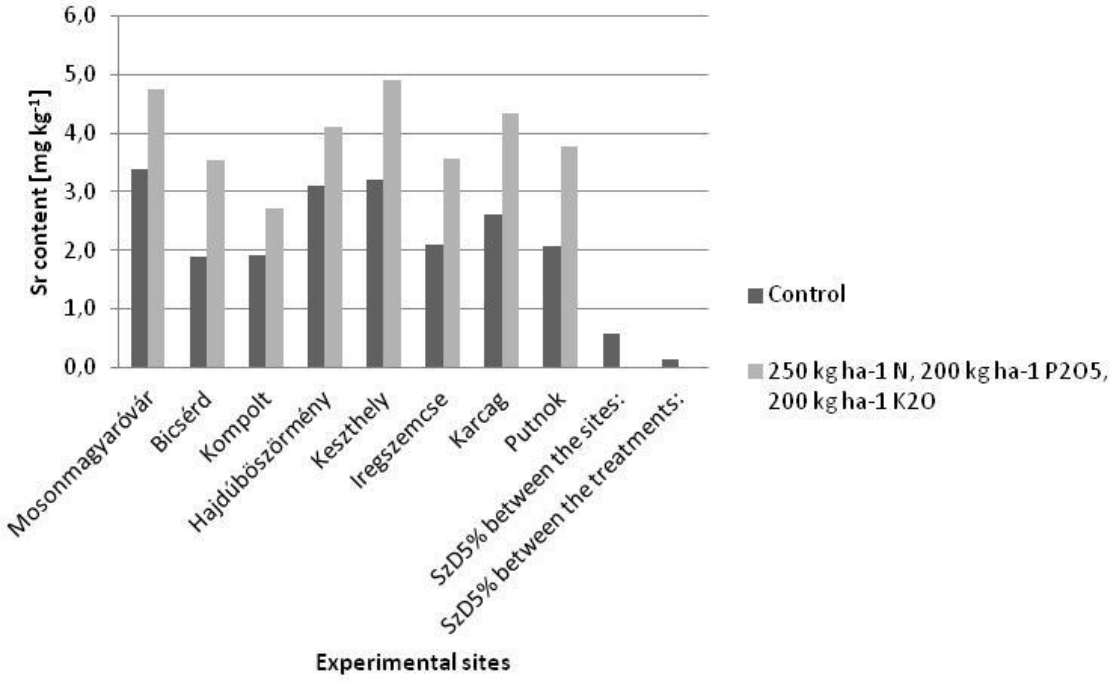

Figure 1.: Effect of mineral fertilization on the Sr content of winter wheat grains on different experimental sites

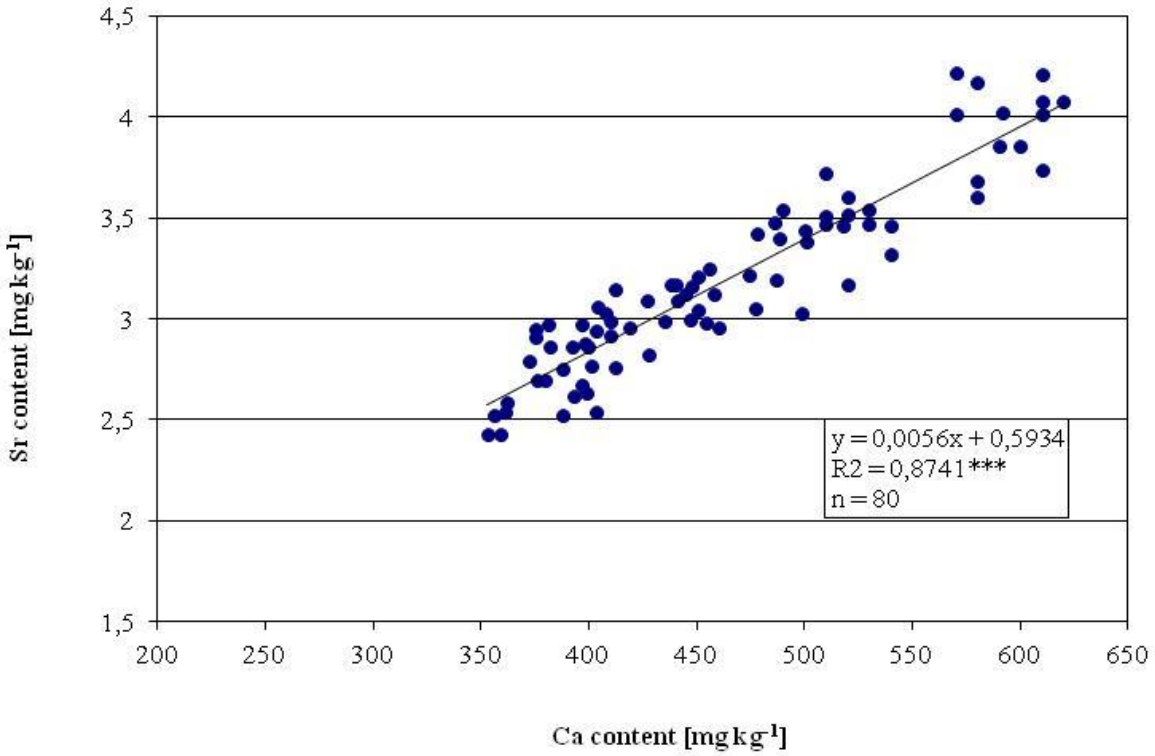

Figure 2.: Connection between Ca and Sr content of winter wheat grains (in 1995)

Ten years later, according to the data of different winter wheat varieties the strontium content slightly decreased (Table 1). This result can be explained by the greatly reduced dosages of mineral fertilizers in the new crop production practice. The Ca/Sr ratio is about 200 for the studied winter wheat varieties. This is consistent with Rosental (1981) who reported data about Sr content in the Eastern European diets. He published that typical diets in this area may contain over 5mg Sr/1000mg Ca. The effects of weather may be significant between the two copping years.

Table 1.: Sr content of different winter wheat varieties in two harvest years

Varieties	2003	2004
GK Kalász	1.49 ± 0.27	1.42 ± 0.37
Mv Magdaléna	2.11± 0.47	1.86 ± 0.52
Ludwig	2.60 ± 0.73	1.93 ± 0.44
Jubilejnaja 50	2.03 ± 0.45	1.81 ± 0.33
Lupus	2.40 ± 0.40	2.14 ± 0.46
GK Öthalom		2.00 ± 0.35

I have tried to estimate the change in strontium contents in the past 170 years by using a trend line. The concentrations of strontium have increased in the context of calcium concentration.

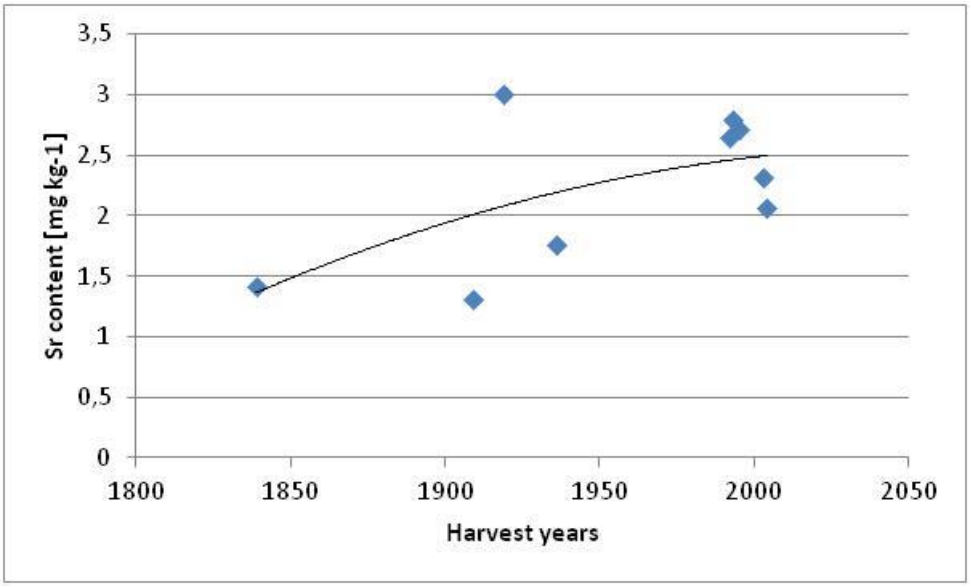

Figure 3.: Changes of Sr content in winter wheat grains in different years

CONCLUSIONS

The main conclusions were as follows: The NPK mineral fertilization increased the Sr contents in winter wheat grains at all experimental locations. Between the calcium and strontium contents of wheat grains are a significantly close relationship. The strontium contents of the wheat grains have increased during the past 170 years.

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DIVERSITY OF *BOTRYTIS CINEREA* POPULATION OF "ASZÚ" BERRIES COMPARE WITH IN TWO DIFFERENT VINTAGE

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SUMMARY

The filamentous ascomycete fungus Botrytis cinerea ('grey rot') has two remarkable characters: on the one hand, it can cause heavy losses in vineyards and in the agriculture in general, but on the other hand, it is the microbe responsible for the noble rot of grape. This economic importance of B. cinerea has inspired an extensive research activity into its biology and disease management, as well. Based on these considerations, phenotypic and genetic properties of Botrytis cinerea and the microbial diversity of noble rot 'aszú' berries were studied in two different vintage. The isolates originated from vineyards where only two single grape varieties, Olaszrizling (János et al., 2012) and Turán (Hajdú E., 2015), have been cultivated. Genetic diversity of Botrytis cinerea population was determined by the analysis of MSB1 minisatellite sequences located within the intron of the ATP synthase.

INTRODUCTION

Botrytis cinerea be a species complex, the cryptic species *Botrytis pseudocinerea* (teleomorph: *Botryotinia pseudofuckeliana*) being found in sympatry with *B. cinerea* (teleomorph: *Botrytinia fuckeliana*), but at low frequency (Walker et al., 2014).

This fungus is a cosmopolitan haploid ascomycetous and heterothallic microorganism. Botrytis infection requires moist conditions and if the weather stays wet, the malevolent form, "grey rot" can destroy crops of grapes. Grapes typically become infected with botrytis when they ripen, but when they are exposed to drier conditions, they become partially dried and the form of infection is brought about by the partial drying process which is known as "noble rot", "botrytization" or "over maturation". When grapes are picked at a certain point during infection, they can produce particularly fine and concentrated sweet wine. The clue to these strategies lies in the understanding of genetic structure and dynamics of its populations (Fournier et al., 2012).

A plant pathogenic fungal population with a high level of genetic variation is likely to adapt more rapidly to fungicides or resistant host plants. On the level of migration between populations and on the presence or absence of sexual reproduction within a population may indicate how rapidly will novel (fungicide resistant, more pathogenic) genotypes spread between populations (Fournier et Giraud, 2008).The pathogen spreads through asexual cycles in spring and summer, mostly dispersed by wind and human activities. Although signatures of recombination indicate the existence of sexual reproduction in overwintering field population (Walker et al., 2014.).

Population surveys often using repetitive elements like microsatellite, transposable element, diverse retron and minisatellite markers have reported diversification of a population. Microsatellites need for sophisticated separation and visualization apparatus due to the small size of the individual repeats. In contrast to microsatellites, minisatellites can be separated and visualized by conventional gel techniques, therefore the minisatellite typing remains an important fingerprinting techniques.

Minisatellites, a class of tandem repeats of 6-120 bp in arrays that are 0,5-30 kb long. They are ubiquitous, rapidly evolving sequences with a high degree of length polymorphism, but their position appears to be essentially stable (Wöstemeyer et al., 2002). The first

minisatellite sequence was identified in a human chromosome. The meiotic recombination causes the instability of this GC rich tandem repeats. In contrast AT rich minisatellites – like the MSB1 - appear to evolve by intra-allelic processes such as replication slippage (Giraud, 1998), (Bois et al., 1999), (Attard et al., 2001). Out of the identified minisatellites four were isolated in filamentous fungi, one of these the MSB1, which can found in the Botrytis cinerea. The basic repeat unit of MSB1 has a length of 37 bp; and the number of repeats from different isolates varies between 5 and 11. The repeat units differ somewhat in sequence and can, therefore, be recognized individually as variants of a common motif. These variants map in identical order in all fungal isolates (Wöstemeyer et al., 2002). MSB1 is a single-locus minisatellite which can be found in the mitocondrium in one loci in the intron of ATP synthase gene (Giraud 1998).

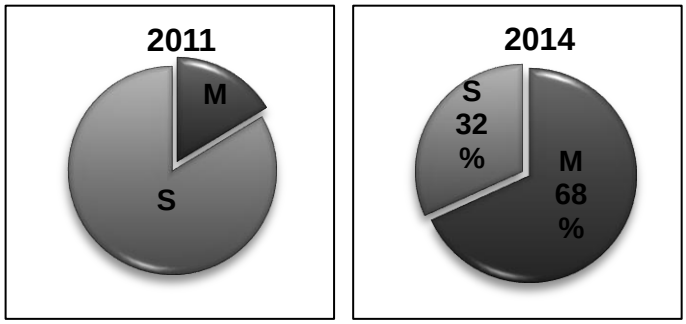
MATERIALS AND METHODS

B. cinerea strains were collected from two small vineyards of the Eger wine district during the vintage period (September-November) in 2011 and 2014 from two dedicated grape varieties Turán and Olaszrizling. Single-spore isolates were prepared from each isolation, and maintained on potato dextrose agar (PDA). The isolates were identified by their common morphologic properties. Morphological characterization was done after a 21-day 20 ^{0}C dark incubation on potato dextrose agar (PDA) by monitoring of the sporulation, mycelium and sclerotium production (Martinez et al, 2003). Genetic characterization was done by analyzing the MSB1 minisatellite sequence. Total genomic DNA was extracted from aerial mycelium of *B. cinerea* strains grown on PDA. MagnaLyser (Roche Applied Science) was used for the disruption of fungal cells, and DNA was isolated with the DNEasy Plant Mini Kit (Qiagen). The amplification of the intron of the ATP synthase gene containing the microsatellite MSB1 (Giraud et al, 1998) was carried out by using primers MSB1fw and MSB1rev. The fragment analysis was performed in an automated single-capillary genetic analyzer (Qiagen). PCR products were purified and sequences were analyzed by LGC Genomics (Berlin, Germany). Editing and alignment of sequences were done manually by Jelwiev (Waterhaus et al., 2009).

RESULTS AND DISCUSSION

Morphological variability

The sclerotium production of *B. cinerea* is the most common phenotypic feature (Elmer et al., 2004). During morphological examination, the isolates presented a high sclerotium production and a low mycelium production ability in 2011. In contrast high mycelium production and low sclerotium production was identified in 2014 (Figure 1.). No significant differences were found between the samples from the different grape varieties or the samples from the different vineyards. In the base of sequential collecting times, differences were found between the isolates, because the sclerotium production rate of isolates collected in late autumn (October, November) was higher than in isolates which were collected earlier (September). But in 2014 significant differs were analyzed. The high proportion of sclerotium production demonstrates the good adaptation ability of *B. cinerea* to the environmental effects in the base of late harvesting technology. It means vintage of 2011 was suitable for the noble rot, but the isolates from 2014 have shown gray rot symptoms.



Dry, warm autumn | Wet September and October, warm autumn

Figure 1: Distributiun of the two morphological group (mycelial and sclerotial) in the two different vintage

3.2. Genetic variability

Table 1. : Alleles of MSB1 Minisatellite sequences according to MVR (Minisatellite variant repeats)

Numbers of alleles	**Numbers of repeats**	Numbers of isolates in **2011**	Different MSB1Variants								
1	**7**	11	a11	b111			b115	**D2H**	b174	c121	c3231
2	**8**	1	a11	b111	b112		b12	**b18H**	b1712	c121	c321
3		82	a11	b111	b112		b12	b2	b1712	c121	c321
4	**9**	2	a11	b111	b112	b112	b12	b2	b1712	c121	c321

Numbers of allels	**Numbers of repeats**	Numbers of isolates in **2014**	Different MSB1 Variants							
1	**8**	10	a11	b111	b112	b12	b2	b1712	c121	c321
3	**7**	2	a11	b111	b115	**D2H**	b174		c121	c3231
4		2	a11	b111	b112	b12		b1712	c121	c321
5		1	a11		b112	b12	b2	b1712	c121	c321
6	**4**	1	a11					b176	c121	c321
7	**7**	1		a12	b112	új	b2	b1712	c121	c321
8		5		b111	b112	új	b2	b1712	c121	c321
9		4		b111	b112	b12	b2	b1712	c121	c321
10	**6**	6			b112	új	b2	b1712	c121	c321
11	**5**	4				új	b2	b1712	c121	c321
12	**3**	1				új		b1712		c321
13	**4**	4					b2	b1712	c121	c321
14	**2**	3							c121	c321

In 2011 sequences of 96 isolates were identified and in the other vintage (2014) 44 sequences were analyzed. In former studies high genetic variability was noticed in the case of genetically informative marker MSB1 in average and in samples from these territories (Váczy et al, 2007; Váczy et al, 2008). Contrary to earlier results, in the case of MSB1 minisatellite a low allele number and variability was found in 2011, as the 1. Table is shown. But in 2014 more allele number and higher variability was identified (1. Table). In 2011 one variant was identified only in one isolate (b18H), which was also identified earlier but only in the Tokaji wine region, as well from one isolate. In the two vintage D2H variant was appeared, this variant have detected only in Hungarian isolates. Among our MVR sequences in 2014 a novel variant can be discovered, which has shown the following sequence:

AAATCATGATTGTTTGACTAATTGTTGGACATTGAT. Between this novel sequence and the sequence of b12 variant only one nucleotide difference can be appeared.

4. CONCLUSION

In summary, the morphological analyses show significant differences between the two different vintages. The unequal distribution of precipitation can cause the different compound of the two main morphological group, the sclerotial and the mycelial. The genetic diversity was higher in 2014, when not only noble rot isolates were identified, contrary in 2011 when the proportion of noble rot isolates were higher, thus a homogenous population structure was demonstrated. Among the MVRs of MSB1 minisatellite in 2014 a novel variant can be appeared.

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APPLICATION OF 1-MCP ON APRICOT AT DIFFERENT TEMPERATURES AND DAYS AFTER HARVEST

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SUMMARY

The efficacy of 1-MCP on apricot at different treatment temperatures and days after harvest during 6 weeks of storage at 1°C was investigated. On the 1st, 3rd or 5th day after harvest, fruits were treated with 1-MCP at 1°C for 24 hours. In order to evaluate the effect of temperature, apricots were exposed to 1-MCP (625–650 ppb) at 1, 10 and 20°C (on the 1st day after harvest). 1-MCP application reduced the ethylene, CO_2 production and delayed softening. Fruits treated on the 1st day after harvest presented the highest firmness at the end of experiment. No significant effect of treatment temperatures on apricot quality was observed. The time period between harvest and 1-MCP application could be delayed till the 5th day after harvest. Results showed that 1-MCP extended the storage life and maintained the overall quality of apricot during storage and shelf-life.

INTRODUCTION

The highly appreciated and popular apricot's (*Prunus armeniaca* L.) fame and value is due to its attractive orange colored appearance, unique flavor and nutritional value. However, because of its short season and postharvest shelf-life, the time for transportation, sale and consumption is limited in time. The postharvest ripening process of apricot is relatively quick, so the quality marks of the fruit decrease significantly including wrinkled skin, flesh softening, juiceless and nutrition losses (Wu et al., 2014).

The efficacy of 1-methylcyclopropene (1-MCP) in maintaining fruit quality in case of tomato, apple, pear, plum, avocado and melon (Blankenship and Dole, 2003) were widely reported. However, in the commercial utilization strategy, exposure temperature and delays of treatment play an important role in a close relation to the features of the different postharvest storage facilities. So, the aim of this study was to examine 1-MCP efficacy and response of apricot to the applications concerning the delays of treatment after harvest and different application temperatures during cold storage and shelf-life.

MATERIALS AND METHODS

Materials

Apricots were harvested at light yellow from an experienced grower in Hungary, in July 2015. Fruits were transported to the Faculty of Food Science, Corvinus University of Budapest, Hungary.

1-MCP (SmartFresh®, AgroFresh, Philadelphia, USA) as an application of SmartFresh® system was provided by Rohm and Haas Polska Sp.z.o.o.

Methods

Experimental design. Fruits were selected for uniformity of size, shape and freedom from external damage. Samples were selected randomly into 8boxes, each box including one hundred and fifty fruits. All the 6 boxes were treated with 625–650 ppb 1-MCP (standard commercial application rate) for 24 hours using an air-tight plastic box in both experiments. During the 24-h long treatments, control boxes (untreated) were kept at 1°C. After 1-MCP application, all of the 8 boxes were kept at 1°C and at RH 95%.

- For different temperature treatment. Apricots were kept at 1, 10 and 20°C before treatment. 3 boxes were treated with 1-MCP at 1, 10 and 20°C after 1 day of harvest.
- For different day treatment.

Table 1. Application time for different day treatments (C: Cooling at 1°C; T: Treated with 1-MCP, 24 hs at 1°C)

Day / Sample	0 (Harvest)	1	2	3	4	5
1st	C	T	C	C	C	C
3rd	C	C	C	T	C	C
5th	C	C	C	C	C	T
Control	C	C	C	C	C	C

Table 2. Measurement time (ST: storage. SL: shelf-life)

Day	0 (Harvest)	14d ST	14d ST + 7d SL	28d ST	28d ST + 7d SL	42d ST

Every two weeks, 25 samples of each box were removed from the fridge for 1 week of shelf-life at 20°C.

Measurements

- **Acoustic firmness.** Acoustic firmness was measured at two opposite sides on each fruit, using acoustic firmness sensor model DTF V0.0.0.105 (AWETA, Nootdorp, The Netherlands).
- **Ethylene production.** Ethylene production was determined by an ICA-56 hand-held ethylene analyzer (International Controlled Atmosphere Ltd., UK) being held for a given time in ahermetically closed plastic container. Results were expressed as microliter of ethylene produced per kilogram of fruit in 1 h ($\mu L \cdot kg^{-1} \cdot h^{-1}$).
- **Respiration rate.** Respiratory intensity as carbon dioxide production was measured by FY A600-CO2H carbon dioxide sensors (Ahlborn Mess-und Regelungstechnik GmbH, Germany) connected to an Almemo 3290-8 data logger (Ahlborn Mess-und Regelungstechnik GmbH, Germany). Results were expressed as milliliter of CO_2 produced per kilogram of fruit in 1 h ($mL \cdot kg^{-1} \cdot h^{-1}$).
- **Surface color.** Apricot peel color was measured with a Minolta Chroma Meter CR-400 portable tristimulus colorimeter (Minolta Corporation, Osaka, Japan). CIE L^*, a^* and b^* color characteristics were determined at two opposite points on the equator of each fruit. Hue angle (H^o) value was calculated as arctangent (b/a).
- **Soluble solids content.** The juice that was released by the firmness test was used to measure soluble solids content (SSC) with a hand-held temperature-compensated ATAGO PAL-1 digital refractometer (Atago Co. Ltd., Tokyo, Japan).
- **Statistical analysis.** All data were analyzed using analysis of variance (ANOVA) via SPSS version 11.0.1 (SPSS Inc, USA). The results were reported as means with standard deviations (95% confidence interval).

RESULTS AND DISCUSSION

Results

Application at different temperatures

Ethylene production of all samples increased during the storage period at 1°C and 1 week of shelf-life at 20°C. Ethylene production of control group showed a sharp increase during cold storage and 7 days of shelf-life (Table 3). The respiration rate of all samples rose dramatically throughout shelf-life period. Control fruits showed a much higher level of carbon dioxide production than treated fruits (Table 3). At the end of cold storage, the respiration rate of fruits treated at 1°C was lower than others. Differences in ethylene and CO_2 production for fruits treated at different temperatures were minor after 2 weeks and 4 weeks of storage plus 7 days of shelf-life.

Table 3. Quality of apricot at different application temperatures during storage and shelf-life

Time	Samples	Ethylene ($\mu L \cdot kg^{-1} \cdot h^{-1}$)	Respiration ($mL \cdot kg^{-1} \cdot h^{-1}$)	Firmness ($10^6 Hz^2 g^{2/3}$)
Initial		2.6	31.4	5.10
14d ST	Untreated	0.50a	9.1a	2.30 a
	App. temp: 1°C	0.30 b	5.8b	3.10 b
	App. temp: 10°C	0.25 b	5.4b	3.15 b
	App. temp: 20°C	0.23 b	4.7b	2.98 b
14d ST + 7d SL	Untreated	41.51 a	92.7 a	0.82 a
	App. temp: 1°C	3.72 b	50.2 b	1.45 b
	App. temp: 10°C	3.33 b	48.2 b	1.39 b
	App. temp: 20°C	4.06 b	47.7 b	1.44 b
28d ST	Untreated	1.11 a	11.1 a	1.31 a
	App. temp: 1°C	0.61 b	8.2 b	2.65 b
	App. temp: 10°C	0.43 b	8.7 b	2.70 b
	App. temp: 20°C	0.52 b	9.4 b	2.45 b
28d ST + 7d SL	Untreated	79.95 a	126.8 a	0.65 a
	App. temp: 1°C	33.72 b	52.6 b	1.24 b
	App. temp: 10°C	35.38 b	50.9 b	1.22 b
	App. temp: 20°C	34.03 b	51.5 b	1.30 b
42d ST	Untreated	4.46 a	15.1 a	0.95 a
	App. temp: 1°C	0.74 b	9.2 b	1.85 b
	App. temp: 10°C	0.69 b	11.9 b	1.79 b
	App. temp: 20°C	0.72 b	11.7 b	1.74 b

App.temp: Application temperature

Means followed by the same letters are not significantly different at $P \leq 0.05$ (at the same measurement time)

The firmness of treated apricots decreased gradually during 6 weeks of storage at 1°C. In contrast, apricot softened rapidly throughout shelf-life at 20°C (Table 3). The softening of fruits took place very quickly at 20°C during shelf-life. No significant effect of treatment temperatures was observed for firmness. But storage period and storage temperature had a significant influence on firmness.

During storage, the skin color of apricot turned from light yellow to orange. The color change was often reported as the sign of ripening (Dong et al., 2002). Hue angle value showed the same trend at different treatment temperatures (Fig.1). No significant difference between treatment temperatures was detected for SSC values.

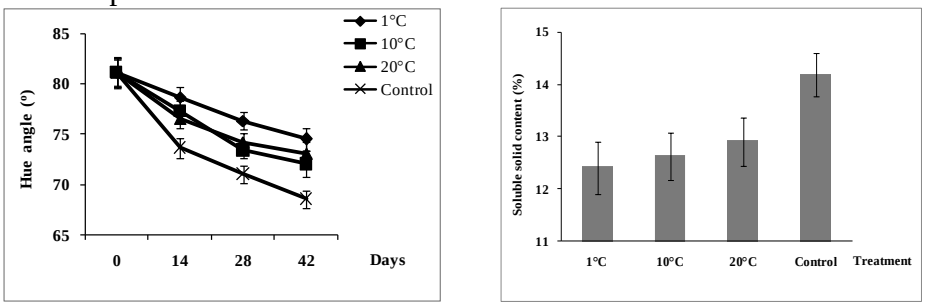

Figure 1. Hue angle (H°) and SSC (after 42 days of storage) of apricot treated at different temperatures. Values are the mean ± SD

Application delays of 1-MCP after harvest

The ethylene and respiratory production of fruits rose as the storage period increased, but at different rates. Apricot treated on the 1st day after harvest produced the lowest amount of ethylene and CO_2 on the 42nd day of the cold storage period (Table 4). Differences in firmness between fruits treated on the 1st, 3rd and 5th day after harvest were minor after 2 weeks of cold storage and shelf-life (Table 4). During the cold storage period, firmness of fruits declined gradually, but at 20°C for shelf-life the softening of samples occurred rapidly. Apricots treated on the 1st day after harvest were firmer than the samples of other treatment

days, but the firmness of samples treated on the 3rd and 5th day after harvest still remained high. No significant difference in ethylene, respiratory CO_2 production, firmness and hue angle values between treatment on the 3rd and 5th day after harvest was detected in case of 1-MCP treated samples.

Table 4. Quality of apricot at different application days during storage and shelf-life

Time	Samples	Ethylene ($\mu L \cdot kg^{-1} \cdot h^{-1}$)	Respiration ($mL \cdot kg^{-1} \cdot h^{-1}$)	Firmness ($10^6 Hz^2 g^{2/3}$)
Initial		2.6	31.4	5.10
14d ST	Untreated	0.50 a	9.1 a	2.30 a
	App. day: 1st	0.30 b	5.8 b	3.10 b
	App. day: 3rd	0.32 b	6.7 b	2.85 b
	App. day: 5th	0.35 b	6.2 b	2.5 b
14d ST + 7d SL	Untreated	41.51 a	92.7 a	0.82 a
	App. day: 1st	3.72 b	50.2 b	1.45 b
	App. day: 3rd	5.34 c	63.0 c	1.06 b
	App. day: 5th	7.95 c	64.6 c	1.08 b
28d ST	Untreated	1.11 a	11.1 a	1.31 a
	App. day: 1st	0.61 b	8.2 b	2.65 b
	App. day: 3rd	0.56 b	8.1 b	2.27 b
	App. day: 5th	0.68 b	9.2 b	2.04 b
28d ST + 7d SL	Untreated	79.95 a	126.8 a	0.65 a
	App. day: 1st	33.72 b	52.6 b	1.24 b
	App. day: 3rd	40.35 b	94.4 c	0.92 c
	App. day: 5th	47.43 c	92.3 c	0.88 c
42d ST	Untreated	4.46 a	15.1 a	0.95 a
	App. day: 1st	0.74 b	9.2 b	1.85 b
	App. day: 3rd	1.22 c	11.6 c	1.45 c
	App. day: 5th	1.38 c	12.4 c	1.40 c

App. day: Application day

Means followed by the same letters are not significantly different at $P \leq 0.05$ (at the same measurement time)

Hue angle values of apricot treated on the 1st day had a higher level than those of fruits treated on the 3rd and 5th day after harvest during the storage period. The skin of control fruits changed to orange much more rapidly than those of treated samples (Fig. 2). There was no significant effect of delays of treatment on SSC.

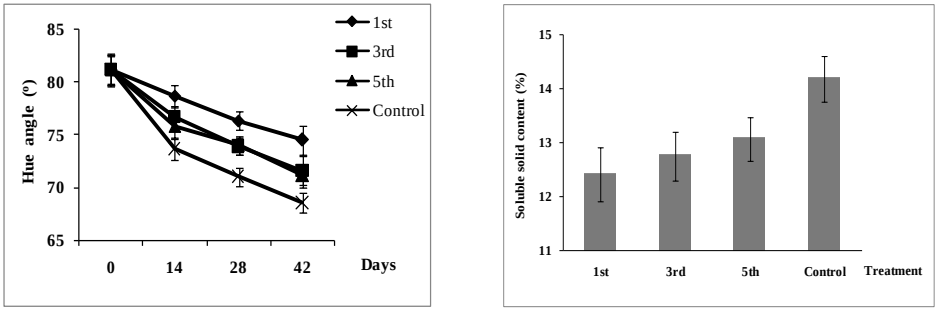

Figure 2. Hue angle (H°) and SSC (after 42 days of storage) of apricot treated at different days after harvest. Values are the mean ± SD

Discussion

In order to prolong the possible period of filling of a storage room, fruits should be cooled after harvest rapidly and then 1-MCP application should be carried out at cold temperature. In practice, application could be delayed with several days depending on room loading period. So, precooling fruits to suitable temperature in order to suppress the ethylene production and 1-MCP treatment at that specific temperature are really necessary. However, many studies reported that 1-MCP application at cold temperature was not as effective as carried out at warmer temperature and sometimes 1-MCP did not exert its action on some

crops. It was assumed, that the binding between 1-MCP and receptors was low at cold temperature (Macnish et al., 2000). For example, 1-MCP treatment at 20°C was more effective than 5°C for broccoli (Ku and Wills, 1999; Able et al., 2002). On the other hand, the effectiveness of 1-MCP depends not only on treatment temperature, but also on cultivar, maturity, concentration and treatment time (Watkins, 2006). There were several reports about the correlation between treatment period and temperature. DeEll et al. (2002) found that corresponding exposure duration of 'Cortland' apples was 6hs at 23°C or 9 hs at 3°C. Another research also indicated that there was no difference depending on treatment temperature between 20°C and 0.5°C for 24hs (Watkins and Nock, 2005). Treatment duration from 12 to 24 hs was enough to maintain the quality of fruits (Blankenship and Dole, 2003).

In this study, 1-MCP had strongly affected quality of apricot during cold storage and shelf-life. Ethylene and CO_2 production were lower in case of treated apricot than control samples. Moreover, the softening and color change of treated fruits also slowed down compared to the control. However, no effect of different treatment temperatures was observed, which coincided with the results found for apple (Dauny and Joyce et al., 2002).

Most changes in ethylene, CO_2 production, firmness and hue angle values were associated with the duration of storage periods and temperature rather than treatment temperatures and delays of application. The softening of fruits during shelf-life was faster than when kept cold. This study suggested that 1-MCP treatment on the 5th day after harvest or slightly earlier can maintain the quality of apricot in agreement with the results of previous reports (Kubo et al., 2003; Watkins and Nock, 2005).

CONCLUSION

Our results confirmed that 1-MCP treatment has a potential in delaying the ripening of 'Zebra' apricot. Different treatment temperatures did not affect the efficiency of 1-MCP on apricot quality during storage and shelf-life. In addition, earlier treatment could extend the storage life of apricot. Application of 1-MCP on the 1st day after harvest showed the highest effectiveness. However, 1-MCP treatment could be postponed till the 5th day after harvest, and the quality of apricot was still preferable.

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EFFECT OF ETHYLENE ABSORBER ON CUCUMBER AND TOMATO QUALITY DURING SIMULATED RETAIL STORAGE

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SUMMARY

This study was aimed to investigate the effect of ethylene absorber (EA) on postharvest life of cucumber and tomato at 20°C and 14°C, 95% relative humidity. Cucumber and tomato samples were stored with 6 sachets of Ethyl Stopper containing $KMnO_4$ (Bioconservacion S.A., Spain). EA could postpone the senescence of cucumber and tomato by reducing ethylene concentration in the storage chambers. Samples stored with EA retained firmness and decay incidence was decreased. Moreover, the use of EA maintained green surface of cucumber compared to the control after 16 days of storage. No chilling injury was observed throughout storage at 14°C. By the combination of cold temperature and EA better results were achieved than by other treatments during the 16 days of shelf-life. Results presented a potential of using EA in storage room where ethylene-producing and ethylene-sensitive vegetables were stored together.

INTRODUCTION

In practice, ethylene-sensitive and ethylene-producing horticultural products are usually shipped and stored together. Therefore, ethylene damage and decay could increase quickly during storage. By decreasing the level of ethylene in the chamber, ethylene-sensitive vegetables can maintain the quality for longer period (Skog and Chu, 2001).

Tomato is a climacteric and perishable vegetable (Tadesse et al., 2012). Cucumber is one of the most important vegetables in the world but it is very sensitive to ethylene (Zhang et al., 2004). In the market, cucumbers are often on shelves together with ethylene producing commodities that makes cucumber senesce rapidly.

The aim of this study was to evaluate the effectiveness of ethylene absorber in preventing postharvest loss for both ethylene-sensitive and ethylene-producing vegetables, when stored together at 14°C and 20°C in simulated retail storage.

MATERIALS AND METHODS

Materials

Cucumber (*Cucumis sativus* L.) and tomato (*Solanum lycopersicum* L.) were bought from a wholesale fruit and vegetable distributor in Hungary, in May, 2015. All vegetables were transported to the Faculty of Food Science, Corvinus University of Budapest, Hungary. Vegetables were selected for uniformity of size, shape and freedom from external damage.

Sachets of Ethyl Stopper containing $KMnO_4$ were provided by Bioconservacion S.A., Spain.

Methods

Experimental design

Samples were selected randomly into 4 boxes, each box containing 15 cucumbers and 15 tomatoes. The weight of each piece was 80 ± 2g for cucumber and 120 ±2 g for tomato. Cucumber was at green stage. Tomato was at red stage 5 of ripening according to tomato ripeness chart (Postharvest Technology center, UC Davis).

Two experiments were conducted on cucumber and tomato with varying storage conditions. In experiment Nr.1, cucumber and tomato were stored together at 20°C, RH 95% for 16 days in the presence and absence of 6 sachets of Ethyl Stopper. In experiment Nr.2, cucumber and tomato were stored together at 14°C, RH 95% for 16 days in the presence and absence of 6 sachets of Ethyl Stopper.

Table 1. Experimental design

Temperature	14°C	20°C
Cucumber and tomato	C	C
Cucumber and tomato	EA	EA

Table 2. Measurement time

Day	0 (Initial)	4	8	12	16

Measurement

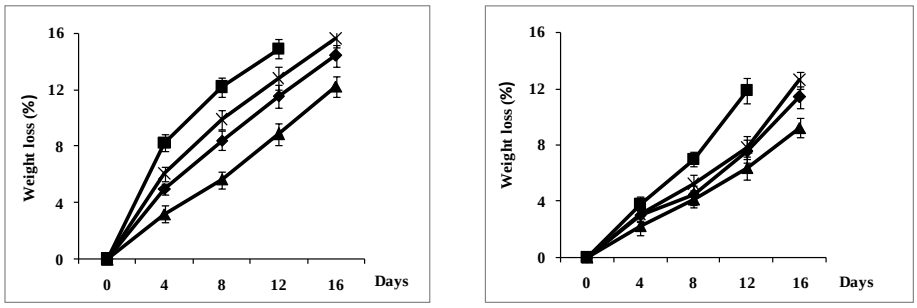
- **Acoustic firmness.** Acoustic firmness was measured at two opposite sides on each piece, using acoustic firmness sensor model DTF V0.0.0.105 (AWETA, Nootdorp, The Netherlands).
- **Surface color.** Vegetable peel color was measured with a Minolta Chroma Meter CR-400 portable tristimulus colorimeter (Minolta Corporation, Osaka, Japan). CIE L^*, a^* and b^* color characteristics were determined at two opposite points on the equator of each sample. Hue angle (H^o) value was calculated as arctangent (b/a).
- **Weight loss.** Vegetables were weighed at day 0 and at the end of each storage interval. The difference between initial and each storage period was considered as total weight loss during that interval and calculated as percentages on a fresh weight.
- **Decay percentage**. Decay was evaluated as fungal mycelia appeared on stem and vegetable surface and calculated as the number of decayed samples divided by initial number of samples multiplied by 100.

Statistical analysis. All data were analyzed using analysis of variance (ANOVA) via SPSS version 11.0.1 (SPSS Inc, USA). The results were reported as means with standard deviations (95% confidence interval).

RESULTS AND DISCUSSION

Weight loss

Weight loss of samples increased during the storage period but at different rates (Fig.1). Control samples stored at 14°C showed lower weight loss than those at 20°C. Vegetables stored with EA at 14°C had less weight loss than the ones with other treatments. The reduction in weight loss was probably due to the effect of temperature on respiration and ethylene absorber by delaying the ripening. The results were in agreement with the report of Silva et al. (2009) for papaya. This could be explained that storing at cold temperature decelerates the metabolism. Moreover, when ripening occurs, the degradation of cellular membranes takes place that causes the increase of transpiration.



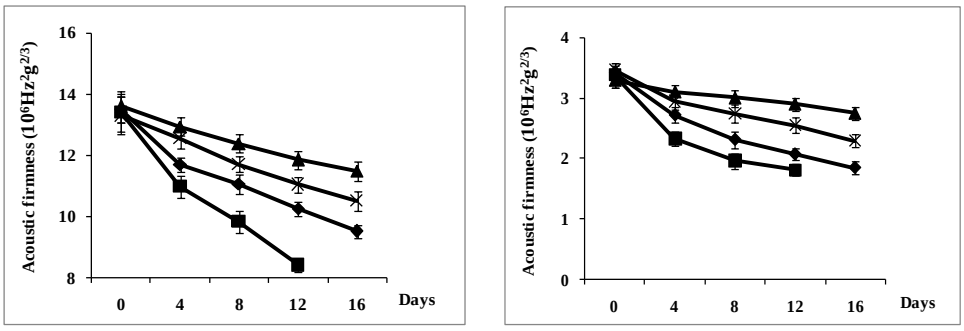
a) Cucumber b) Tomato

Figure 1. Effect of temperature and EA on weight loss of vegetables (■ Control 20°C; ♦ Control 14°C; × EA 20°C; ▲ EA 14°C). Values are the mean ± SD

Acoustic firmness

Firmness of cucumber and tomato reduced throughout storage period both in the presence and absence of ethylene absorber. However, vegetables kept at 14°C were firmer than those stored at 20°C (Fig. 2). After 16 days of storage, the control vegetable at 20°C showed the lowest firmness.

Softening of produce is due to biochemical processes such as hydrolysis of pectin and starch by enzyme during ripening (Ali et al., 2010). The ethylene absorber could limit the activity of ethylene in ripening (Aharoni et al., 1993), so firmness of EA vegetables was maintained during storage.



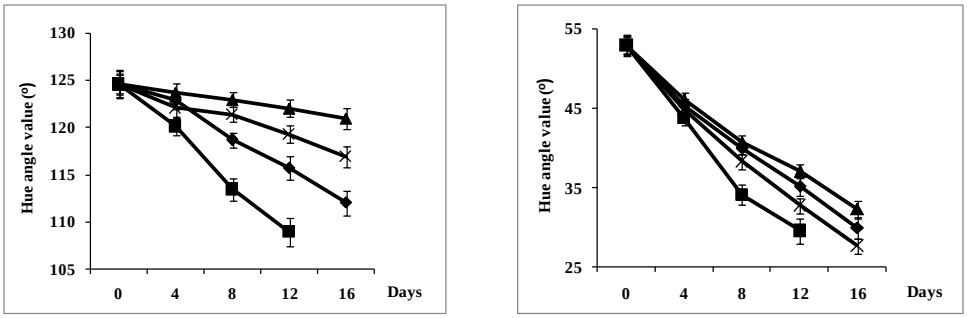
a) Cucumber b) Tomato

Figure 2. Effect of temperature and EA on acoustic firmness of vegetables (■ Control 20°C; ♦ Control 14°C; × EA 20°C; ▲ EA 14°C). Values are the mean ± SD

When stored in combination of low temperature with EA, there were significantly reduced percentage of weight loss and maintained vegetable firmness as compared to the control.

Surface color

There was a significant change in hue angle during storage. Hue angle value of vegetables reduced dramatically in control cucumber at 20°C as well as 14°C (Fig. 3a). Cucumber is an ethylene-sensitive commodity so the senescence took place when tomato produced high ethylene level in the chamber. The surface color is the indicator of ripening. The skin of control cucumber turned from green to yellow rapidly after 8 days of storage, whereas samples stored with EA were still green. EA could retard the decline in hue angle of commodities during storage.



a) Cucumber b) Tomato

Figure 3. Effect of temperature and EA on surface color of vegetables (■ Control 20°C; ♦ Control 14°C; × EA 20°C; ▲ EA 14°C). Values are the mean ± SD

Decay percentage

Fewer samples were lost due to decay in EA groups than control groups (Table 3). EA was effective in postponing the fungal growth on the surface of cucumber and on the stem of tomato. The decline in decay of EA groups was probably due to preventing the ethylene effect on senescence that makes produce more susceptible to infection. Storing at 20°C, the mould development appeared at 4th day for both control cucumber and tomato, while the early signs of decay appeared at 8th day in samples stored with EA. The vulnerability of vegetable also depended on temperature.

Table 3. The percentage of decayed samples during storage

Vegetable	Sample	Decay (%)			
		4days	8days	12days	16days
Cucumber	Control 14°C	6.7a	26.7a	66.7a	86.7a
	Control 20°C	20.0b	51.4b	93.3b	-
	EA 14°C	0	0	6.7c	13.3b
	EA 20°C	0	6.7c	20.0d	33.3c
Tomato	Control 14°C	6.7a	13.3a	33.3a	66.7a
	Control 20°C	13.3b	33.3b	86.7b	-
	EA 14°C	0	0	6.7a	20.0b
	EA 20°C	0	13.3a	26.7d	33.3c

Means followed by the same letters are not significantly different at $P \leq 0.05$ (at the same measurement time)

CONCLUSION

The result of this work indicated that storing cucumber and tomato with ethylene absorber can reduce the weight loss, softening and the change of color during storage. In addition, postharvest decay also decreased when vegetables were stored with EA. The combination between ethylene absorber and cold storage has potential in maintaining the quality of commodities throughout storage. Further study should be carried out to investigate the effect of dosage of Ethyl Stopper on produce quality.

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RESEARCH OF CHEMICAL PARAMETERS OF RED CURRANT, JOSTA AND SOUR CHERRY

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SUMMARY

Fruits have an important role in nutrition and prevention. They are rich in vitamins such as Vitamin B1, Vitamin B2, Vitamin B6 and Vitamin C. In addition, they are also contains Vitamin A and E, minerals, such as Se, Fe, Cu, Zn, and carotenoids and flavones. Carotenoids and flavones are antioxidants, which protect cells membranes. Due to these effects inhibit some diseases, such as atherosclerosis and several types of cancer. However, the quality of fruits also depends on the method of processing and storage. In our experiment we evaluated the effects of processing on the nutritional properties of red currant, josta and sour cherry. We measured vitamin C contents, total acid contents, total phenolic contents and total flavonoid contents of fresh fruits and jam and syrup made from them. It was found that most of these parameters were decreased with processing and the highest losses were found in the case of jam making.

INTRODUCTION

The name of red currant comes from the arabic culture. Rinio Benedetto gave the name „ribes" to this fruit in the XVth century (Koháry, 2004). The cultivation started in Netherlands, Denmark and North Germany. Red currant belongs to the Saxifragales, the family of Grassulariaceae (Theresia, 2009). The name of cultivated currant is Ribes rubrum, which is bred from two wild species (Horánszky – Járainé Komlódi, 2002). The red currant is rich in vitamins. First of all, in vitamin B_6 (28-58 µg) and vitamin C (26-47 mg), but it also contains vitamin B_1 (15-60 µg), vitamin B_2 (20-40 µg) and 18-72 µg of provitamin A (carotene) in 100 g. The acidity is also high, citric acid is 2 g, malic acid is 0,29 g. The currant has a lot of dietary fiber (3,5 g), mainly pectin and cellulose. This berry has a positive effect for the organism, since it stimulate the metabolism, improves the endocrine system and funtions of organs (Belucz, 2007).

The breeding of josta started in Germany in the Institute of Max Planc in 1922. Lorenz was the first, who did crossings (Papp-Porpáczy, 1999). The name of this species is *Ribes nigrolaria*. This name came from crossing of the *Ribes nigrum* (black currant) and *Ribes grossularia* (goosberry) (Géczy, 2000). In Hungary the josta got state recognition in 1983 (Belucz, 2007). This vitamin C concentration of berry is between 35 and 100 mg/100g, depending on the available references (Christine, 1997). Josta has a high acid content, it is 2,49 % (Géczy, 2000).

The sour cherry is originated from North India (Bálint, 1993). This cultivated species came from the crossing of *Prunus avium* and *Prunus fruticosa* (G. Tóth, 2001). The name of sour cherry is *Prunus cerasus*. This fruit is rich in vitamin A and C, in P, K and Ca (Christine, 1990). Sour cherry contains a lot of sugar: 6,1 g/100g glucose and 3,1 g/100g fructose (Pappné Tarányi, 2000).

MATERIAL AND METHODS

Our materials were fresh fruits: red currant, josta and sour cherry and raw syrup and jam made from them. The place of purchase was the village Palló in Transcarpathia. The laboratory analysis of fresh fruits and the preparation of products were performed in June 2013. After 8 month of storage we measured the syrups and jams in February 2014.
Our measured parameters:

- Dry matter content and moisture (MSZ 6367-3, 1983)
- Vitamin C contents (MSZ EN 12147, 1998)
- Total acid contents (α-α-dipyrydyl method)
- Total flavonoid contents (Meda et al., 2005)
- Total phenolic contents (Kim et al., 2003)

RESULTS AND DISCUSSION

Dry matter contents of fresh fruits, syrups and jams.

Fresh fruits had the lowest dry matter contents, which was 19.1 % in red currant, 18 % in sour cherry and only 14.4 % in josta. In syrups this number was between 28.6-38.8 %. The dry matter in the jams grown significantly. The dry matter content of red currant jam was 75.2%, sour cherry jam had 69.5% and josta jam had 64.45% (Figure 1.).

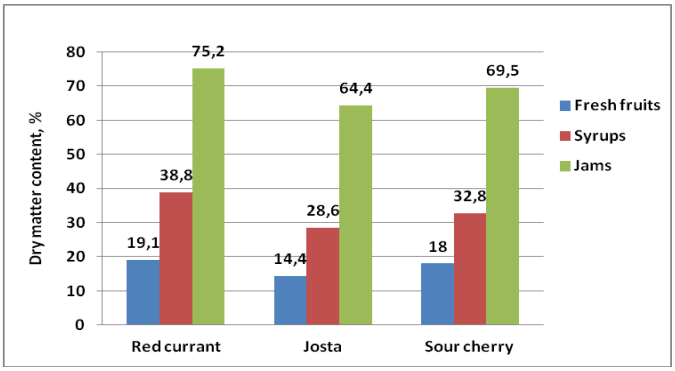

Figure 1.: Dry matter contents of fresh fruits, syrups and jams

Vitamin C content of fresh fruits, syrups and jams.

Vitamin C content was the highest in josta, 115 mg/100g. In the case of red currant it was 38.2 mg/100g and in the case of sour cherry 20.9 mg/100g. Due to the processing in syrups the concentration of vitamin C content decreased to 13.6-16.3 mg/100g. Jams also had low values, it was between 17.1-22.8 mg/100g. Interestingly, in sour cherry jam the vitamin C content increased as an effect of processing, due to the concentration (Figure 2.).

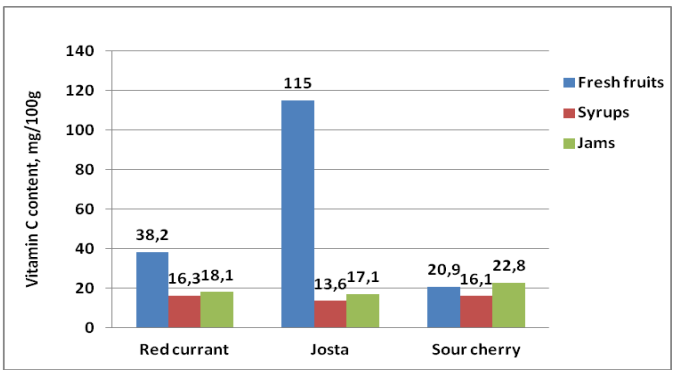

Figure 2.: Vitamin C contents of fresh fruits, syrups and jams

Acid contents of fresh fruits, syrups and jams.

The acid content in fresh fruits was between 1.15-2.1%. Due to the processing the acid content in red currant syrup reduced to one third compared with fresh fruit, in josta and sour cherry syrup decreased nearly to the half. Heating also reduced the acid content in jams, it is among 0.55-0.9% (Figure 3.).

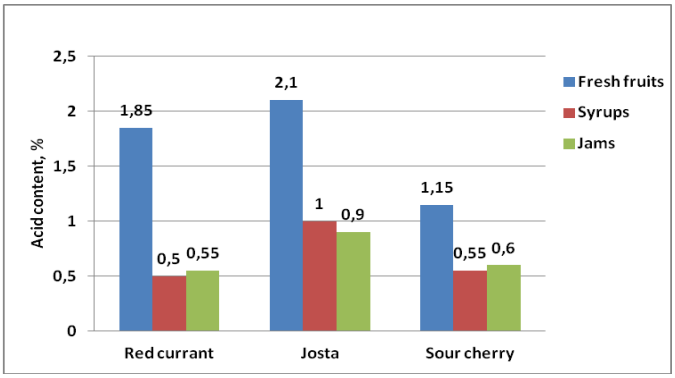

Figure 3.: Acid contents of fresh fruits, syrups and jams

The total flavonoid contents of fresh fruits, syrups and jams.

The total flavonoid content was the highest in sour cherry, it was 320.7 mg CE/100g. In josta this number was 145.3 mg CE/100g and in the red currant it was 35.6 mg CE/100g. In syrups this value significantly decreased resulting values between 5.83-34.7 mg CE/100g. In jams these values were higher than in syrups, it was 30.9-42.6 mg CE/100g (Figure 4.).

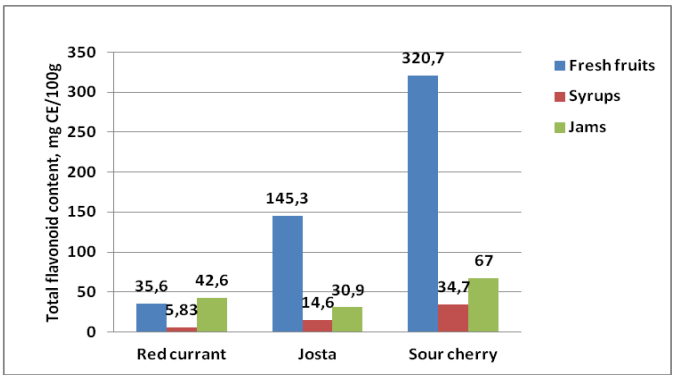

Figure4.: Total flavonoid contents of fresh fruits, syrups and jams

The total phenol contents of fresh fruits, syrups and jams.

The total phenol contents were variable. In fresh fruits it was 62.2-198.7 mg GAE/100g. The processing reduced the phenol content in josta syrup, but this value increased in the red currant syrup (158 mg GE/100g) and in the sour cherry syrup (192.5). In jams these values were between 73.7-79.1, but in red currant jam this number was higher than in fresh fruit (Figure 5.).

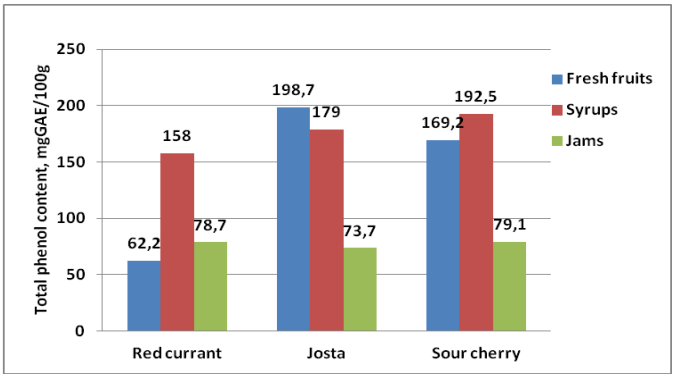

Figure 5.: Total phenol contents of fresh fruits, syrups and jams

CONCLUSIONS

It was found that most of the evaluated parameters were decreased with processing and storage, except the total flavonoid content in red currant jam, which was higher than in fresh currant and except the total phenol content in red currant jam compared to the fresh currant. This was due to the reduction of moisture content, resulting more concentrated products. The highest losses were found in the case of jam making. Therefore, if it is possible, we should eat only fresh fruits, without heating and they will keep all of their benefits and usefulness.

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HIGH HYDROSTATIC PRESSURE TREATMENT AND NITRITE CONCENTRATION EFFECTS ON FUNCTIONAL PROPERTIES OF MEAT BATTER

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SUMMARY
Aim of this study was to investigate the effect of 450 and 600 MPa hydrostatic pressure on color, water holding capacity (WHC) and protein solubilization of raw meat batter containing 50, 75, 100 and 125 ppm nitrite. High pressure alone increased the lightness, decreased the redness and had no effect on the yellowness of the raw meat batters. The nitrite reduction resulted in less red and darker color irrespective of the pressure treatments and pressure levels. Pressure treatment at 450 and 600 MPa significantly enhanced the WHC however, nitrite reduction decreased the water holding property. High pressure treatment denaturated the sarcoplasmic proteins in the range of 100-250 kDa and induced a reduction of protein solubility in case of albumins (60-70 kDa). Pressure treatments had no significant effect on myoglobin solubilization. Nitrite concentrations did not affect the solubilization of sarcoplasmic proteins in pressure treated raw meat batters.

INTRODUCTION
The stage after comminuting and mixing of the meat and other ingredients is known as meat batter (raw process phase). Functional properties of raw meat batter basically determined the quality of meat product. High hydrostatic pressure process can enhance the functional properties of muscle-based meat products (Buckow, 2013). Pressure induced gel from raw meat batter results in smoother, more glossy, less firm and more elastic gel with improved water holding capacity, compared to thermally induced gel (Cheftel, 1997; Jimenez, 2002). Nitrite is an important additive of meat products to provide desired color, flavor, antioxidant and antimicrobial activity and textural properties. However, the free nitrite in meat products have been identified as potential health risk; nitrites can generate methemoglobin, which is a recognizable sign of nitrite toxicity in humans (Chow, 2002), and may react with certain amines in foods to produce carcinogenic nitrosamines (Cassens, 1997). In view of the potential health risks of nitrite, the utilization of nitrite in meat products faces a challenge (Cunliu, 2012) to reduce the amount of nitrite. High hydrostatic pressure can affect the texture and gel forming properties of myofibrillar proteins and, hence, has been suggested as a process to produce additive-free meat products (Jung, 2000; Sun, 2010).

MATERIAL AND METHODS
Meat batter preparation
Meat batters were prepared from pork muscle (pork shoulder). The muscle was ground chopped (diameter 4.0 mm) and mixed with salt (sodium chloride, NaCl; 2.0% weight of meat), polyphosphate (tetrasodium pyrophosphate, $Na_4P_2O_7$; 0.4% of meat), ascorbate (sodium L ascorbate; 0.5% weight of meat), ice (70% weight of meat) and fat (belly flank; 40% weight of meat). Nitrite (sodium nitrite, $NaNO_2$) was used 0.005% (50 ppm) 0.0075% (75 ppm) 0.01% (100 ppm), 0.0125% (125 ppm) weight of meat. The 100 ppm nitrite concentration is normally used in the meat processing in Hungary. The raw meat batters were vacuum packed in polyethylene pouches and stored between 4-6°C before pressure treatment. Three batter samples were pressurized and analyzed (without heat treatment) for each nitrite concentration.

Pressure treatment

The vacuum packaged raw meat batters were treated with high pressure at 450 and 600 MPa for 5 minutes at room temperature in a Resato FPU-2000 high pressure equipment. Pressure gradient was 100 MPa/min. 0 MPa means unpressurized raw meat batters.

Color measurement

Color of pressure treated raw meat batters was measured using Minolta ChromaMeter CR-400 (Konica Minolta Inc., Japan). The measured values expressed in CIE Lab as L* (lightness), a* (redness) and b* (yellowness). Nine replicates were analyzed from each combination.

Water holding capacity

The water holding capacity of raw meat batters was determined by Grau method (1953) after the pressure treatment. 200-300 mg meat batter was put and exactly weighted on a known weight 2500 mm^2 (50x50 mm) area filter paper. Filter paper and batter was placed between two glass plates and pressurized with 0.5 kg weigth for 5 mins then the filter paper was dried. The appeared spot (moisture from meat batter) was cut out from the filter paper and the paper without spot was weighted. The water holding capacity (WHC) was calculated as the proportion of area and weight of spot and meat batter (WHC = area of spot / weight of meat batter). The WHC of meat batter was expressed in [mm^2/mg meat batter] dimension. Three replicates were analyzed from each HHP-nitrite combinations.

SDS-PAGE analysis

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was used to qualify soluble proteins. The BioRad Mini Protean (BioRad, Munchen, Germany) electrophoresis cell was used. Samples were pre-treated according to the Laemmli method (Laemmli, 1970). The molecular weight of each band was estimated by comparing the migration distance with proteins of known molecular weight (Precision Plus Protein All Blue Standards). Standard was run beside the other samples. After fixation and staining each gel was scanned and analyzed with image analysis software (Gel Doc XR Scanner, BioRad, Munchen, Germany).

Statistical analysis

The data were analyzed using IBM SPSS 22.0. Statistical analysis was performed by analysis of variance (ANOVA**)** and Tukey-test. Results were expressed as mean ± standard deviation (SD), and difference was considered to be statistically significant at $p<0.05$.

RESULTS AND DISCUSSION

Color of high pressure treated raw meat batters

Table 1. shows the lightness (L*), redness (a*) and yellowness (b*) of the raw meat batter samples. High pressure alone increased the lightness, decreased the redness and had no effect on the yellowness of the raw meat batters. All of the pressure treated meat batters at same nitrite concentrations were ’whiter’ than the unpressurized (0 MPa) samples. The ’whitening’ effect was significant at 600 MPa. Furthermore, the nitrite reduction resulted in less red and darker color whatever the pressure treatment and/or pressure levels. The ’whitening’ effect of high pressure treated raw meat batter can be explained by changes of the myofibrillar and sarcoplasmatic proteins (Jung, 2003) and decreasing of redness caused by the oxidation of myoglobin (Carlez, 1995).

Table 1.: Objective color (CIELab) of raw meat batters after high pressure treatment at different nitrite concentrations

Pressure [MPa]	Nitrite [ppm]	L*	a*	b*
0	50	$59,95^{d} \pm 1,01$	$8,79^{b,c} \pm 0,19$	$15,06^{a} \pm 0,43$
	75	$62,2^{b,c} \pm 0,21$	$8,64^{b,c,d} \pm 0,44$	$14,83^{a,b} \pm 0,50$
	100	$62,75^{b,c} \pm 0,50$	$9,59^{a,b} \pm 0,41$	$14,82^{a,b} \pm 0,54$
	125	$65,83^{a} \pm 0,66$	$9,9^{a} \pm 0,77$	$13,94^{a,b} \pm 0,69$
450	50	$61,86^{d} \pm 0,26$	$7,99^{c,d,e} \pm 0,17$	$13,7^{a,b} \pm 0,55$
	75	$63,15^{b,c} \pm 0,56$	$8,36^{c,d,e} \pm 0,28$	$13,72^{a,b} \pm 0,93$
	100	$63,19^{b,c} \pm 0,83$	$8,61^{b,c,d,e} \pm 0,17$	$14,12^{a,b} \pm 0,52$
	125	$65,16^{a} \pm 0,72$	$8,84^{b,c} \pm 0,32$	$13,49^{b} \pm 0,35$
600	50	$62,31^{b,c} \pm 0,38$	$7,65^{d,e} \pm 0,22$	$14,99^{a,b} \pm 0,71$
	75	$64,06^{a,b} \pm 0,99$	$7,59^{e} \pm 0,29$	$14,41^{a,b} \pm 0,42$
	100	$63,92^{a,b} \pm 0,50$	$8,27^{c,d,e} \pm 0,19$	$15,13^{a} \pm 0,59$
	125	$65,85^{a} \pm 0,62$	$8,35^{c,d,e} \pm 0,18$	$13,89^{a,b} \pm 0,58$

Mean ± standard deviation (n=9)
Different letters in the same column indicate significant difference ($p<0.05$)

Water holding capacity (WHC) of high pressure treated raw meat batters

Table 2. presents the water holding capacity (WHC) of raw meat batters after high pressure treatment at different nitrite concentrations. Water holding capacity is presented in mm^2/mg dimension. The lower value means better water holding capacity.

High pressure treatment alone resulted in better WHC. The 450 and 600 MPa pressure treatments significantly enhanced the WHC of raw meat batters. It is related to the denaturation of sarcoplasmic proteins that could have a positive effect on enhancing the WHC of high pressure treated meat batter (Marcos, 2010.) However, the nitrite reduction increased the value of WHC that is debased the water holding property of raw meat batters. The WHC values of meat batters prepared with 50 or 75 ppm nitrite were higher than that of 100 and 125 ppm samples. In case of 50 ppm nitrite samples the difference was significant. Decreasing difference was seen at 600 MPa pressure treatment compared to 75 and 100 ppm. Based on statistical analysis interaction between pressure levels and nitrite concentrations was not observed.

Table 2.: Water holding capacity of raw meat batters after high pressure treatment at different nitrite concentrations

Pressure [MPa]	Nitrite [ppm]	Water holding capacity [mm^2/mg]
0	50	$3,135^{a} \pm 0,05$
	75	$3,046^{a,b,c} \pm 0,04$
	100	$3,031^{a,b,c} \pm 0,05$
	125	$2,972^{c} \pm 0,02$
450	50	$3,099^{a,b} \pm 0,01$
	75	$2,997^{b,c} \pm 0,03$
	100	$2,964^{c} \pm 0,08$
	125	$2,956^{b,c} \pm 0,02$
600	50	$2,959^{c} \pm 0,01$
	75	$2,962^{c} \pm 0,02$
	100	$2,939^{c,d} \pm 0,03$
	125	$2,836^{d} \pm 0,02$

Mean ± standard deviation (n=3)
Different letters in the same column indicate significant difference ($p<0.05$)

Protein solubilization of high pressure treated raw meat batters at different nitrite concentrations

The sarcoplasm contains significant amounts of myoglobin and mostly miofibrils. They are responsible for the color and binding (water, fat) properties of meat products. Figure 1. presents the results of SDS-PAGE electrophoresis of the sarcoplasmic proteins.

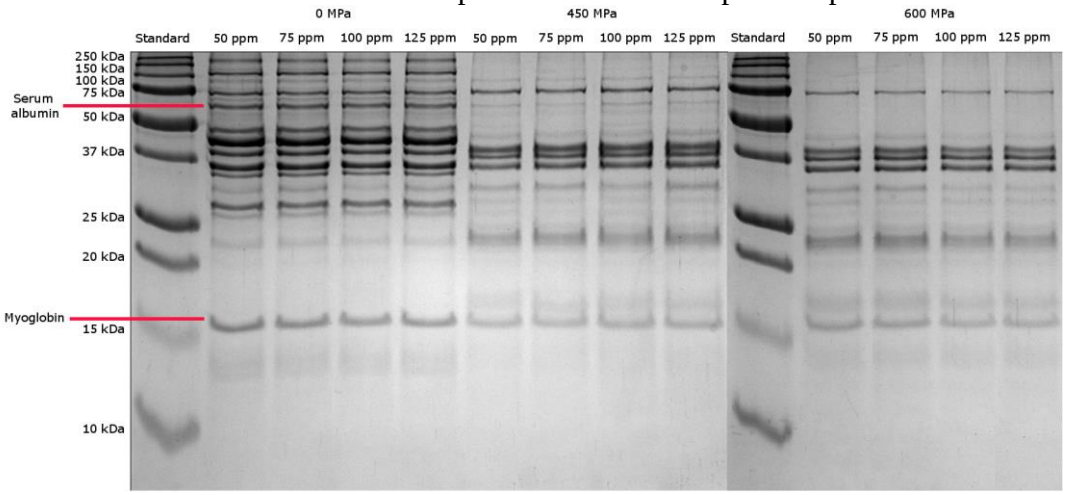

Figure 1. SDS PAGE gel electrophoresis pattern of raw meat batters after pressure treatment at different nitrite concentrations

A significant difference was seen in raw meat batters protein solubilization after pressure treatments. As the SDS-PAGE pattern presents (Figure 1.) intensity of sarcoplasmic protein bands significantly decreased by the pressure treatments. Sarcoplasmic proteins above 100 kDa mostly denaturated and aggregated due to pressure treatments. Solubility of these proteins was less affected by pressure treatment at 450 MPa than at 600 MPa where the bands of proteins (100-250 kDa) fully disappeared. High pressure had impact on the albumins (60-70 kDa). Pressure treatment at 450 MPa or above significantly decreased the intensity of albumin band. Intensity of myoglobin at 16,9 kDa slightly decreased due to pressure treatments. Its solubility was less affected by pressure treatment than that of 'heavier' proteins. Based on SDS-PAGE results the different nitrite concentrations did not affect the solubilization of sarcoplasmic proteins in pressure treated raw meat batters.

CONCLUSIONS

Application of high hydrostatic pressure resulted in lighter meat batters. However the nitrite reduction decreased the lightness at all pressure levels. Pressure treatments and nitrite reduction resulted in less red appearance of meat batter without any interaction between the factors. High pressure treatment modified the structure of meat batter sarcoplasmic proteins mainly above 60 kDa. The sarcoplasmic protein denaturation enhanced the water holding capacity of meat batters independetly of the utilized nitrite concentration. The nitrite reduction had no effect on the sarcoplasmic protein denaturation.

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EFFECT OF MIXING RATIO AND THE TEMPERATURE GRADIENT ON THE AVRAMI PARAMETERS DURING SOLIDIFICATION OF DIFFERENT FAT BLENDS

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SUMMARY

The aim of the work was to monitor the crystallization of palm mid fraction (PMF)-sunflower oil (SF) and coconut oil (COC)-sunflower oil (SF) fat blends (blending ratio: 100%-0%; 90%-10%; 80%-20%; 70%-30%; 60%-40%; 50%-50%). Measurements were performed during a 120 minute period with measurements taken every three minutes. The blends were cooled. Temperature gradients were 75°C, 70°C, 65°C and 60°C. Based on the measured SFC values, parameters of the scientifically accepted Avrami model ('k' and 'n') were calculated. The result were that the temperature gap and the ratio of the SF in the blends did not change the type of the nucleation. However, these factors had a significant effect on the 'k' parameter which indicates the rate of crystallization. We have also found that the ratio of the SF had a more significant effect than the temperature gap. As a result of comparing the two types of fat blends, we concluded that the lauric coconut oil and the palmitic acid dominated palm mid fraction had similar crystallization properties.

INTRODUCTION

Most food products get their final structure after going through a cooling precedure. Consequently understanding of solidification process of fats and lipid blends is important in food technology. Since fat containing food are very complex systems interactions between fat and oil components can play important role in modifying physical properties. Previous results show that several factors are responsible in solidification of fats (e.g. Sato 2001). Of those the effect of liquid oil has a great impact on the solidification kinetics of different fats. (Braipson-Danthine, 2004). Some of the earlier reported data suggest that the magnitude of the temperature gap fundamentally influences the characteristics of the solidification (Campos et al, 2002). Of the several possibilities , Avrami model proved to be the most suitable to describe the kinetiks of solidification (Marangoni, 1998). Based on these experiences the aim of our work was to study the solidification process of fat-oil blends by means of Avrami model.

MATERIALS AND METHODS

The vegetable fats and oils used for the experiment were palm mid fraction (PMF, IOI Loders Crocklaan Netherlands), coconut fat (COC, Barco Mimaropa Ventures, Indonesia), and Vénusz refined sunflower oil (SF, Bunge Zrt. Hungary). Blends of PMF-SF and COC-SF were prepared in fat-oil order m/m % :100%-0%; 90%-10%; 80%-20%; 70%-30%; 60%-40%; and 50%-50%. Three glass NMR tubes (10 mm diameter) were filled (halfway) for each blend. Before the measurements, the tubes were put into water baths at different temperatures, namely 75°C, 70°C, 65°C, and 60°C. The samples were tempered in the water baths for 30 minutes. The kinetics of the crystallization was studied by measuring the solid fat content of the samples using Bruker Minispec120 NMR spectroscope. After the first measurement, the samples were put into a cooler thermostat which circulated ethylene glycol at 0°C. Each sample was measured every 3 minutes for 120 minutes. Solid fat content (SFC) data were plotted as a function of time. Parameters of Avrami model (n and k) were calculated from the results,,using the following formula:

$$\frac{SFC_t}{SFC_{eq}} = 1 - e^{-kt^n}$$

(Campos et al, 2002)
where SFC_t is the solid fat content % at t minutes, while SFC_{eq} is the maximum solid fat content a sample reaches during crystallization, k is the Avrami constant which shows the crystallization rate, and n is the Avrami exponent, which indicates the mechanism of the crystal growth.

RESULTS AND DISCUSSION

Solidification process of COC-SF blends were similar for each temperature gap. As an example we illustrate the phenomenon in Figure 1. The curves belong to the Δt=65°C.

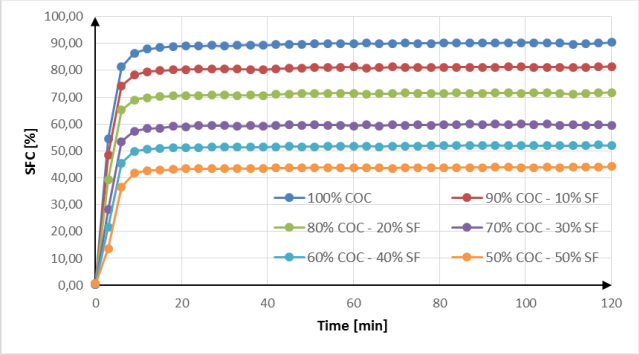

Figure 1.: Typical solidification curve for COC-SF blends (Δt =65°C)

Results in the Figure 1 show that the solidification was rather fast. Within the first ten minutes of the process the SFC values approached the equilibrium. Equilibrium SFC values were in accordance with the amount of liquid oil in the blend.
Calculated Avrami parameters are summarized in Table 1.

Table 1.: Calculated Avrami parameters for COC-SF blends

		Mixing Ratio					
	Avrami 'n'	100%	90%	80%	70%	60%	50%
Temperature gradient	80°C	0,41	0,41	0,43	0,45	0,50	0,49
	75°C	0,42	0,41	0,42	0,41	0,46	0,50
	70°C	0,40	0,40	0,37	0,44	0,46	0,47
	65°C	0,39	0,40	0,39	0,38	0,42	0,45
	60°C	0,35	0,36	0,32	0,40	0,39	0,42
	Avrami 'k'	100%	90%	80%	70%	60%	50%
Temperature gradient	80°C	1,14	1,17	0,99	0,82	0,69	0,73
	75°C	1,01	1,02	1,02	0,88	0,74	0,60
	70°C	1,07	1,09	1,18	0,95	0,85	0,74
	65°C	1,10	1,10	1,06	1,07	0,89	0,76
	60°C	1,27	1,20	1,36	0,96	0,97	0,82

Avrami exponent (n) varied between 0.32-0.50 proved that the nucleation was one dimensional and instantaneous. In case of Avrami constant (k) a decreasing tendecy was observable. Higher amount of oil as well as bigger temperature gap caused smaller k values. Effects of these factors on the parameters are shown in Figure 2 and Figure 3.

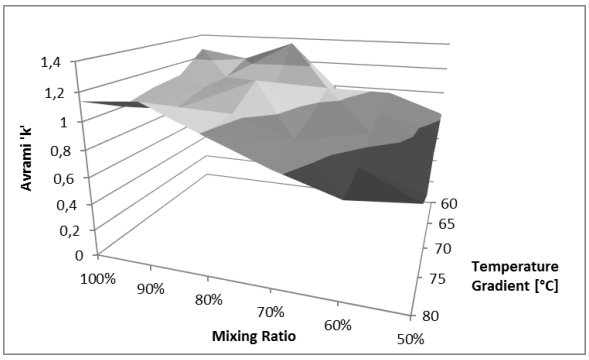

Figure 2.: Changes of Avrami constant (k) of COC-SF blends

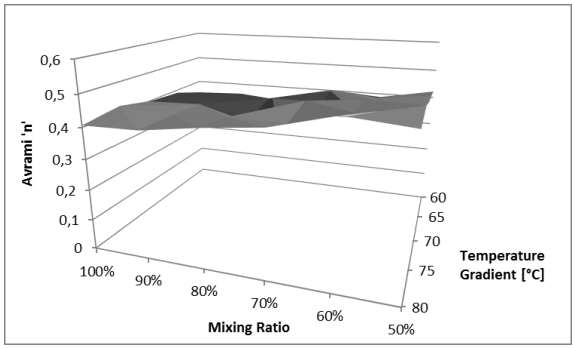

Figure 3.: Changes of Avrami exponent (n) of COC-SF blends

Solidification process of PMF-SF blends is demonstrated in Figure 4. Because of the similarity of the results, Figure 4 shows only the curves belong to the 65°C temperature gap.

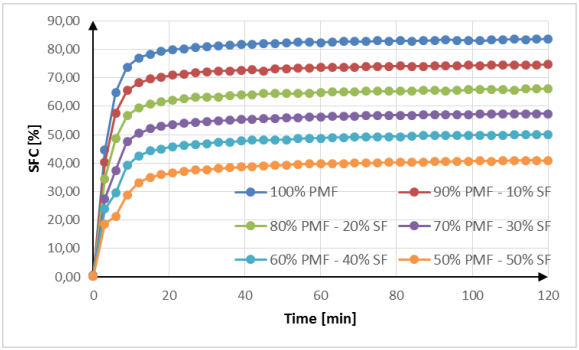

Figure 4.: Typical solidification curve for PMF-SF blends (65°C)

Table 2.: Calculated Avrami parameters for PMF-SF blends

		Mixing Ratio					
	Avrami 'n'	100%	90%	80%	70%	60%	50%
Temperature gradient	80°C	0,62	0,61	0,57	0,62	0,61	0,65
	75°C	0,52	0,50	0,49	0,55	0,59	0,64
	70°C	0,52	0,52	0,53	0,56	0,57	0,63
	65°C	0,50	0,50	0,50	0,59	0,58	0,63
	60°C	0,52	0,48	0,51	0,54	0,57	0,61
	Avrami 'k'	100%	90%	80%	70%	60%	50%
Temperature gradient	80°C	0,36	0,35	0,44	0,35	0,34	0,27
	75°C	0,61	0,64	0,61	0,43	0,36	0,28
	70°C	0,57	0,55	0,51	0,42	0,36	0,28
	65°C	0,61	0,59	0,56	0,40	0,38	0,30
	60°C	0,65	0,66	0,57	0,47	0,40	0,32

Calculated Avrami parameters are summarized in Table 2. Values of Avrami exponent were between 0.48-0.65 and values of Avrami constant varied from 0.27 to 0.66. While

Avrami exponent was closely constant, a clear decrease in Avrami constant was observable. These findings are illustrated on Figure 5 and Figure 6.

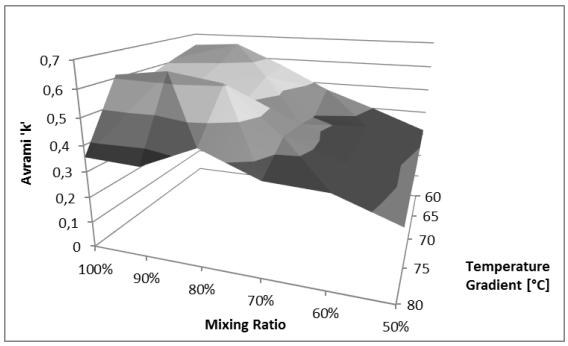

Figure 5.: Changes of Avrami constant of PMF-SF blends

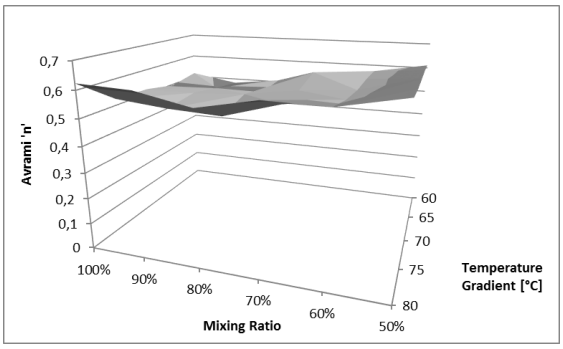

Figure 6.: Changes of Avrami exponent of PMF-SF blends

CONCLUSIONS

Presence of SF doesnt modify the mechanism of the crystal growth proved by the closely constant n parameter. Value of n was close to 1, that suggests an instant and one dimensional crystallization (Widlak ,2001) Decrease in k parameter shows the temperature dependence of the solidification process. This is in accordance with the earlier reported data (Marangoni , Narine 2002). Smaller figures indicate the retardation of crystalization procedure. It comes through the increased amount of liquid oil in the blend

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NIR SPECTRUM OF THE COMBINED LTLT AND HHP TREATED LONGISSIMUS DORSI OF PORK

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SUMMARY
Sliced pork meat was used as raw material to establish the effects of the sous-vide technology and the high hydrostatic pressure treatments (and their combinations) on meat samples. The slices were cooked at 60°C sous-vide and treated at two different high hydrostatic pressure (300 MPa and 600 MPa) for five minutes pre-, or post-heat treatment. Near infrared spectroscopy (NIR) is a widely used non-destructive method to determine quality parameters in food analysis. In our study we wanted to monitor the technology induced changes of quality parameters by a Metrinir apparatus in the range of 700-1700 nm. Samples were in their native state and after removing the moisture by liofilisation.

INTRODUCTION
Food-quality and food-safety are the most important parameters influencing consumer choices, and are also primary factors for food manufacturers and distributors.. High hydrostatic pressure (HHP) technology, as a non-thermal preserving technique, applies 100-1000 MPa of pressure to inactivate pathogenic and food spoilage microorganisms while retaining the valuable components of foods. Sous-vide cooking is a current preservation technique in the catering industry to prepare ready to eat meals. It is gaining popularity due to the products with enhanced shelf-life and added special sensory properties. (Polyákné-Dalmadi, 2007) This cooking method where food is sealed in airtight plastic bags then placed in a water bath or in a temperature-controlled steam environment for longer than normal cooking times at an accurately regulated temperature much lower than normally used for cooking. After cooking if the meal is not consumed immediately it has to be cooled down as fast as possible and be kept under 3°C.

Changes in the volatiles of minced pork and beef meat could be monitored well with the electronic nose. (Dalmadi et al.,2015) In this study our aim was to investigate the effect of the same combined minimal processing technologies from another point of view. Near Infrared (NIR) spectroscopy is using the near-infrared region of the electromagnetic spectrum is a widely used quality control method in several fields of food and agricultural industry. It can be used to quantify the composition of agricultural products because it meets the criteria of being accurate, reliable, rapid, non-destructive, and inexpensive. (Burns et al., 2007)

MATERIAL AND METHODS
Sample preparation
A whole pork chop (Longissimus dorsi) was purchased in a wholesale supermarket (m_1= 3,78 kg) After being prepared to be free of surface fat, ligament and connective tissue each meat was cut into slices (2 cm) transversally to the fibre direction. The weight of each sample was 90-100g. Samples were vacuum sealed in 90 µm PA/PE plastic poaches using a Multivac C100 V.S. machine. (**Figure 1.**)

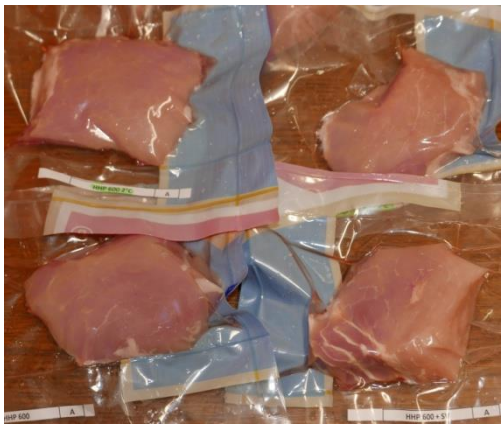

Figure 1. Vacuum packed samples

High hydrostatic pressure treatment

Meat samples were pressurized at 300 and 600 MPa for 5 minutes at room temperature in a RESATO FPU-100-2000 HHP unit (Resato International B.V., the Netherlands). The pressure transmission fluid was glycol-oil mixture (Resato PG fluid, Roden, Holland). The pressure build-up rate was 100 MPa/min. Build-up and decompression times were not included in the treatment time. The initial temperature of the pressure transmission fluid was 20,5°C, the adiabatic temperature change of the system (samples and the pressure transmission fluid) was under 12°C.

Sous-vide (LT-LT) treatment

Heat trreatment was carried out in a water bath (Labor Műszeripari Művek LP507/1). Temperature set at 60°C, the duration was 1 hour. Samples were cooled without in iced water right after cooking.

Treatments

We used single (heat or pressure) treatments and combined (heat after pressure and pressure after heat) double treatments. (**Table 1.**)

Table 1. The applied treatment combinations

Treatment	Parameters
Sous- vide (SV)	60 °C, 1 h
HHP 300	300 MPa, 5 min
HHP 600	600 MPa, 5 min
SV+ HHP 300	60 °C, 1 h + 300 MPa, 5 min
SV+ HHP 600	60 °C, 1 h + 600 MPa, 5 min
HHP 300+ SV	300 MPa, 5 min + 60 °C, 1 h
HHP 600+ SV	600 MPa, 5 min + 60 °C, 1 h

Storage

Samples were stored in a cooling cabinet (J 600-2, Thermotechnika Ker. Ltd., Hungary) for 3 weeks at 2°C and 8°C. These temperatures were chosen to be below and to exceed the recommended <3°C storage parameter. Samples were taken for analyses on day 0 and at the end of the 3rd week.

Liofilisation

Moisture was removed from the samples by freeze-drying so the water peaks will not disturb the spectral information in the “-OH” ranges.

NIR analysis

The measurements were carried out with a METRIKA apparatus (type METRINIR 10-17 PR) in the range of 740-1700 nm. (**Figure 2.**)Two independent filling was prepared with three repeated measures with turning the tray 120° each time. This gave us six spectrum which was used for the statistical analysis. Moist samples were cut into small cubes so it could fill the sample holder properly. Freeze dried samples were milled after lyophilisation so the powder of the samples were filled into the trays of the NIR instrument.

Figure 2. Milling of the freeze-dried samples

Data processing and statistical analysis

The measured spectral data was exported and processed (smoothing left-right 2-2; derived 2nd) using Unscrambler software. Data were evaluated by principal component analysis followed by a discriminant analysis (CDA) using IBM SPSS (Version 20, SPSS Inc. Chicago, Illinois, USA)

RESULTS AND DISCUSSION

Figure 3.a. and b. show the results of CDA of near infrared measurements of moist and lyophilised samples. We aimed to compare treatments so we used all data for this analysis regardless of storage or temperature. As it can be seen the NIR analysis followed by this statistical method separated our groups by the applied procedure quite well. Three subgroups were formed at the moist samples (1.HHP followed by heat treatment, 2. Heat treatment followed by HHP, 3. simple HHP), while we can observe four at the freeze-dried ones (1.HHP followed by heat treatment, 2. Heat treatment followed by HHP, 3. simple HHP and 4. simple heat treatment forms a new group here). The order of the treatments seems to have a critical role as they are constantly forming two well defined subgroups in both cases. Regarding the heat treatment – at the moist samples it is joined t the heat followed by HHP combined group. The explanation might be that the first impact determines the characteristics of the sample. As the moisture was removed (figure 3.b) the only heat treated sample came out of this subgroup. The results of the PCA followed by a CDA analysis show only a slight difference between the two sample types although the freeze-dried samples show more profound pattern on the CDA map as the groups are better formed by the applied treatments.

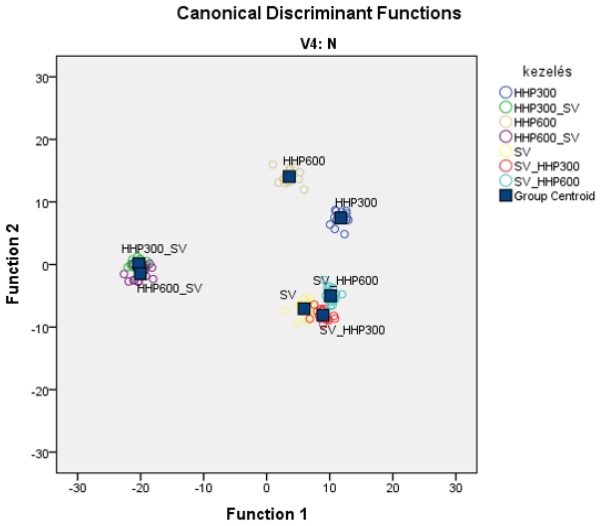

Figure 3. a. PCA-CDA result plot moist samples

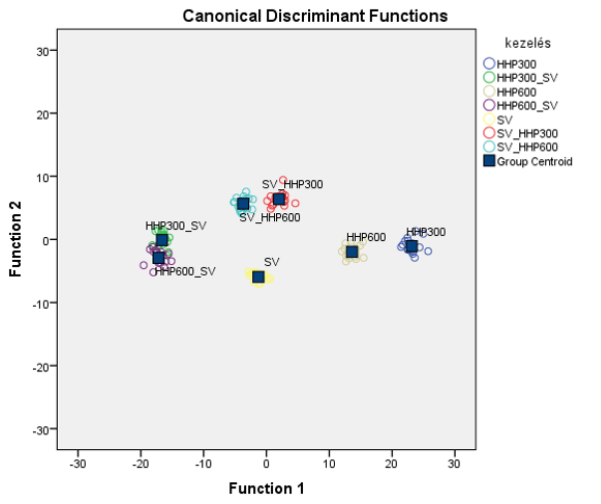

Figure 3. b. PCA-CDA result plot lyophilised samples

CONCLUSIONS

Based on our results we can say that the consecutive application of two minimal processing technologies has promising future. The samples that underwent the combined treatment form clearly separated subgroups. Moreover we can state that the order of the treatments has a major role in the quality parameters of the products. We are keen on continuing our research monitoring the characteristics of the changes caused by the minimal processing techniques.

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SENSORY ANALYSIS AND ELECTRONIC NOSE OF PORK MEAT PATTIES TREATED BY HEAT AND/OR HIGH HYDROSTATIC PRESSURE

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SUMMARY

The aim of this work was to study the effect of low temperature-long time heat treatment and high hydrostatic pressure treatment on the aroma components of the pork meat patties using electronic nose and sensory panel. The background of this study is to map the changes for further use and comparison with other parameters of the samples when applying combined treatments. Ground pork meat was cooked sous-vide and was HHP treated at different pressure levels or was treated with their combinations. Electronic nose was used to monitor the most important changes primary and a sensory panel to examine the odour differences in those cases where the electronic nose showed the most important changes in the volatile compounds. Both methods successfully detected the differences between treatments.

INTRODUCTION

Quality and safety are two basic requirements that should be taken into account during food processing. Both high hydrostatic pressure treatment and the sous-vide technology as minimal processing methods are very effective in their field but beside the advantages we still face several problems. (Polyák–Dalamadi, 2007) Among others the survival of psychrofil and psychrotolerant microorganismes or the incomplete inactivation of enzymes. The combined application of these technologies could be beneficial both on the quality and the shelf-life of the products.

Scientists, manufacturers and consumers agree that among other quality parameters odour has high importance in the food industry. (Ohlsson, 1994; Cardello et al., 2007).

Sous-vide cooking is well known of preserving all natural flavours and odours of the raw material. The vacuum packaging behaves as an artificial skin and prevents valuable components leaving the product. The low temperature - long time heat treatment gives a special character to the treated raw materials should it be from animal or plant origin. (Vaudagna, 2002; Baldwin, 2012) The HHP (High Hydrostatic Pressure) as a minimal processing technology has beneficial effect on the microbiological state of the products while it has only a slight effect on the structure – covalent bonds remain intact thus aroma components and colour are well preserved. (Hogan et al., 2005; Cheftel-Culioli, 1997)

These technologies are applied on a daily basis. Numerous publications were released mentioning positive effects and disadvantages. Their combined application following the hurdle principle is not thoroughly investigated yet, very few references could be found in this field.

MATERIAL AND METHODS

Sample preparation

Pork chop was purchased at a local market and was grinded using a Robot-Coupe grinder until it reached a homogenous-smooth texture. The half of the meat pulp was removed and paprika powder (3g/kg meat) was added to the rest and was turned again to mix it well. Meat patties were formed 40-50g each. (**Figure 1.)** Samples were vacuum sealed in 90 µm PA/PE poaches using a Multivac C100 V.S.machine.

High hydrostatic pressure treatment

Meat samples were pressurized at 300 and 600 MPa for 5 minutes at room temperature in a RESATO FPU-100-2000 HHP unit (Resato International B.V., the Netherlands). The pressure transmission fluid was glycol-oil mixture (Resato PG fluid, Roden, Holland). The pressure build-up rate was 100 MPa/min. Build-up and decompression times were not included in the treatment time. The initial temperature of the pressure transmission fluid was 21 °C, the maximal temperature in the pressure chamber was 28,2 °C.

Figure 1. Vacuum-packed meat patties ready for the treatments

Sous-vide (LT-LT) treatment

It was carried out in a water bath (Labor Műszeripari Művek LP507/1). Temperature set at 60°C, duration of heat treatment was 1 hour. Samples were cooled in iced water after cooking.

Treatments

We used simple (heat or pressure) treatments and combined (heat after pressure and pressure after heat) treatments. The same combination of treatments (**Table 1**.) were applied on the control (without paprika powder) and on the spicy (paprika powder added) sample sets.

Table 1. The treatments applied in this study

Treatment	Parameters
Sous- vide (SV)	60 °C, 1 h
HHP 300	300 MPa, 5 min
HHP 600	600 MPa, 5 min
SV+ HHP 300	60 °C, 1 h + 300 MPa, 5 min
SV+ HHP 600	60 °C, 1 h + 600 MPa, 5 min
HHP 300+ SV	300 MPa, 5 min + 60 °C, 1 h
HHP 600+ SV	600 MPa, 5 min + 60 °C, 1 h

Storage

Control and spicy samples were put on trays and placed in a cooling cabinet (J 600-2, Thermotechnika Ker. Ltd., Hungary) and stored for 3 weeks at 2°C and 8°C. These temperatures were chosen to be below and to exceed the recommended <3°C storage parameter. Samples were taken for analyses on day 0 and at the end of the 3rd week.

The electronic nose

Measurements were carried out using an Applied Sensor NST 3320 apparatus (Applied Sensor Technology, Sweden) 3,5g of sample was measured into the sample holder. (Figure 2.) The head space of the samples were analysed at 30°C.

Figure 2. Sample preparation for the E-nose analysis

Sensory analysis – triangle test

A sensory panel of 74 members evaluated the samples using the triangle test method. This difference test is simple: we wanted to know whether or not some specific attribute exist between the two samples. The test was carried out in a three hour period. However neither the e-nose nor this sensory method can be used to determine how large is the difference between the samples. Three samples (SV, HHP600, SV+HHP600) were chosen for the sensory test - using the results of the e-nose. These samples were arranged in six sets. (AAB, ABB, AAC, ACC, BCC, BBC)

Statistical analysis

Data were evaluated by discriminant analysis (CDA) using IBM SPSS (Version 20, SPSS Inc. Chicago, Illinois, USA)

RESULTS AND DISCUSSION

The results of the CDA show that the model made three well separated groups by the storage parameters. (**Figure 3.A and 3.B**) The 2°C samples were closer to the day0 samples in both cases. This means the effect of temperature during storage has an important role – changes at 2°C are obviously less important than at 8°C.

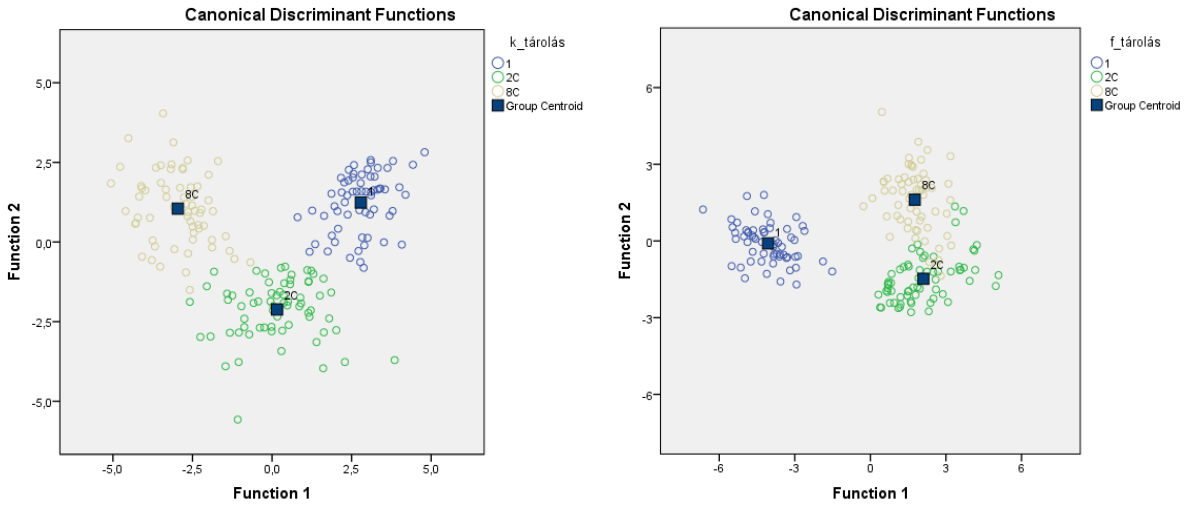

Figure3.A and **3.B** classification Plot of CDA result by storage

The model established for the classification by the treatment type is also very informative. The difference can be seen (**Figure 4.A and 4.B**) between the HHP treated samples and all other samples that suffered any form of heat treatment (by the discriminant

function 2). Samples treated with HHP at 600 MPa form a separate group regardless of the succession of treatments. Samples treated with HHP 300 MPa and the sous-vide sample form another group that can be explained by the minimal effect of the 300 MPa treatment.

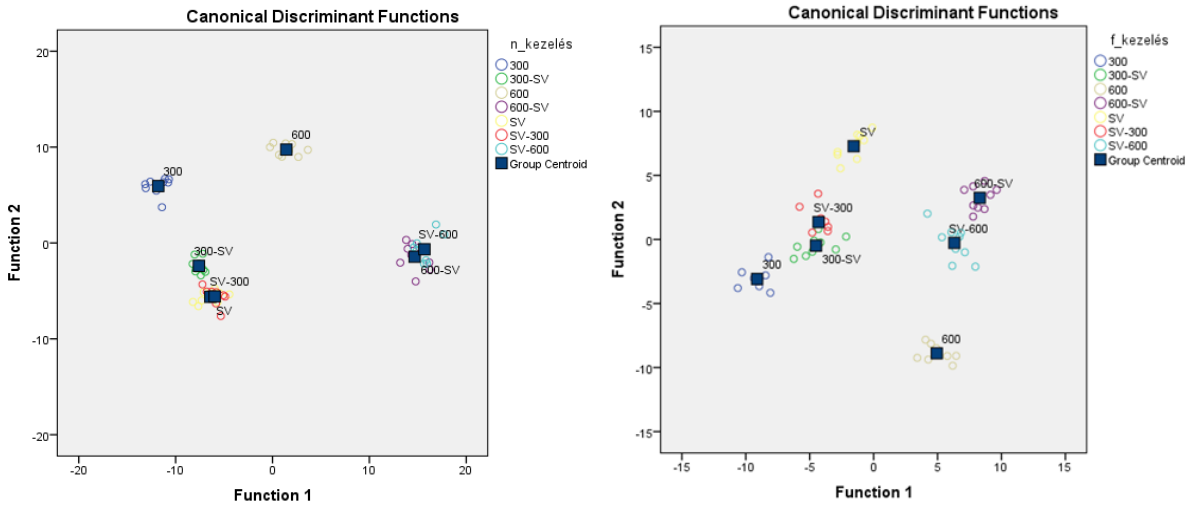

Figure 4.A and 4.B classification Plot of CDA result by storage

At 74 assessors at least 38 correct answers are required (binomial distribution statistics) to find a significant ($p<0,001$) difference between the samples. The result of the triangle test is showing a significant ($p<0,001$) differrence in all cases. (**Table 2.)**

Table 2. Correct answers at the triangle test (n=74)

SV and HHP600		HHP600 and SV-HHP600		SV and SV-HHP600	
set 1/1	set 2/1	set 1/2	set 2/2	set 1/3	set 2/3
43	**39**	**44**	**40**	**44**	**45**

At the SV and HHP600 test panelists gave the least correct answers. However the difference is significant, these samples show more similarities than the other two pairs - where finding the different one was easier as the results prove.

CONCLUSIONS

We can confirm the diverse flavour characteristics caused by the treatments applied in this study. Both the e-nose and the sensory panel could make the difference between the samples, This should be taken into consideration in the future when using these technologies in the research and in the industry as well.

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VESAGE – A PRODUCT INNOVATION PROJECT

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SUMMARY

There is an increasing demand for functional food products, including foodstuffs with health benefits and practical formulation as well. VeSage is the result of a product development project at Corvinus University of Budapest, Faculty of Food Science, Deák Tibor College of Advanced Studies. The name is a portmanteau of VEgetable suaSAGE, and it contains mainly vegetables and egg white powder, these ingredients create a cold cut with high nutritional value, and a sliceable replacer for the less practical fresh vegetables, that soak sandwiches. The red and the green variants of VeSage are both ovo-lacto vegetarian, soy-free, gluten-free lactose-free, and eco-friendly thank to the polylactic acid (PLA) tray packaging. The project won the first prize at the Ecotrophélia Europe 2015 product development competition, at which one of the requirements is the eco-innovative aspect of the presented product.

INTRODUCTION

VeSage is a novel approach to cross nutritional functionality with practicality, creating not only an innovative food product, but a new category. It is a sliceable, vegetable based cold cuts with high nutritional value. Due to its ingredients it is also fit to eat for vegetarians, individuals suffering from lactose or gluten intolerance. It does not contain soy and artificial additives, has a high vitamin and fiber content and at the same time it is low on calories, therefore perfect for consumers on a diet, or people simply looking for a convenient product with numerous health benefits.

The idea of VeSage occurred when we realized, that there are no vegetable based products that are not meant to be meat replacers, but are as practical as cold cuts, sausages or bolognas in case of meat products; hence the portmanteau VEgetable SauSAGE. The above mentioned are basically cheap sources of essential amino acids, which is the nutritional functionality of the food, and they are easy to handle, therefore practical. It is certain, that most people do not think about them as functional foods, but if we consider them as such, we realize that there is a scarcity in similar products based on fruits or vegetables. There are patés and spreads with such ingredients, but they lack the property of easy handling because of their rheological characteristics. Having a firm, sliceable texture is key to convenient everyday use for example in sandwiches or cold buffets.

MATERIAL AND METHODS

Composition

Due to trade secrets, exact composition of the product and critical parameters of the process line cannot be listed. The main ingredients of the two variants are listed in table 1 (not in order). As stated in the submission of the patent, the weight ratio of the vegetables has to be at least 70% of the total weight.

Table 1.: Ingredients of the products (not in order of weight ratio)

Green	Red
Ingredient	
salt	paprika powder
potato	tomato
garlic	garlic
green pea flour	green pea flour
green peas	egg white powder
onion	carrot
spices	spices
celery (allergenic)	salt
water	tomato purée
egg white powder	water
spinach	beetroot
	pepper

The product has a generally low fat, salt and sugar content, but has a high amount of protein incorporating essential amino acids as well. Raw materials are rich in vitamins, and even after heat treatment, levels of vitamin C (which is one of the most heat-sensitive nutrients) are very high, we estimated that almost half of the recommended daily value (DV%) is covered by 100 grams of the product. Detailed estimated nutritional data can be found in table 2.

Table 2.: Nutritional data for 100 g of the product

green		red	
Energy	419 kJ/100kcal	Energy	348 kJ/83kcal
Fat	0.8 g	Fat	0.5 g
Saturated fats	0.0 g	Saturated fats	0.0 g
Carbohydrates	9.1 g	Carbohydrates	7.6 g
Sugars	1.4 g	Sugars	5.5 g
Fiber	4.0 g	Fiber	2.7 g
Protein	20.1 g	Protein	17.3 g
Salt	0.8 g	Salt	0.8 g

Processing plan

Processing of the product is carried out in the following steps:

Acceptance and quality control of raw materials

Every shipment of raw ingredients have to be examined for defects. This does not apply to the non-raw ingredients such as salt or powders in air-tight packaging. Methods and circumstances of the investigation are regulated by the Codex Alimentarius Hungaricus.

Cleaning

This step requires a suitable equipment, that rinses the ingredient that are likely to be unclean, these are mostly matching with the ones that need to be investigated upon acceptance.

Precutting

This step is required to secure the constant quality of freezing and defrosting of unused raw materials. The increased homogeneity affects all further processes of manufacturing. Cutting into 8-10mm pieces is sufficient.

Frozen storage

One of the main advantages of VeSage is the constant nutritional value offered without being affected by seasonality. This requires a freezing of the precut raw materials between (-20) - (-18) °C until further processing.

Measuring of the ingredients

Preheating

This step basically means defrostation and a cooking step of the vegetables, which makes the subsequent cutting and homogenization simpler and more effective. This means that a lower rpm and cutting time will be required, preventing the formation of air pockets that could favor the growth of microbes leading to a shorter shelf-life, also the segment of the product would become less pleasant. Temperature and length of the process is set to a time-temperature combination, which is enough to soften the texture of the vegetables and has a pasteurization effect to some extent, but is low enough not to compromise the thermal stability of vitamins.

Shredding, mixing, homogenization

This is one of the most important steps of manufacturing, optimal process parameters assure the right texture, stability of the emulsion, and expected appearance of the product. These parameters were found to be optimal at low rpm (revolutions per minute) at first (~1000) and after proper mixing of every ingredient 5000 rpm for 3 minutes. These parameters also prevent exuding moisture after filling, a vacuum-cutter is required to prevent foaming as well.

Formulation

Filling is carried out in a polyamide casing, for this purpose a filling machine that can perform the procedure is important, again, to prevent bubble and air pocket formation. Slicing and distribution to the PLA plates is carried out after heat treatment.

Heat treatment

Another important procedure is pasteurization, also heating contributes to characterization of the final product by coagulation of the egg proteins, creating the matrix. The parameters for the heat treatment was calculated to balance the decay of *C. botulinum* ($F_{121.5°C}$=2.52min) and vitamin loss. The process is followed by rapid cooling.

Cold storage

It is important to decrease the core temperature of the product to 4°C max., at which 3 weeks of shelf-life was measured, therefore we suggest to apply 2 weeks of shelf-life.

Eco-innovative aspect of the manufacturing process

The processing steps requiring the most energy, and therefore the least eco-friendly are heating procedures. Waste energy generation can be minimized by engineering tools, such as proper sizing of heat treatments, also there is a powerful tool that is rarely used and give precise calculations on the optimal reuse of waste energy from heating. This is called the pinch technology, which is generally an optimization algorithm, which connects all heat-exchange technological steps to a maximum efficiency. Although this kind of optimization is relatively common, it is mostly restricted to single processes, and manufacturing is not recognized as a whole. Complexity of today's processing plants makes it difficult to design advanced heat-exchange systems to minimize energy waste, but at such small scale of manufacturing it is well feasible. Also, pinch technology was designed for continuous processes, therefore the applied method (especially mass flows) has to be controlled by a sensitive automation equipment.

Packaging

In terms of eco-awareness, we have been searching for a packaging material with the property of fast degradation and recyclability, therefore providing appropriate solution for reducing the ecological footprint of our society, and is potentially proper for packaging and storing VeSage, practically and aesthetically alike.

PLA is a versatile polymer. It is produced from lactic acid the following way: lactic acid is produced by the fermentation of dextrose, gained from starch. Starch can be gained from many sources, mainly by the utilization of industrial plants, such as maize or sugar cane. The organization of Biodegradable Products Institute (BPI) certifies the PLA-based products according to international standard (ASTM 6400) for degradability in industrial composting facilities, in regulated temperature, moisture and other parameters of the conversion. With appropriate treatment at temperature 65.5°C and 90% relative humidity, products are fully degraded within approximately 50 days. According to the *Allied Market Research*, by 2020 the global PLA-market is expected to reach $5.2bn. The expenses of PLA are considerably competitive compared to other alternatives, such as PET packaging materials, considering that its production is at far lower price. Raw materials required for the production of PLA, (eg. maize, sugar cane and other starch-rich plants) are present galore, and at lower price, compared to oil-based packaging, resulting in major and highly considerable margin for companies interested in innovative packaging. Furthermore, due to the depletion of the petrochemical resources, notable increase is expected in the price of PET packaging. Product presented in the designed packaging is shown in figure 1.

Figure 1.: Packaging design

DISCUSSION

The product won the first prize at the Ecotrophélia Europe 2015. competition out of 16 products from different European countries. Main aspects of the evaluation grid were (among others) total coherence of the product, innovativeness and marketability. The interest from different sectors of the food industry is high, and the product has gained great attention in a short period of time, meaning VeSage is a product with high market potential.

EFFECT OF EDIBLE COATING ON SHELF LIFE OF SOUR CHERRY

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SUMMARY

Sour cherry is a popular fruit in Europe, mainly cultivated in the Eastern part of the continent. These fruits are considered as perishable commodities, which cannot be stored for any length of time. A useful and ecofriendly technique for postharvest protection of fruits and extending of their shelf-life is the application of edible coatings and films containing chitosan. In this study the antimicrobial effect of edible coatings (chitosan, chitosan combined with Ca-lactate, or with alginate) was investigated in order to evaluate its applicability for extending the shelf life of sour cherry. The microbiological status of the samples during refrigerated storage were determined through assessment of the total viable counts, yeast counts and presence of coliforms. Chitosan or chitosan with Ca-lactate reduced the microbiological load of sour cherries by 1-2 log CFU/sour cherry, which was more pronaunced in case of yeasts.

INTRODUCTION

Sour cherry is very popular and the most produced fruit after apple in the Eastern part of Europe. As it is perishable it can not be stored for long time. Application of chitosan edible coatings is one possibilty to increase the shelf life of this product. To improve their shelflife, minimally processed fruits and vegetables such as strawberries (Campaniello et al., 2008), apples (Assis, 2008), broccoli (Moreira et al,. 2011), mango (Chien et al., 2007) have been treated with chitosan efficiently.

The aim of this study was to extend the shelf life of sour cherry by the application of chitosan edible coatings with or whout the combination of Ca-lactate, or alginate.

MATERIALS AND METHODS

Materials

Érdi ipari sour cherries, a Hungarian variety, were used in the experiments. Samples were purchased at a local store and processed immediately after buying. The fruits were washed with tap water and dried at room temperature.

Edible coatings

The following edible coatings were prepared for the experiments: 1g/100 ml water soluble low molecular weight chitosan was dissolved in distilled water (Ch); 1g/100 ml water soluble low molecular chitosan and 1g/100 ml calcium lactate were dissolved in distilled water (Ch + Ca); the third edible coating contained two layers, prepared by 1g/100 ml solution of sodium alginate in distilled water and 1g/100 ml solution of water soluble low molecular weight chitosan in distilled water (Ch +Al). All ingredients were prepared by stirring the polymers with magnetic stirrer overnight until they were completely dissolved.

The fruits (Ch or Ch + Ca) were immersed into the corresponding solution for two minutes and air dried at room temperature (25°C) for 30 minutes. After that they were placed into plastic containers (10 pieces into each) with punched cups and stored at refrigerated temperature (6±1°C) for 13 days.

The layer-by-layer polyelectrolyte deposition process was used to prepare double-layer coating. First the sour cherries were dipped into alginate solution for two minutes, then they

were dried at room temperature for 60 minutes, and finally were immersed into chitosan solution for another two minutes. After the second drying the fruits were packed into plastic containers and stored at refrigerated temperature (6±1°C) for 13 days.

Microbiological analysis

During refrigerated storage total aerobic plate count, coliforms, yeast and mold counts were determined on days 0, 2, 5, 8 and 13 of storage at 6 °C. Total aerobic plate counts were determined by pour plating with TGE (Trypton, glucose, yeast extract) agar. Plates were incubated at 30 °C and evaluated after 24-48 hours. Coliforms were grown on VRBG agar by double pour plating. Plates were incubated at 37 °C for 24-48 hours. The number of yeast and molds were counted on RBC agar by spread plateing after 3-5 days incubation at 25 °C.

RESULTS AND DISCUSSIONS

Gradual increase of the total viable counts of untreated sour cherries could be observed during the examined storage period, reaching the level of 7 log CFU/1 piece of sour cherry (Figure 1.). Yeasts dominated in control samples. The number of coliforms was below the detection limit during the whole experiment.

Within the initial day of the experiment chitosan coating resulted in an initial reduction in total counts of about 1 order of magnitude (Figure 1). This difference remained constant during the whole storage time. About 2 order of magnitude reduction in the number of yeasts could be observed at the beginning of the experiment, which was followed by re-growth to about 6,5 log CFU/1 piece of sour cherry within 8 days where they remained steady until Day 13 (Figure 2).

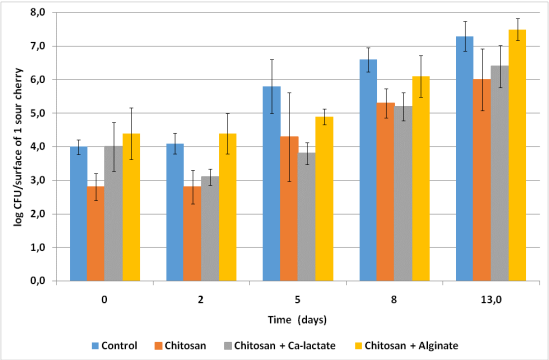

Figure 1.: Total viable count of coated samples

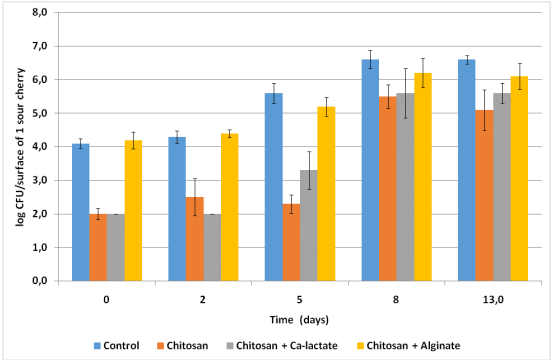

Figure 2.: Yeast count of coated samples

Antimicrobial activity of chitosan on different fruits (eg. strawberries, apples, kiwifruit, pears) has been shown previously (El Ghaouth, 1991,; Du et al., 1997, Bautista-Baños et al., 2004). The results of this experiment gives further confirmation to the quality maintaining effect of chitosan.

Addition of Ca-lactate to the edible film does not changed the antimicrobial action of chitosan. There was no significant difference in the results of the combination of chitosan with Ca-lactate and chitosan used alone. But the addition of alginate significantly reduced the inhibitory effect of chitosan resulting similar TPC and yeast counts as in control samples (Figure 1 and 2). These differences could not be observed in case of moulds (Figure 3). The effect of alginate combination on mould counts was very similar to the effect of chitosan alone.

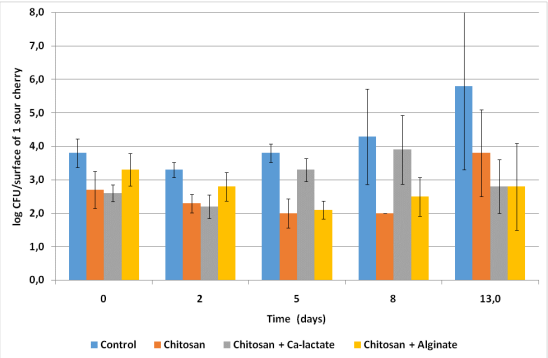

Figure 3.: Mould count of coated samples

Chitosan acts in different ways as natural antimicrobial agent: as a semi-peremeable barrier; reduces respiration, oxidation and waterloss (Maqbool et al., 2011). Its further function is the direct interference of fungal growth (Bai et al., 1988). It is also reported as a chelating agent (Cuero et al., 1991). It was demonstrated that binding of chitosan with DNA and inhibition of mRNA synthesis may also occur in microorganisms (Sudarshan et al., 1992).

CONCLUSION

The use of chitosan as a natural preservative can improve the microbiological quality of foods and delay their spoilage.

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TRITICALE IN SALTY SNACK PRODUCT

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SUMMARY

Triticale is a hybrid of Triticum and Secale, mainly used in animal feed. However its good nutritional value made it useable in bread production. It has not been tried up till now in salty snack products. Our aim was to investigate applicability of this raw material in real manufacturing process of a salty snack. Organoleptic measurements were done on salty sticks containing 100% triticale (Hungaro durumrye), 100% wheat and 100% triticum spelta as the flour part in the recipe. As the gluten development capacity of these cereals are very different, main production points were the kneading and the extrusion. We were able to produce good quality products without manufacturing issues (such as inhomogeneity in structure or length). In organoleptic evaluations triticale got first place, spelt became second, and the conventionally used wheat got 3rd place.

INTRODUCTION

Triticale is a hybrid of wheat (Triticum) and rye (Secale) first bred in laboratories during the late 19th century in Scotland and Sweden. Commercially available triticale is almost always a second generation hybrid, i.e., a cross between two kinds of primary (first cross) triticales. As a rule, triticale combines the yield potential and grain quality of wheat with the disease and environmental tolerance (including soil conditions) of rye. Only recently has it been developed into a commercially viable crop. Depending on the cultivar, triticale can more or less resemble either of its parents. In 2013, according to the Food and Agriculture Organization (FAO), 14,5 million tons were harvested in 29 countries across the world. (Hungary was the 15th in 2013 with its production)

The protein content is higher than that of wheat, although the glutenin fraction is less. Therefore it can develop less gluten, than normal wheat flour. Triticale has also been stated to have higher levels of lysine than wheat.

Spelt (Triticum spelta) is a species of wheat cultivated since 5000 BC. It is richer in protein and many vitamins than wheat. However its baking value is not as good as that of wheat, because of less gluten. In the cultivation of spelt less pesticide is needed, therefore it is more suitable for “nature focused” consumers. Figure 1. shows the used grains.

a) **b)** **c)**

Figure 1: a)Triticum aestivum, b) Triticum spelta, c) Durumrye

The traditional way of producing salty stick is to extrude them, and cut them for a certain length.

In this trials common wheat, triticale (a new breed called Hungaro durumrye) and spelt was used as only flour part. Manufacturing parameters were looked at, and physical and organoleptic evaluations were done on the finished products.

MATERIALS AND METHODS

Materials

The main ingredient of a salty stick recipe is flour (approx. 70%). Three types of flour was used: wheat flour (type BL55), Hungaro durumrye, and spelt flour.

Beside flour the same amount of water, sugar, palm fat, salt, yeast, whey powder and backing powder was used in all three samples (the exact recipe is confidential).

Production of salty sticks

After weighing flour has been sifted in the kneading bowl. Water, other powdered ingredients has been given to the mixture. The dough was mixed for 10-15 minutes in the z blade mixer. After kneading 30 minutes of resting is implanted. After leavening the dough will be put through the pasta machine, which extrudes a continuous rope of dough. Rotating blades will cut the dough ropes in the desired length. The formed pieces will be immersed in 2% NaOH solution before baking. This gives the sticks the shiny brownish color after baking. Salt crystals are sprinkled on the top of the wetted sticks as decorations. The baking happens at 240°C in pre-heated oven. After baking the sticks will be cooled and packed (Figure 2.).

Figure 2.: Salty sticks

Methods

The determination of water absorption and rheological properties were done according to MSZ ISO 5530-3 using a valorigraph.

In the organoleptic evaluation 20 judges (non-trained consumers) have tested to products according to MSZ 20501/2-82 (Hungarian standard). According to this descriptive analysis the following parameters were judged: shape, color, texture, fragrance, taste. Parameters get a weight factor and the total point of a product is calculated as a sum of individual points multiplied with its weight factor. (Table 1.) The total point for a product can reach 20. The product will be accepted if a minimum of 6, 4 points in total. If one parameter gets 0 point, the product failed.

Table 1.: Organoleptic parameters

Parameter	Characteristics	Weight factor	Maximum points
shape	straightness	0,6	5
color	light- dark	0,6	5
texture	soft-hard	0,8	5
fragrance	pleasant-unpleasant	1,0	5
taste	salty-blend	1,0	5
		Total points	**20**

RESULTS

According to the literature statements, water adsorption of durumrye was found to be the smallest. The dough developed from durumrye showed fast softening.

Looking at the values of the wheat flour, one can state, that the quality of the wheat was not the best. The results of the valorigraph measurements is summarized in Table 2.

Table 2: Results of the Valorigraph measurements

Samples	Water weight loss **(cm^3)**	Water adsorption **(%)**	Dough development time **(min)**	Dough softening **(VE)**
Wheat flour	**31,3**	**62,6**	**2**	**80**
Spelt flour	**28,9**	**57,8**	**4**	**120**
Durumrye flour	**25,7**	**51,4**	**0,5**	**300**

During production the differences in the gluten developments of the flor types did not cause any difficulties. In the dough mixing less water was needed in case of durumrye, to get the same consistency as wheat flour (in line with the measured water adsorption values).The dough became a little bit less shiny and less elastic after extrusion, but the dough was still formable, and breakage was not an issue.

Figure 3 shows the results of the organoleptic evaluation. In total points product made of Hungaro durumrye got the most points (17,68 out of 20), second was spelt (16,25 points) and surprisingly last was wheat (10,1 points).

Triticale containing salty sticks got higher points in parameters as texture, smell and taste, which are very promising results in terms of further product developments.

Salty stick made with spelt showed similar characteristics as wheat product.

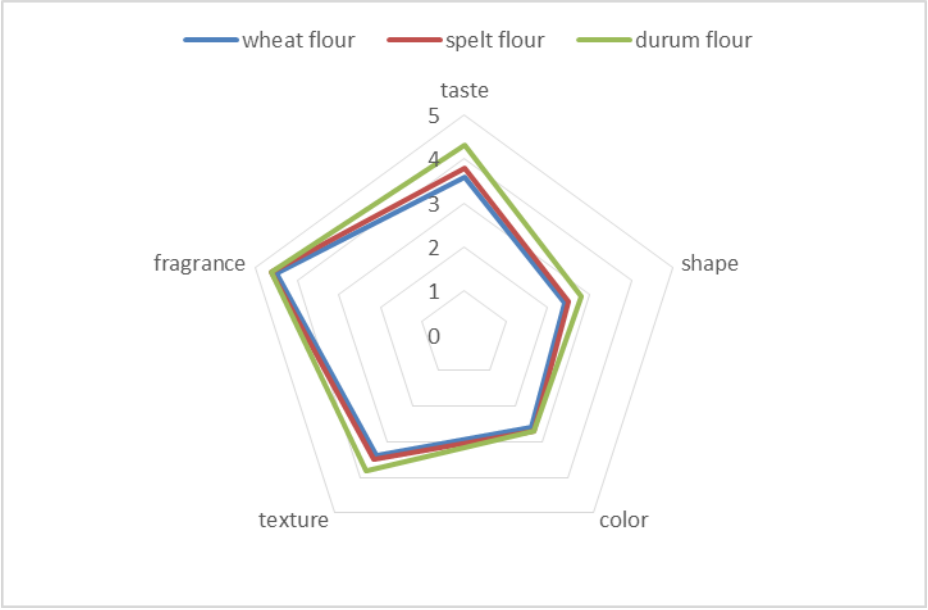

Figure 3. Sensory evaluation summary

CONCLUSIONS

Triticale has been developed as a hybrid of triticum sorts and secale. It is not a GMO product, and therefore focus is now higher on its application into food products. In the cross breeding of Hungaro durumrye (a sort of triticale), durum wheat and rye were used. It has more protein than wheat, but can develop less gluten as the glutenin fraction of it is less. As a benefit it has more lysine, than wheat. It has been tested before in bakery applications (breads, rolls), but not in snack foods.

In our trials salty sticks were produced using durumrye or spelt flours, and the finished products were compared to the standard product made with wheat flour.

The different flours gave no production problems, such as breakage or inhomogeneous texture. In final organoleptic tests consumers preferred durumrye salty sticks to spelt and wheat, even though its taste, smell, textural properties were different.

Although currently the price of durumrye is 3 times higher, then the price of conventional wheat, it does have a market niche.

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CURRENT TRENDS AND CHALLENGES IN MYCOTOXIN RESEARCH

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SUMMARY

The Carpathian Basin belongs to the particularly sensitive regions of Europe related to climate change, which has a major impact on agricultural production, food security and food safety. As a result of global warming as well as the contaminations and stress factors associated with climatic extremities, chemical and microbiological food and feed safety is facing new challenges. The growth of the mycotoxin producing moulds is influenced by numerous environmental factors. As a result of the climate change, the possibility of mould infection and mycotoxin contamination of foods and feeds changes, both before the harvesting and storage of crops. The paper gives a summary on the most important consequences of global warning on aflatoxin and fusarium toxin production. It also gives an outlook on some new challenges, as the co-occurrence and thus interaction between mycotoxins, and the importance of modified and masked mycotoxins.

INTRODUCTION

Predictions indicate that European areas will encounter different effects of the changing climate and its impact on agriculture and food safety will vary for the different regions. The Carpathian Basin belongs to the particularly sensitive regions of Europe. Climate change manifests in increase of the temperature, variation in precipitation, drought and change in the atmospheric carbon dioxide (IPCC, 2007).

EFFECT OF CLIMATE CHANGE ON MOULD INFECTION

Moulds are heterotrophic organisms widespread in nature. Their growth is influenced by numerous environmental factors, among others the nutrient medium, moisture content, oxygen content, and temperature. As the growth and toxin production of fungi strongly depend on the weather conditions (temperature, relative humidity), the degree of mould infection and mycotoxin contamination may vary from year to year, and it may show substantial regional differences as well.

Species of the most important moulds belong to the *Aspergillus*, *Fusarium* and *Penicillium* genera. Regarding temperature the optimum for growth is 25–30 °C for most *Penicillium* species and 37–40 °C for most *Aspergillus* species. The temperature optimum of the majority of *Fusarium* species is between 20 and 32 °C, but these moulds easily tolerate a colder climate as well.

Due to the 'mediterraneanization' of the climate of our region, the thermophilic *Aspergillus* species may become increasingly prevalent in our region, while the *Penicillium* species preferring the temperate climate may move north (Farkas and Beczner, 2010). Some years ago the countries neighbouring Hungary from the south already faced this phenomenon. As one of the most important consequences of global warning, aflatoxin-producing *Aspergillus* species are predicted to appear in countries of temperate climate, which could result in aflatoxin contamination of the agricultural crops grown there (Paterson and Lima, 2010). Recently, numerous papers have reported the occurrence of aflatoxin-producing moulds and higher than permissible levels of aflatoxins in European countries of temperate climate. Dobolyi et al. (2011) have conducted a comprehensive survey series to determine the presence of aflatoxin-producing moulds in maize-growing areas of Hungary. According to their conclusions, aflatoxin contamination of agricultural crops, such as maize, is not a serious threat in Hungary yet, but its spread is considered likely.

Between 2005 and 2008, Borbély et al. (2010) measured the mycotoxin content of cereal samples originating from the eastern part of Hungary, and found aflatoxin B_1 (AFB_1) in 4 out of the 83 samples analysed, in 3 out of which samples an aflatoxin contamination exceeding the maximum limits set by the EU was detected.

Fusarium head blight is one of the most dangerous diseases of cereals all over the world. Of the causative agents, the most pathogenic are *Fusarium graminearum* and *F. culmorum*, which mainly infect wheat, maize and barley. As a result of the hot summers serially occurring in consecutive years, the prevalence of the previously dominant *F. culmorum* has decreased and *F. graminearum* has become dominant in Europe. Of the *Fusarium* toxins, fumonisins and the species *F. verticillioides* producing them can also be expected to become more common as a result of rainfall following the dry weather. It is also likely that mycotoxins currently less known to have a human health impact or considered to be less dangerous to human health (e.g. moniliformin) have will acquire a higher importance in the future.

EFFECT OF CLIMATE CHANGE ON MYCOTOXIN CONTAMINATION

Mycotoxins are secondary metabolites of moulds. Numerous compounds have been identified as secondary metabolites. About 100 of them have been demonstrated to have toxic effect and approximately 20 mycotoxins exert pronounced deleterious effects on human and animal health. Among them, AFB_1 and fumonisin B_1 (FB_1) have carcinogenic effects, numerous mycotoxins (AFB_1, trichothecenes) exert immunosuppressive actions, others (AFB_1, FB_1, ochratoxin A) are nephrotoxic and hepatotoxic, while zearalenone causes reproductive disturbances.

According to an EFSA report (EFSA, 2012), a survey of the literature and mathematical modelling indicate that in case of a predicted temperature increase of 2 °C the risk of aflatoxin contamination of the maize crop may substantially increase in certain regions of Southern Europe (in Central and Southern Spain, Southeast Portugal, Southern Italy, the Balkan countries and the European part of Turkey). A moderate increase of aflatoxin risk can be predicted in Romania, France, Hungary and North-eastern Italy. Reckoning with a +5 °C temperature increase scenario, a further extension of the area of risk increase can be expected.

As mentioned before, as a consequence of warm summers, *F. graminearum* has become dominant in Europe instead of the previously dominant *F. culmorum*. Since the latter generally produces less mycotoxin than *F. graminearum*, mycotoxin concentrations, and the total amount of mycotoxin produced may consequently increase. Besides DON and ZEA, *F. graminearum* may produce NIV as well, however there is a big regional difference in NIV production (Miller et al., 2008). If a shift in DON/ZEA co-contamination to NIV/ZEA co-contamination can be predicted, depends on the occurrence of the chemotype of the mould. Only a very few isolates have been proved to be NIV producers in the USA. In the UK 71% DON and 25% NIV producing *F. graminearum* chemotypes were isolated and identified from wheat ears showing classic symptoms of fusarium head blight (Jennings et al., 2004). According to a Hungarian survey the occurrence of NIV in Hungary could be linked exclusively with *F. culmorum (*Xu et al., 2008).

CO-OCCURRENCE AND INTERACTION OF MYCOTOXINS

Humans and animals are generally exposed to multiple mycotoxins in parallel, as some mycotoxins typically co-occur in cereals. However, toxicological data, risk assessments are based on, are provided by studies in which only the individual effects of certain toxins are investigated.

The simultaneous exposure of animals to more than one toxin is of concern and requires more study. Synergistic effects may explain why animals sometimes respond negatively to mycotoxin levels much lower than those reported in scientific studies as able to cause mycotoxicoses.

In a survey conducted over a period of 4.5 years in countries of Southern Europe the *Fusarium* mycotoxins were found to be the major contaminants (fumonisins, type B trichothecenes and ZEA) of feed material and compounded feed samples (Griessler et al., 2010). Streit et al. (2013) analyzed 83 feed samples for a total of 139 mycotoxins and metabolites (including masked mycotoxins) using a multi-mycotoxin LC-MS/MS assay. Of the samples tested, 85.5% originated from Europe (19, 17 and 15 samples were submitted from Hungary, Austria and Denmark, respectively). As few as two samples contained less than 10 mycotoxins and metabolites, while the majority (66%) of the samples were contaminated by 16–30 toxins and metabolites. *Fusarium* toxins were the most commonly found mycotoxins, with DON being detectable in 89% of the samples. With the exception of a few extreme lots, the samples contained the individual mycotoxins in low concentrations, mostly at levels below the maximum allowable limits recommended by the EU. In a three-year (2009–2011) analysis comprising a total of 7049 feed samples from several continents for 5 mycotoxins (aflatoxin, DON, zearalenone, fumonisin and ochratoxin A), 48% of the samples contained two or more mycotoxins (considering only the European samples, this ratio was lower, 39%). The most commonly found contaminants were DON and fumonisin (Rodrigues and Naehrer, 2012).

There are several studies about mycotoxins' interaction (mainly in vitro), but only a few report the combined effects of Fusarium mycotoxins which are more likely to co-occur (i.e. FB1, DON and ZEA). *Grenier and Oswald (2011) performed a meta-analysis of published raw data on mycotoxin interactions in vivo* - which varies according to the animal species, the dose of toxins, the length of exposure, but also the parameters measured.

MODIFIED AND MASKED MYCOTOXINS

In the past decade more studies have been published concerning the formation and role of 'masked' mycotoxins in naturally infected and contaminated foods and feeds. Mycotoxins that are undetectable by conventional, extraction-based analytical methods are known as masked mycotoxins (Berthiller *et al*., 2013). While extractable mycotoxins can be easily detected, bound mycotoxins are not directly detectable, they have to be liberated from the matrix by chemical or enzymatic pre-treatment prior to chemical analysis. Dall'Asta et al., (2010), who reported the occurrence of non-covalently bound fumonisins in raw maize, suggested an *in vitro* digestion model to evaluate their levels. With this method after an enzymatic pre-treatment significantly more (30-40%) fumonisin was detected compared to that measured after the conventional extraction method.

The term 'masked' mycotoxins was introduced by Gareis et al. (1990) to describe zearalenone-glycoside. The International Life Science Institute (ILSI) adopted the definition for masked mycotoxins as: "Mycotoxin derivates that are undetectable by conventional analytical techniques because their structure has been changed in the plant". Rychlik et al. (2014) a systematic definition for the two different modifications of mycotoxins: matrix associated and (biologically or chemically) modified mycotoxins. According to the suggestion the term 'masked' mycotoxin should be kept exclusively for the biologically modified mycotoxins that were conjugated by the plants.

From the consumer health point of view risk assessment should take all mycotoxin compounds into consideration, regarding both exposure and toxicity data.

To assess exposure data reliable analytical methods have to be developed measuring not only free but also modified and matrix associated mycotoxins.

The harmful effect of these compounds depend also on their bioavailability and toxicity, which is also less known. Conjugated and matrix-associated mycotoxins can be cleaved by the gut microbiota or digestive enzymes. Only very few data on the absorption, distribution, metabolism, elimination and toxicity of them is available.

CONCLUSIONS

Mycotoxins are still unavoidable in our environment. In spite of the very strict control of contamination and the highly developed very sensitive analytical methods to detect them, we have to face new challenges, like climatic change, new emerging mycotoxins, few knowledge in interactions and metabolism and also the toxicity of new metabolites and modifications.

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COLONIZATION AND ADAPTATION OF ENDOPHYTIC AND ENTEROPATHOGENIC BACTERIA IN PLANTS

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SUMMARY
Plant associated endophytic bacteria are living inside the tissues and their colonization has been proven by both cultivation and microscopic analysis. They are in close interaction with certain enteral pathogenic bacteria, which could be either beneficial or unfavourable for the survival and colonization of the pathogens.
Our research work extended to the molecular analysis of the endophytic bacterial populations of sweet pepper (Capsicum annuum L. var. grossum) cultivated in hydroponics or soil, and several putative endophytic strains have been isolated and characterized. Internalisation of selected endophytic and food-borne bacteria has been studied by FISH technique.

INTRODUCTION
Endophytic bacteria are living inside the plant tissues without doing harm to the host plant or triggering its defence mechanism, even their presence is beneficial to the host. This association is different from the endosymbiosis as the endophytic bacterial cells are not able to enter into the plant cells or they are not separated into membrane compartments.
Positive effect of the endophytic association to the plant is very wide including the growth promotion by hormone production, ACC deaminase activity, iron uptake with the help of bacterial siderophores or prevention of the infection by plant pathogenic bacteria, fungi, insects and viruses (Mendes et al., 2011; Reinhold-Hurek and Hurek, 2011; Ryan et al., 2008).

Root associated endophytic bacteria are represented by the highest number of species and populations but colonization of the phyllosphere by endophytes is also considerable. Because of the close plant-bacteria interactions these endophytes represent the plant's second genome (Berg et al., 2014; Garbeva et al., 2001). Rhizosphere is, however, the proven source of several opportunistic human pathogenic bacteria including *Burkholderia, Enterobacter, Pseudomonas, Staphylococcus* and *Stenotrophomonas*, therefore application of potential epiphytic or endophytic biocontrol bacteria belonging to these genera needs careful risk assessment prior to registration (Berg et al., 2005).

Outbreaks connected to consumption of leafy green vegetables and sprouts highlighted that certain enteric pathogens especially *Escherichia coli* and *Salmonella enterica* serovars are responsible for majority of these outbreaks. At the same time there are some proofs indicating that epiphytic or even the endophytic bacteria are in interactions with the pathogens, which could be either beneficial or detrimental for the persistence and colonization of the pathogenic bacteria on the surface or inside the plant. Endophytic bacteria may either help or inhibit survival and internalization of the pathogens (Cooley et al, 2006; Poza-Carion et al., 2013).

Main sources of enteric pathogens are human and animal feces, which come at the plants generally through water, raw or inadequately amended manure and insect vectors (Martinez-Vaz et al., 2014). Survival and persistence of enteropathogenic bacteria on the surface or inside the plants are determined by several ecological factors. Dinu et al. (2011) proved that Shiga-toxin producing *E. coli* (STEC) O157:H7 turned into viable but nonculturable (VBNC) state under stressful condition and verotoxin production was detected even after 3 days of turning the cells into this state. Adaptation of *E. coli* strains to the plant host was indicated by phenotypic and phylogenetic analysis of *E. coli* strains isolated from crops and mammalian hosts (Méric et al., 2013). Plant derived isolates displayed higher biofilm and extracellular

matrix production, and they utilized sucrose and p-hydroxyphenylacetic acid in higher frequency than the mammalian isolates.

Gene expression profiles of *E. coli* O157:H7 isolates linked to the consumption of ready-to-eat bagged spinach in the United States during 2006 were compared with other, geographically associated isolates by Parker et al. (2012). They found that several spinach and clinical isolates showed decreased expression of many *rpo*S regulated genes than their environmental counterparts. These isolates had a mutation in the *rpo*S gene which rendered them more sensitive to the oxidative, acid and osmotic stresses, and at the same time they have got an increased nutrient scavenging activity. The last mentioned characteristic of the isolates allowed them to survive and adapt to new environments characteristic to the bagged spinach. These findings support the hypothesis that such "preconditioned" *E. coli* O157:H7 mutants have got the ability to multiply in new environments and infect humans.

Martinez-Vaz et al. (2014) made a survey of the molecular genetic aspects which mediated attachment, colonization and biofilm formation of human enteric pathogens, especially *E. coli* and *S. enterica* on leafy greens and constructed a model for the association and establishment with such kind of vegetables. One of the most important groups of genes includes stress response genes, which allow the pathogens to cope with nutrient limitations, oxidative stress and defence mechanisms. As the result bacteria adjust their metabolism to scavenge nutrients and develop more favourable energy conservation mechanisms.

MATERIAL AND METHODS

Putative endophytic bacterial isolates from different sweet pepper variants (*Capsicum annuum* var. *grossum* L., HO and KAPIA) grown in soil and hydroponic systems were analysed by phenotypic and genotypic methods followed by molecular identification and phylogenetic analysis. Internalization of selected endophytic isolates in pepper seedlings was investigated by fluorescent in situ hybridization (FISH).

RESULTS AND DISCUSSION

Examples of phenetic and genetic typing of the putative endophytic isolates followed by molecular identification and phylogenetic analysis will be introduced.

Adaptation and application of the FISH method allowed visualization of the endophytic bacteria applied for inoculation of pepper seeds inside the plant tissues.

CONCLUSIONS

Better understanding of the plant-microbiome interactions as well as interactions among the different plant associated bacterial populations including human enteric pathogens may promote combating against vegetable and sprouts associated outbreaks and development of different strategies for raising food safety along the agri-food chain.

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THE IMPACT OF DIFFERENT STARTER CULTURES AND CHEESE COAGULANTS ON BIOGENIC AMINE FORMATION IN CAMEMBERT.

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SUMMARY
Consuming cheese containing higher levels of biogenic amines is associated with food intoxication. Free amino acids are substrates for biogenic amine synthesis. Proteolysis plays a crucial role because it is directly related to free amino acid availability. Therefore, the effects of three Penicillium candidum strains (PCA-1, PCA-3, TT-033, with increasing proteolytic activity) and three coagulants types (CHY-MAX® M 1000, CHY-MAX® Plus, HANNILASE® XP 750 NB) on the biogenic amine content in camembert was investigated. Lower proteolytic activity was expected to produce lower biogenic amine concentrations. The biogenic amine levels were determined using reverse-phase high performance liquid chromatography (RP-HPLC). TT033 produced significantly ($p<0.05$) more spermidine compared with PCA-1 and PCA-3. The histamine concentration was higher ($p<0.05$) with PCA-1 than TT-033. Applying different coagulants yielded an insignificant effect on the levels of biogenic amines. No clear correlation was observed between proteolytic activity and the biogenic amine concentration in the tested cheeses.

INTRODUCTION

Proteolysis plays an important role in food fermentation. In addition to starter cultures, coagulants and ripening cultures induce proteolytic processes in cheese. Proteolysis leads to positive effects, such as the development of texture and flavour, but it also can produce undesired substances, such as biogenic amines (Fox & McSweeney, 1996). Biogenic amines in food are mainly formed by decarboxylating the corresponding amino acid (Ten Brink et al., 1990). Therefore, proteolysis plays a crucial role because it is directly related to free amino acid availability (Beutling, 1996).

Biogenic amines are associated with food intoxication (Giraffa, 2002). Based on the mean content and consumer exposure, cheese is one of the most relevant food products for histamine and tyramine exposure (BIOHAZ, 2011).

Researchers have investigated the effects of proteolytic activity and free amino acids on biogenic amine content in food. Herbert et al. (2005) confirms the assumption that free amino acid availability is related to biogenic amine formation. Enhanced proteolysis increases biogenic amine content in Gouda and Manchego cheese (Fernández-García et al., 1999, Leuschner et al., 1998). Researchers have also studied the influence and proteolytic activity of rennet on biogenic amine content in cheese (Ordiales et al., 2013).

This work aims to investigate the influence of mould with different proteolytic activities and different types of coagulants on the biogenic amine content in camembert cheese.

MATERIAL AND METHODS

Moulds and Coagulants

The moulds and coagulants used in this study were classified based on their proteolytic activity as low = (o), moderate = (+) and high (++). Three different moulds and coagulants were obtained from Chr. Hansen (Nienburg, Germany): PCA-1 (o), PCA-3 (+), TT-033 (++), CHY-MAX® M 1000 (o), CHY-MAX® Plus (+), and HANNILASE® XP 750 NB (++).

Cheese Manufacturing

Camembert cheese was produced in six batches on a pilot-plant scale. Pasteurised, unskimmed milk (Hochwald Nahrungsmittelwerke GmbH, Hünfeld, Germany) was heated at 38 °C. Starters were then inoculated with 0.015 % lactic acid culture F-DVS SSC-100 (Chr. Hansen GmbH, Nienburg, Germany) and moulds (dosage: 4 U/1000 l milk), and 0.15 g/l calcium chloride was added (Bunte Kuh, Hosenfeld Hainzell, Germany). After one hour of acidification, 45 IMCU/l of clotting agent was added. Table 1 provides an overview of the combination of starters and enzymes used for each batch. Following a 45-min set, the milk coagulum was cut into cubes of 2.5 x 2.5 cm and allowed to heal for 8 minutes. The curds were stirred gently for 25 minutes and then poured into 10 cm diameter hoops. The hoops were turned 10, 20, 50 and 110 minutes after filling. After draining, the overnight cheeses were salted in brine (pH: 4.77, salt: 17 %) for one hour, and the hoops were removed (pH before (mean): 5.05, pH after (mean): 5.14). The cheese wheels ripened at 16 °C with at least 85 % relative humidity for 7 days, were wrapped into duplex paper and stored at 6 °C for 21 additional days.

Table 1: Mould and coagulant combinations

Batch	Mould	Coagulant	Proteolytic activity	
			Mould	Coagulant
Ia	PCA-1	CHY-MAX® Plus	o	+
Ib	PCA-3	CHY-MAX® Plus	+	+
Ic	TT-033	CHY-MAX® Plus	++	+
IIa	PCA-3	CHY-MAX® M 1000	+	o
IIb	PCA-3	CHY-MAX® Plus	+	+
IIc	PCA-3	HANNILASE® XP 750 NB	+	++

RP-HPLC Analysis

For sample preparation, the cheese was homogenised with a blender for 20 seconds at 2000 rpm. Ten grams was weighed in a beaker and acidified by adding 90 ml trichloracetic acid solution (10 %). The sample was finely dispersed using an Ultra-Turrax homogeniser (T25, IKA® Inc., Cincinnati, OH) for 60 seconds at 13000 rpm. Dispersion was filtered twice (MN 615 ¼ Ø 185 mm, membrane filter CHROMAFIL Xtra Typ PET-20/2 0,2 µm, MACHEREY-NAGEL, Düren, Germany). For derivatisation, 0.4 ml of the extract was mixed (pear shaped flask) with 1 ml of saturated sodium bicarbonate solution and 1 ml of dansyl chloride solution (5 mg/ml) freshly prepared in acetone. A heat treatment was then used for 10 minutes at 70 °C. The liquid phase was evaporated to dryness in the rotary evaporator at 40 °C. The residual derivatives were dissolved by adding 2 ml acetonitrile. The sample was transferred to Eppendorf caps and centrifuged at 14 °C-16 °C and 16000 rpm for 30 minutes. A volume of 25 µl was analysed using a Waters 2695 HPLC (Waters Corporation, Milford, Massachusetts, USA) equipped with an RP-18 column (ACE C18-3 µm 125x3 mm, Fa. Advanced Chromatography Technologies, Aberdeen, Scotland. Precolumn: Symmetry C18 Sentry Guard Cartridge, 100Å, 5 µm, 3.9 mm X 20 mm, 2/pkg, Waters Corporation, Milford, Massachusetts, USA). The samples were detected using a fluorescence spectrophotometer F 1050 (Hitachi Ltd. Corporation, Tokyo, Japan) at EX 330 and EM 500 nm and a Waters 996 PDA UV detector (Waters Corporation, Milford, Massachusetts, USA) at 252 nm. The dansylated samples were eluted using a step gradient.

Gradient: 50 % B for 2 min, 50 to 100 % B in 12 min, 100 to 50 % B in 10 min and 50 % for 2 min.

Eluent A: 0.02 N acetic acid - acetonitrile (9 : 1), Eluent B: acetonitrile : methanol : 0.02 N acetic acid (4.5 : 4.5 : 1). Flow rate: 0.5 ml/min.

Statistical Analysis

The data were evaluated for statistical significance using SPSS 20.0 for Windows. The mean values were compared using a one-way analysis of variance (ANOVA). The Tukey-HSD multiple comparison test was then used to determine differences between the sample means with significance at $p < 0.05$. For unequal variances, the Games-Howell test was applied.

RESULTS AND DISCUSSION

The concentration of biogenic amines for the batches with different moulds are shown in table 3. Histamine production was significantly lower in cheeses with TT-033 (++) than with PCA-1 (o). Significantly higher spermidine accumulation was observed in TT-033 cheeses compared with PCA-1 (o) and PCA-3 (+) cheeses.

Table 2: Biogenic amine concentrations (ppm) in cheeses produced using different mould strains1

	PCA-1 (o^2) n = 9	PCA-3 ($+^2$) n = 10	TT-033 ($++^2$) n = 9
Histamine	12.34±1.02[a]	11.57±0.59[b]	11.23±0.66[a,b]
Tyramine	ND	ND	ND
Cadaverine	12.61±0.81	11.92±0.44	12.21±0.33
Putrescine	15.60±0.90	15.83±7.51	14.55±0.76
Spermidine	18.13±1.24[a]	17.32±1.45[a]	20.43±1.38[b]

[1]The data are the mean ± standard deviation, [2]Proteolytic activity: o = low, + = moderate, ++ = high
n = number of values being averaged. ND= not detected.
The means in the same row that do not share the same superscripts differ at $p<0.05$ using Tukey's HSD (for homogeneity of variances) or Games-Howell (for inhomogeneity of variances) multiple comparison.

Table 3 provides an overview of the analytical results for cheeses produced using different types of clotting agents. No significant difference was observed among the three batches. These results are consistent with the findings from a recent study that examined the influence of rennet proteolytic activity on biogenic amine concentration in "Torta del Casar" cheese (Ordiales et al., 2013).

Table 3: Biogenic amine concentrations (ppm) in cheeses using different coagulants1

	CHY-MAX® M 1000 (o^2) n = 9	CHY-MAX® Plus ($+^2$) n = 9	HANNILASE® XP 750 NB ($++^2$) n = 9
Histamine	10.59 ± 0.18	10.75 ± 0.30	11.23 ± 1.28
Tyramine	ND	19.38 ± 0.27[*]	20.38 ± 2,06[**]
Cadaverine	13.55 ± 0.54	13.27 ± 0.82	14.71 ± 4.28
Putrescine	15.40 ± 0.79	15.46 ± 0.52	16.12 ± 3.78
Spermidine	20.45 ± 0.89	20.05 ± 0.77	21.69 ± 2.99

[1]The data are the mean ± standard deviation. [2]Proteolytic activity: o = low, + = moderate, ++ = high
n = number of values being averaged, [*] n = 5. [**] n = 3, ND = not detected
The means in the same row that do not share the same superscripts differ at $p<0.05$ using Tukey's HSD (for homogeneity of variances) or Games-Howell (for inhomogeneity of variances) multiple comparison.

The data on the mean content of histamine, tyramine, cadaverine and putrescine in cheeses ranges between 20.9 and 62 mg/kg, 68.5 and 104 mg/kg, 72 and 109 mg/kg and 25.4 and 65, respectively (BIOHAZ, 2011). In this study, even high proteolytic moulds and rennets produced relatively low levels of biogenic amines. As cadaverine, putrescine and alcohol consumption or intake of certain drugs may exert a potential effect (Chu & Bjeldanes, (1981), Sattler et al., (1988), Sessa et al., (1984)), even low levels of histamine and tyramine may

provoke adverse health effects. Therefore, the biogenic amine concentration in food should be as low as possible.

CONCLUSIONS

The results from this study show that the *Penicillium candidum* strains PCA-1 (o) and PCA-3 (+) reduced the spermidine concentration in camembert cheeses because they produced significantly lower levels compared with TT-033 (++). Using PCA-1 (o) increased histamine accumulation compared with TT-033 (++). In general, the levels of biogenic amines were relatively low. The selected production and characteristic features of camembert cheese did not create an environment favourable for biogenic amine formation. To better understand the relationship between proteolytic activity in starters and clotting agents as well as biogenic amine accumulation, further research should be performed using cheese with longer maturation times.

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DYNAMIC NETWORK ANALYSIS: NEW RISK ASSESSMENT METHODOLOGY FOR FOOD CHAIN SAFETY

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SUMMARY

The Hungarian cattle production and export has been an important part of national economy since five centuries. The traditional static views of the displacement network hide important patterns of structural changes affecting nodes 'centrality and farms' spreading potential, thus limiting the required information for the effective risk assessment. Taking advantage of the network science approach, here we are able to the first time to analyse and display temporal data of movements of Hungarian cattle. The processing of such high quantity of data demanded the application of sophisticated network science tools and algorithms. By fully taking into account the temporal changes of nodes and edges, that is able to uncover the development of the Hungarian cattle network and reveal on mechanisms. Our aim has been to establish such a data-based methodology, which is able to determine the most vulnerable parts of a cattle farm network, to work out an optimised strategy of the inspection of herds, control of cattle-traffic as well as strategies in case of epidemiological crisis situations.

INTRODUCTION

The globalization, particularly its sociological and commercial aspects has started the research of complex networks in the late 1990's (Baranyi et al., 2013). The pioneers of the graph theory represented the nodes and the edges as in the sociology the individuals and their relationships (Stanley and Katherine, 1994; Salathé and Jones, 2010), genes / proteins and their interaction with each other in molecular biology (Barabási and Albert, 1999), the companies and business relationship in the economy (David and Douglas, 1992). It turned out that the structure and evolution of the networks shows many similarities regardless of what they represent.

The network science may have important role in the food science, microbiology and generally bringing security of food supply as well. The results of the network theory can be used in prevention and in the risk-based control and monitoring systems, of the companies for example, by their commercial relations with each other (Chmiel, 2007).

The best-known application of network theory methods in food safety issues is analysis of the viral or bacterial diseases resulting from the transport of infected / contaminated animals and plants. *Lentz et al. (2011)* explored the pig transport routes in Germany and they were able to show the hubs where the cross infection is more likely.

Nepusz et al. (2009) analysed the alerts data of the Rapid Alert System for Food and Feed (RASFF). They inferred patterns with network science methodology and they used the model to forecast as well.

The part of national and international risk analysis and assessment make a connection between the food chain safety measures and public health outcomes and expectations. It is essential to ensure the chemical and microbiological safety of our food, while maintaining the sustainability of its production, trade and distribution. Food chain safety measures should be increasingly objective, scientifically based, for which a risk based approach is needed. In most cases, we are using already existing international risk assessment, risk ranking and risk based priority setting studies, literature data, but the data needed for the substantiated risk assessment are in many cases not available. The lack of data or possible delays in providing updated records may hinder their use, especially for time-varying patterns (Szeitzné, 2008; Valdano et al., 2015). In the traditional risk based planning approach the calculations are based on severity of the hazard and the probability of occurring, however the effectiveness of this targeting mechanism can be questioned.

With application of network analysis tools it is possible to add a new layer of information to the assessment methodology, resulting in different – and presumably more realistic – outcomes.

MATERIAL AND METHODS

Database

The cattle trade network is obtained from the database of the national cattle identification system (ENAR). This system is able to follow the animals along the whole life cycle, from birth to slaughterhouse or from entering to the territory of Hungary to exporting. In this way a continuous dataflow is generated, supplying each day more than 1000 lines of raw data. Each movement record reports the unique identifier of the animal, the codes of the holdings of origin and destination, and the date of the movement.

The cattle holding network has been modelled with those available data on the basis of a network-analysis approach, because this interdisciplinary field of science considers the relationships between organisations as a graph (Albert and Barabási, 2002; Barabási, 2011). This graph G=(V,E) consists of a set of vertices (V) and a set of edges (E) (Tichy et al., 1979). In this case the vertices are (1) the cattle-exporters to Hungary; (2) the importers, buying living cattles from Hungary and (3) the various economic organizations (including farms, slaughterhouses, markets, distribution centres etc.). The edges of network are the cattle-flows between vertices. We consider animal movements during a 3 year time period, from 2012 to 2014, involving about 50 000 premises. The above mentioned trade routes represent approximately 510 000 movements a year. In the network the nodes may be active or inactive depending whether farms sell/buy cattle in a given timeframe, so if the quantity of the animals on the farms is zero, than it is inactive node in the graph.

The static network was broken down into monthly representations to analyse the behaviour of the network over time. The basic network parameters were calculated for each month, resulting in a dynamic network.

Network analysis

The network of cattle holdings and movements were first analysed to investigate the type of structure and to calculate the main parameters. For each node the following measures were calculated:

The node degree is the number of relations / edges of the nodes. However, in the case of the directed networks the in-degree and the out-degree values are important as well. Degree has generally been extended to the sum of weights when analysing weighted networks and labelled node strength, so the weighted degree and the weighted in- or out degree was calculated as well (Barrat et al., 2004; Newman, 2001; Opsahl et al., 2010).

In certain networks the nodes with the most important role are the high degree nodes (hubs). However, this is quite simplistic approach and if the network has a strongly inhomogeneous structure (clusters structures to show), it is certainly false: nodes with low degrees, but connecting the clusters in many cases play important role in the network (Jon, 1999).

The HITS algorithm was developed by Jon (1999). This algorithm is a link analysis algorithm that helps in identifying the essential nodes in a graph. It consists of two scores, a hub score and an authority score. The authority score of a node is a measure of the amount of valuable information that this node holds. The hub score of a node shows how many highly informative nodes or authoritative nodes this node is pointing to.

So a node with a high hub score shows that this node is pointing to many other authoritative nodes. On the other hand, a node with a high authoritative score shows that it is pointed to a large number of nodes, thus serves as a node of useful information in the network.

Betweenness centrality is even more an important statistical property of a network. This is applied in a lot of real-world problems, such as finding influential people in a social network, finding crucial hubs in a computer network, finding border crossing points which have a largest traffic or trade flow. The betweenness centrality of a node is an indicator of its centrality or importance in the network. It is described as the number of shortest paths from all the vertices to all the other vertices in the network that pass through the node in consideration. (Brandes, 2001).

We have used Gephi open-source software for network visualization and analysis, making possible to use a lot of algorithms and models, moreover, it is designed to automatically getting the list of plugins available from the Gephi Plugin portal (Devangana, 2015).

RESULTS AND DISCUSSION

The various statistics algorithms of the software tool provided access to characterization of the cattle movement system, exploring both its structural and dynamical properties. We had the opportunity to compare the calculated values in monthly breakdown and with this we could unveil the central farms, logistics centres or slaughterhouse and the peripheral holdings as well. There is a big difference between Betweenness Centrality, Authority and Hub Centrality: in case of the two latter a central node can be any node in the network, but in the case of betweenness centrality (as the name indicates) the central nodes can not be source-vertex or sink-vertex[3] (Okoth and Wagner, 2009). The vertices of high betweenness centrality value are usually logistic centres, transloading places or major livestock farms. These nodes have important role in epidemiological investigations, because of the high risk of cross-infections.

CONCLUSIONS

The globalization, the data explosion, the fast changing trade routes and food technologies are the important drivers which inspire us to develop new lines of the field of food chain safety and new risk assessment methodology (Ercsey-Ravasz, 2012).

There is a strong need for an interdisciplinary approach to monitor, understand, and control the trade-flow in the food chain. Such an approach would facilitate a better mapping of the animals or the plants, especially if it is broken down into time-scales, varieties or values of nodes (Wilkinson et al., 2011). The movements of cattle are increasingly becoming available thanks to monitoring and tracing systems put in place in the European Union. By using the approaches and techniques of network science, it is possible to analyse the dynamical system of cattle movements, going beyond static and simple approximations (Bajardi et al., 2011; Natale et al., 2009). This study opens the road to future work in several directions.

This would: 1) contribute to be able to determine the most vulnerable parts of a cattle holding network; 2) increase effectiveness of the control the cattle-flow; 3) reveal the interdependencies; 4) help in working out an optimised strategy of the inspection of herds; 5)increase preparedness against outbreaks and intentional attacks;

6) enhance epidemiological modeling simulations and 7) provide information on the source of possible infections so that preventive and control measures can be applied (Caporale et al., 2001).

[1]: source vertex is a node with in-degree zero, while a sink vertex is a node with out-degree zero

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METABOLIC PROFILING AND ALLERGENIC PROTEIN INVESTIGATION OF DIFFERENT TOMATO VARIETIES

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SUMMARY

Tomato (Solanum lycopersicum) is a popular and widely consumed vegetable, since it is cultivated worldwide. During the evaluation of breeding materials not only yield and agro-technical properties are important. Crop breeding involves wild and related species as source of resistance genes that can raise questions about the safety of consumption. For this reason the investigation of bioactive metabolites and allergenicity is crucial. This work was conducted to investigate the differences in the metabolite profile and allergenic proteins of different tomato lines, varieties and hybrids by GC-MS, HPLC and spectrophotometric methods, and tools of proteomics. Targeted metabolic profiling was used to analyse vitamin C, carotenoids, tocopherols, phenolic compounds and to measure the antioxidant capacity and total phenolic content. Determination of IgE reactivity was carried out by Western blotting and immunosensor. The aim of this study is to create a metabolomical database for various genotypes by applying validated methods of metabolomics and proteomics.

INTRODUCTION

Tomato (*Solanum lycopersicum*) is cultivated worldwide both for fresh market and processing industries. The contribution of the European Union is 15,338 thousand tons per year, where largest producers are Italy and Spain. Tomatoes contain important vitamins (C, E, pro-A, group B) and minerals (K, Mg, P) and have a low calorie content (23 kcal/100 g), whilst their protective metabolites (especially lycopene) provide protection against cardiovascular, inflammatory and cancer related diseases (Hazewindus, 2012). Climate change poses a new challenge to the realization of agro-technical requirements, while the quality of crops and their nutritional value also need to be paid attention to. Traditional plant breeding uses wild and related species as a source of genes. Along with the resistance genes, other chromosome regions coding potentially toxic metabolites or allergenic proteins can be transferred to the new varieties. Determination of health related metabolites and allergenic proteins is an important task, especially in the case of tomatoes, which are usually eaten fresh without any heat treatment. The prevalence of tomato allergy ranges from 1.5% (Northern Europe; Petersen, 1996) up to more than 16% (Italy; Ortolani, 1989), and may cause serious health risks for susceptible individuals. Several tomato allergens' sequence show homology with other allergens' sequences thus arises the possibility of cross-reactivity. Investigating allergenic proteins is a challenge, not only because of low protein contents or the instability of these macromolecules, but differences between human IgE levels and reactivity, and the non-specific binding of the primary (CCDs) or secondary antibodies are also causing difficulties.

MATERIAL AND METHODS

In the first two years of the project (2014-2015) five tomato varieties, sixteen lines and hybrids from 20 to 140 g fruit size representing processing and fresh market types were involved from field and plastic house cultivation. Genotypes were also grouped to few to

multiresistant by carrying resistances to *Verticillium dahliae*, *Fusarium oxysporum* f. sp. *lycopersici*, *Pseudomonas syringae* pv. *tomato*, Tomato Mosaic Virus or root knot nemathodes.

Metabolomics

The metabolic profiling was carried out by validated methods (Table 1).

Table 1.: Methods used for bioactive metabolite determination

	Method
Vitamin C, carotenoids (HPLC)	Abushita et al. 2000, *J Ag Food Chem*, 48.6. pp. 2075-2081.
Vitamin B_5, B_6 (GC-MS)	Juhász et al. 2014, *Plant Breeding*, 133.4. pp. 515-520.
Tocopherols (HPLC)	Speek et al. 1985, *J Food Sci*, 50.1. pp. 121-124.
Phenols (HPLC)	Gómez-Romero et al. 2010, *Phytochemistry*, 71.16. pp. 1848-1864.
Total polyphenol (spectrophotometry)	MSZ 9474-80
Antioxidant capacity (spectrophotometry)	Brand-Williams et al. 1995, *Lebensm-Wiss Technol*, 8.1. pp. 25-30.

Proteomics

Samples of four tomato varieties have been peeled and *granum*, *pericarpium* and *mesocarpium* tissues were freeze-dried separately. Extraction was carried out with 1% NaCl solution for 1.5 h with gentle agitation. Total salt-soluble protein content was measured with Qubit fluorometer (Invitrogen). Proteins were separated on 15% gel using sodium dodecyl sulphate poliacrylamide gel electrophoresis. Gels have been stained with Coomassie Blue and detection of glycoproteins was conducted by Schiff staining (Zacharius, 1969). To examine antigen-Human IgE binding, Western blotting was implemented using anonym human tomato sensitive sera and horseradish peroxidase conjugated secondary antibody. Soy allergic anonym human serum was used as a negative control. Proteins for the immunosensor have been purified from *granum* extract by ion exchange fast protein liquid chromatography (IE-FPLC) using EnRich Q column and NGC Quest 10 plus instrument (BioRad). Deglycosylation was carried out by the method Woodward et al. (1984). Optical Waveguide Lightmode Spectroscopy (OWLS) technique was used for label-free, highly sensitive determination using OWLS 120 Model (Microvacuum) and integrated optical waveguide sensors (chips) type of OW2400. The systems based on immunoreaction generally rely on highly selective and sensitive devices to translate the biological recognition process (antibody–antigen binding recognition) into a physical magnitude variation in real time. Silanization and immobilisation of purified antigen was carried out by the method of Adányi et al. (2006). For negative control anti-bovine serum albumin and soy allergic human serum were used.

RESULTS AND DISCUSSION

Metabolomics

Significant differences were found between plastic tunnel and open field tomato samples in concentration of bioactive components. The open field tomatoes contained higher values of vitamin C, B_6, antioxidant capacity and phenolic components. There were no correlation between growing technology and the concentrations of tocopherol derivatives, carotenoids and vitamin B_5. The quantity of these components was regulated by genotype. Vitamin C content of fruits was strongly correlated with total phenolic content supporting previous

findings (Guorong, 2009). As we expected, crop technology, size of tomato fruits and the application of technology could be well differentiated by discriminant analysis. Smaller fruits showed higher levels of vitamins and other metabolites because of their relatively higher ratio of skin and dry matter contents. In case of open field cultivation the bigger impact of environmental parameters under maturation resulted in higher antioxidant capacity. Results suggest that multivariate analysis of metabolites can differentiate genotypes carrying different disease resistances indicating that breeding can cause significant changes in plant metabolism (Figure 1).

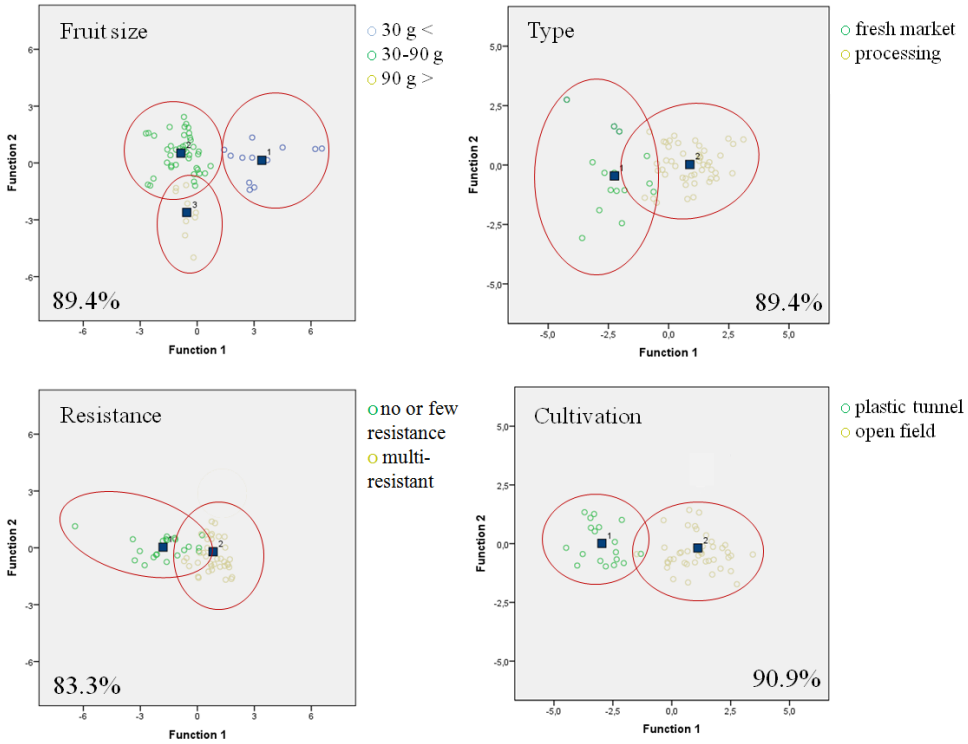

Figure 1.: Multivariate analysis of the measured metabolites

Proteomics

Total protein contents of the *granum* tissues were significantly higher in every variety, followed by *pericarpium* tissue. Separation of proteins on SDS-PAGE revealed protein bands in the molecular weight region of well-known tomato allergens. By visual inspection of the gel, a 50 kDa band was selected for isolation by FPLC from the salt soluble fraction of the granum as it had the highest protein content. After Schiff staining, several glycoproteins were detected, mainly in *pericarpium* and *mesocarpium* extracts. After SDS-PAGE separation of proteins from different extracts (*pericarpium*, *mesocarpium*, *granum*) Western blotting was carried out with four different tomato sensitive human sera, a negative control serum and without serum (only secondary antibody). Results showed different sensitivisation between positive sera, and non-specific binding of the secondary antibody was observed mainly at the 50 kDa molecular weight region in *granum* extract. The Soy allergic anonym human serum used as negative control also showed (although weaker) antigen binding properties.

To check specific antibody-antigen binding OWLS based immunosensor measurements were implemented. The isolated 50 kDa *granum* protein was immobilised. Comparing the anti-human IgE with negative anti-bovine serum albumin, the anti-human IgE provided higher signal, meaning stronger binding capacity (Figure 2). Comparing positive sera with negative control, we also detected differences between the initial reaction rates, indicating stronger

binding with positive sera. Western blotting was repeated with deglycosylation of the proteins. A *pericarpium* originated glycoprotein at 23 kDa was not immunoreactive after deglycosylation, while it showed strong immunoreactivity in glycosylated form.

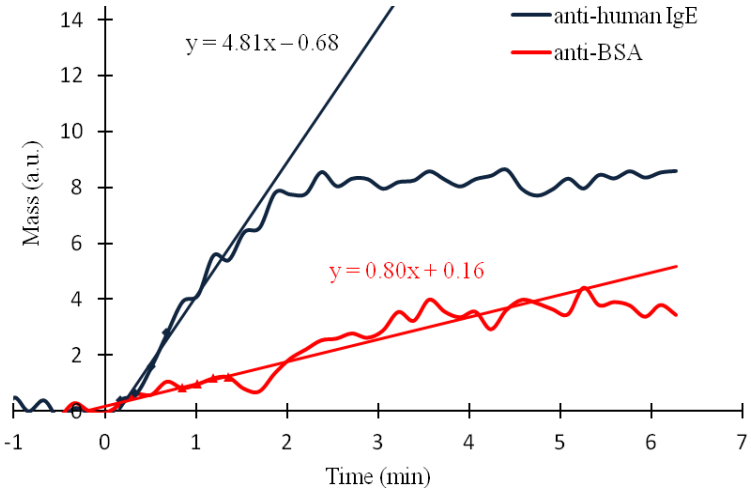

Figure 2.: Differences in initial reaction rates between secondary antibody (anti-human IgE) and negative control (anti-BSA) measured by OWLS immunosensor

CONCLUSIONS

Our methods proved to be accurate and reliable for determining health related metabolite groups from tomato. The attained databank could play an important role in the future, helping the process of selecting new varieties. Cherry type tomatoes and open field cultivated varieties are considered better for fresh consumption, since the higher vitamin and phenolic content. During the investigation of allergenic proteins the main conclusion is that new methods should be implemented to prevent false positive results in Western blotting, which occur by the non specific binding of the secondary antibody. By creating a reliable method there would be no more false positive diagnosis and the eating habits of the patients need not to be drastically changed by total avoidance.

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PREPARATION OF APPLE PÁLINKA BY CERAMIC PERVAPORATION MEMBRANE

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SUMMARY

This study was based on the production of apple pálinka using laboratory pervaporation equipment. Hungarian fruit brandy is called pálinka, which can be made by pot distiller or multistage distiller made of copper. In case of traditional pot still distillation the final product is gained from two separate distillations. Pervaporation is an energy efficient membrane process for separating liquid mixtures. Application of pervaporation to separate the product of the initial distillation leads to lower energy consumption than using double-distillation process. The aims of our experiments were to examine applicability of pervaporation in reference to the preparation of apple pálinka and to compare the products of traditional distillation and pervaporation processes.

INTRODUCTION

The traditional alcoholic beverage of Hungary is called pálinka. The production of this Hungarian speciality is regulated by European Union. Pálinka is a fruit brandy which is made exclusively from fruit grown in Hungary. The fermentation of fruits, distillation and bottling are also done in Hungary. Pálinka can be prepared by two methods of distillation. In case of traditional pot still the mash is boiled to obtain the alcohol and other volatile components through distillation. The alcoholic liquid obtained is refined in a separate phase. Using column distillation apparatus boiling and refining proceed at the same time in a single process called rectification (Harcsa et al, 2014). The major disadvantage of double-distillation and rectification process is the high operating temperature, which causes high energy consumption. Pervaporation is a membrane technique for the separation of liquid mixture by selective transport through a non-porous membrane (Pereira el al, 2006). The application of pervaporation in food industry have been investigated by several researchers as *aroma compounds recovery or concentration in fruit juices (Álvarez et al, 2000, Lipnizki et al, 2002) and dealcoholisation of wine or beer (Takács et al, 2007).* However, the capital cost of pervaporation process can be higher than distillation; pervaporation has many advantages such as high selectivity, low energy consumption and minimum loss of aroma compounds (Isci et al, 2006). The aroma of alcoholic beverages consists of a large number of volatile organic compounds at ppm concentrations. Pervaporation is suitable for the recovery of ethanol and other volatile aroma compounds from aqueous mixtures (Tan et al, 2005). The separation is not based on relative volatilities, as in the case of distillation, but rather on the relative rates of permeation through the membrane (González-Rodríguez et al, 2003). Combining pervaporation and distillation could be a promising hybrid process for substituting double-distillation technique. *The aim of our work was to develop an alternative technology for the production of pálinka which integrates distillation and pervaporation.*

MATERIAL AND METHODS

In our study an apple spirit made by pervaporation was compared with a double-distilled apple pálinka prepared from the same raw material. Fermentation and distillation processes were carried out at the Department of Brewing and Distillation, Corvinus University of Budapest. The double-distilled spirit was produced in pot still by redistilling the product of the first distillation. The pervaporated apple spirit was prepared from the same initial distilled

spirit using laboratory pervaporation equipment. Pervaporation process was performed at the Department of Food Engineering, Corvinus University of Budapest.

The separation of the first distillation was operated by ceramic tube membrane using vacuum pervaporation method. The commercial membrane (Pervatech) with PDMS active layer has an effective membrane area of 0.011 m^2. In the experiment, the feed temperature was maintained at 50 °C and the feed flow-rate was kept at 400 L/h. The pressure on the feed side was atmospheric, and the pressure on the permeate side was 20 mbar during the separation process. Permeate is collected in traps, which at laboratory scale are normally cooled by using liquid nitrogen (-196°C). All samples (permeate of pervaporation process, the first and second distillates) were analysed by GC/MS at Bálint Analytics. The major volatile compounds, beside ethanol, identified and quantified were methanol, 1-propanol, 1-butanol, iso-butanol, 1-hexanol, ethyl acetate, ethyl hexanoate, ethyl octanoate, ethyl decanoate, and acetaldehyde.

RESULTS AND DISCUSSION

The ethanol content of distilled and membrane separated spirits are shown in Table 1. The doubled-distilled apple pálinka consisted of only the middle distillate had an ethanol content of 79.3 % (V/V). The separation of the first distillate by pervaporation membrane leads to an ethanol content of 49.9 % (V/V).

Table 1 Concentrations of pineapple aroma compounds in model solutions

	Initial distillate	Pot still distillate	Permeate
Etanol content (% V/V)	21.7	79.3	49.9

The methanol concentration (figure 1) of all investigated alcoholic samples not exceeding the permitted levels, furthermore the permeate methanol concentration is less than methanol content of real apple pálinka. From the results, it can be concluded that the applied PDMS membrane has very low methanol selectivity. Regarded to the analysis of methanol content we can say that pervaporation appears to be a promising technique to refine the first distillate.

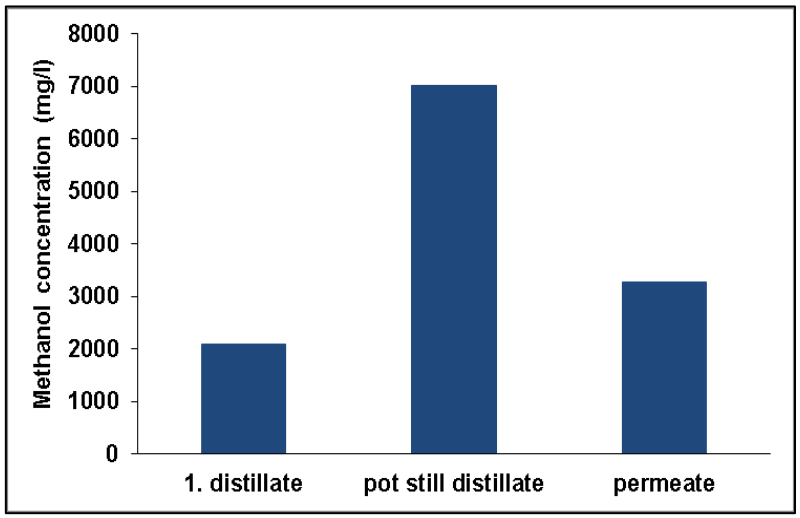

Figure 1: Concentration of methanol in the distilled and pervaporated spirits

Figure 2 shows the amount of higher alcohols such as 1-propanol, iso-butanol, 1-butanol and 1-hexanol. In case of pot still distillation these components are found higher

concentrations in the final fraction because of their higher boiling point than ethanol. The enrichment of these alcohols by pervaporation has a negative influence on the aroma of the final spirit.

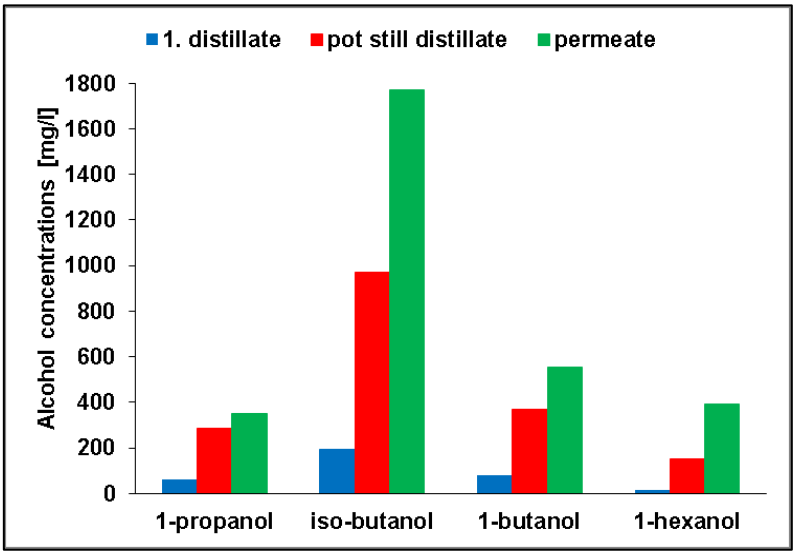

Figure 2: Concentrations of the other alcohols in the distilled and pervaporated spirits

Acetaldehyde and ethyl acetate are presented in higher concentration in the first fraction during batch distillation. It can be seen on figure 3 that concentrations of both compounds are much higher in pervaporated spirit than real apple pálinka. Concentrations of the other esters (figure 4) are also higher in permeate than double-distilled spirit leads to more intensive fruit flavour.

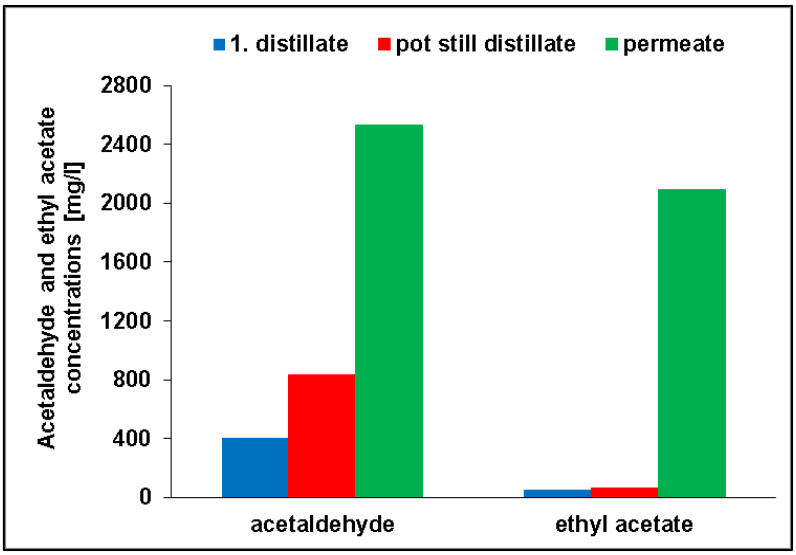

Figure 3: Concentrations of acetaldehyde and ethyl acetate in the distilled and pervaporated spirits

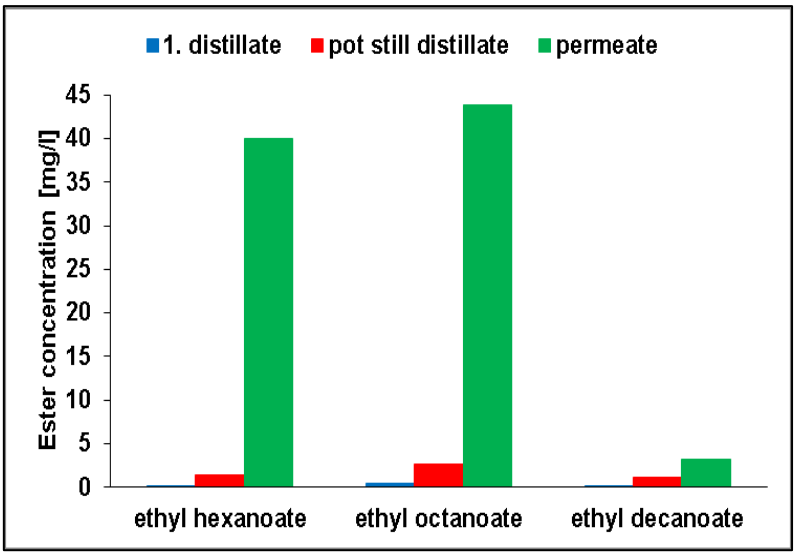

Figure 4: Concentrations of the other esters in the distilled and pervaporated spirits

CONCLUSIONS

As a conclusion it can be revealed, that the applied membrane is suitable for the separation of ethanol and the other volatile components from the first distillate. On the other hand, pervaporation is not capable to refining the initial distillate leads to unpleasant tastes and odour in permeate. Using another pervaporation membrane with lower selectivity of harmful volatile components is needed to produce alcoholic beverage with a pleasure fruit flavour and smell.

ACKNOWLEDGEMENTS: *The authors would like to acknowledge the financial support of the Hungarian National Science Foundation (OTKA CK-81011) and Hungarian Research Institute of Organic Agriculture (ÖMKI).*

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EXAMINATION OF BIOACTIVE COMPONENTS AND AROMA COMPOUNDS IN PAPRIKA POWDERS FROM KALOCSA

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SUMMARY
Five varieties of paprika were investigated to examine the effect of post-ripening and storage. The paprika varieties were taken from Kalocsa and were the following: Kaldóm, Szegedi 178, Szikra, Delikát, Szegedi 80. The samples were prepared as paprika powder with and without stalk. The bioactive components and aroma compounds were determined. The amount of carotenoids, tocopherols and ascorbic acid were detected by HPLC and aroma compounds by GC-MS. The concentration of carotenoids was higher in paprika powders without stalk. Considering tocopherol and ascorbic acid values the same tendency can be observed. The amount of carotenoids and tocopherols significantly increased in post-ripened paprika. In paprika powders without stalk more aroma components were detected. The effect of post-ripening on flavours was examined.

INTRODUCTION
Paprika powder, as natural source of carotenoids, is one of the most familiar natural colourant. Spice red pepper (*Capsicum annuum* L.) is one of the hungaricum products. Because of its colour intensity and aroma profile is excellent, there is increasing demand for Hungarian paprika in world markets. There are some features about the examined paprika varieties: Delikát F1 (D) is a sweet, bacterial leaf spot resistant variety, with semi-determinate growth habit and erect fruits. The fruits are suitable for producing high quality paprika powder. Kaldóm (K) is a sweet, bacterial leaf spot resistant variety with semi-determinate growth habit and erect fruits. This cultivar needs shorter vegetation period and suitable for direct seeding. The fruits has special aroma so this variety is recommended to produce high quality paprika powder. Szegedi 80 (Sz80) is a sweet variety with indeterminate growth habit and pendulous fruits. Nowadays this is the most common growing cultivar. Szegedi 178 (Sz178) is a hot variety with indeterminate growth habit and pendulous fruits. Recently this is the most common growing cultivar for producing hot paprika-cream but it is also suitable for producing hot paprika powder. Szikra F1 (Sz) is a hot, bacterial leaf spot resistant hybrid variety, with indeterminate growth habit and pendulous fruits. The fruits are as good for producing hot paprika-cream as for producing hot paprika powder.

MATERIAL AND METHODS
Samples

The study was carried out from the most grown five paprika varieties, concerning the effect of the post-ripening phase and the presence of stalk (S) in paprika powders on the quality during a three-month storage period. The paprika powders prepared from freshly picked and post-ripened fruits and the same samples after storage were studied and the results compared.

Determination of carotenoid content

Half gram of ground spice paprika sample was extracted by 50 ml of 1,2-dichloroethane-acetone-methanol (2:1:1) mixture for 15 min and filtrated. After evaporation of solvent under vacuum the residue was re-dissolved in 10 ml of isopropanol-acetonitrile-methanol (55:35:10) mixture (Biacs and Daood, 1994). The measurement system consisted of Waters 2695 HPLC with 250 x 4.6 mm, 3 µm Nucleosil C-18 column, 2996 Photodiode Array Detector (470 nm) and gradient elution was applied.

Determination of vitamin C

One gram of ground spice paprika was mixed with 25 ml of 3% meta-phosphoric acid solution, shaken for 15 min at 20 °C, filtered and cleaned up with 0.45 µm filter (Chromafil A-45/25, Cellulose mixed esters) before injection. The measurement system consisted of Agilent Technologies 1200 Series HPLC instrument with Nucleosil C-18, 150 x 4.6 mm, 3 µm column, Diode Array, Multiple Wavelength Detector SL (265 nm) and gradient elution was applied.

Determination of tocopherol content

From 0.5 g of ground paprika tocopherols were extracted with 5 ml 30% KOH/MeOH, 0.5 g ascorbic acid and 20 ml methanol (35 min under reflux, Speek et al., 1985). After cooling, the tocopherol fraction was extracted twice by 40 ml n-hexane. The hexane fractions were pooled and washed with distilled water (3x), dried over anhydrous Na_2SO_4, evaporated and re-dissolved in 5 ml of n-hexane (Daood et al., 2014). The measurement system consisted of Jasco HPLC (880-PU) instrument (Shimadzu C-R6A Integrator) with Nucleosil-100, 250 x 4.6 mm, 5 µm column, RF-535 Fluorescence HPLC Detector (295 nm and 320 nm) and isocratic elution of n-hexane-ethanol, 99.6:0.4 was applied.

Analysis of paprika aroma

One gram of ground paprika was placed in capped headspace vial (40 ml), temperated at 50°C for 30 min, then the SPME needle (100 µm PDMS coated fused silica fibre, Supelco) was exposed at 50°C for 30 min. Volatiles were desorbed at 250°C in the GC injection port and flushed into the GC column (Mazida et al., 2005). HP 5890/II gas chromatograph (Hewlett Packard) equipped with a 30 m x 0.25 mm x 0.25 µm RH-5ms+ capillary GC column and 5971 MS detector were used to analyse the volatile compounds of the red paprika powders. The detection was performed in the 35-350 mass range (Csóka et al., 2013). Compound recognition was based on mass spectra identification spectrum-library (Wiley275.L spectrum library) after individual background correction.

RESULTS AND DISCUSSION

Total carotenoid content in paprika samples

The content of total carotenoids was significantly increased during post-ripening, but during storage it was decreased in all samples. Due to the presence of stalk the concentration was lower with about 10%. During post-ripening the amount of total carotenoids was increased with about 10% in Kaldóm and Szegedi 178 samples. The lowest total carotenoid content was found in fresh Szikra stalk sample (2477 µg/g). After post-ripening, the concentration of total carotenoids was the highest in the Szegedi 80 (5272 µg/g). While the total carotenoid content in post-ripened stored samples decreased with 12%, and in fresh stored samples with 30% (Fig 1).

In each samples the concentration of free capsanthin was greatly decreased during post-ripening and storage, however the total amount changed less. Among fresh samples Szegedi 80 had the highest (286 µg/g), while Delikát had the lowest (180 µg/g) free capsanthin content.

After post-ripening the concentrations changed to 248 and 144 µg/g, respectively. During storage the free capsanthin content further decreased, so in fresh stored Szegedi 80 and Delikát it changed to 201 and 151 µg/g, and the post-ripened stored samples contained 191 and 118 µg/g, respectively. The rate of free capsanthin concentration decrease was the lowest in all Szikra paprika powder (Fig 2).

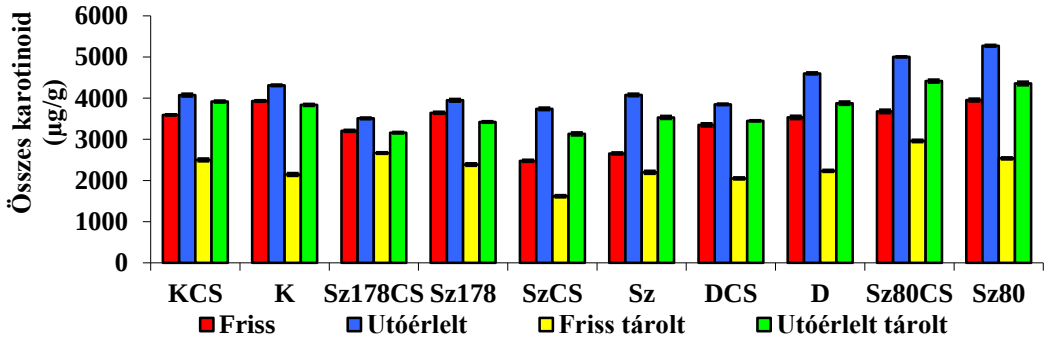

Figure 1. Total carotenoids content of paprika powders

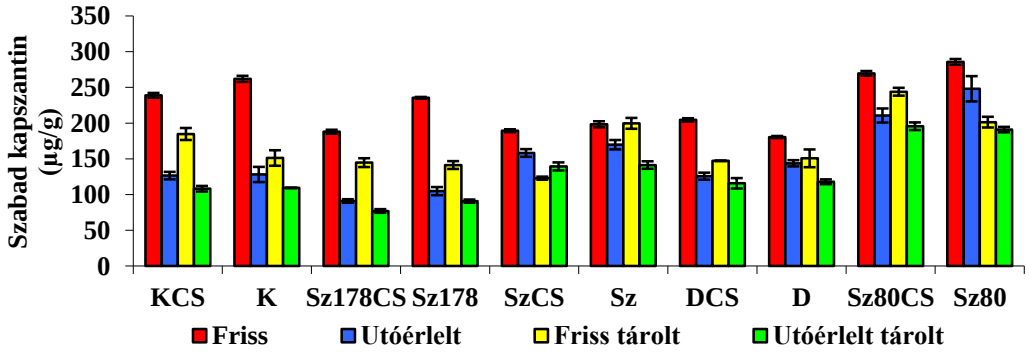

Figure 2. Free capsanthin content of paprika powders

Vitamin C content in paprika samples

During post-ripening the vitamin C content of red paprika fruit was greatly decreased, while during storage only a slight decrease could be observed. As the highest, the quantity of vitamin C was 8548 µg/g in the fresh Kaldóm and 7438 µg/g in the fresh Delikát. In the fresh-stored Szikra paprika powder the value of vitamin C was only 126 µg/g.

Total tocopherol content of paprika samples

Total tocopherol content of paprika powders showed more stability in the samples, than previously mentioned parameters. While during post-ripening it was increased slightly (~8%), during storage it was clearly decreased. The total tocopherol content in fresh stored samples showed higher rate of decrease (~27 %) than in the post-ripened stored ones (~8%, Fig 3).

Aroma compounds in paprika samples

In paprika powders without stalk more aroma components were detected than in paprika powders with stalk. During post-ripening the aromatic compounds were changed and it was further raised during storage.

In case of Szegedi 178 without stalk (Sz178) the following aroma components formed meanwhile post-ripening: 3-hydroxy-2-butanone, heptadecane, 3,8-dimethyl-decane, trans-caryophyllene, α-longipinene, alloaromadendrene, 2-propanone. β-selinene, aristolen, benzaldehyde, eremophilene, β-himachalene and 2-methyl-pentadecane. The ß-ionone terpene compound occured just in stored samples. In Sz178 we detected the α-longipinene and ß-himachalene sesquiterpene aroma components as this sample was a pungent paprika (Fig 4).

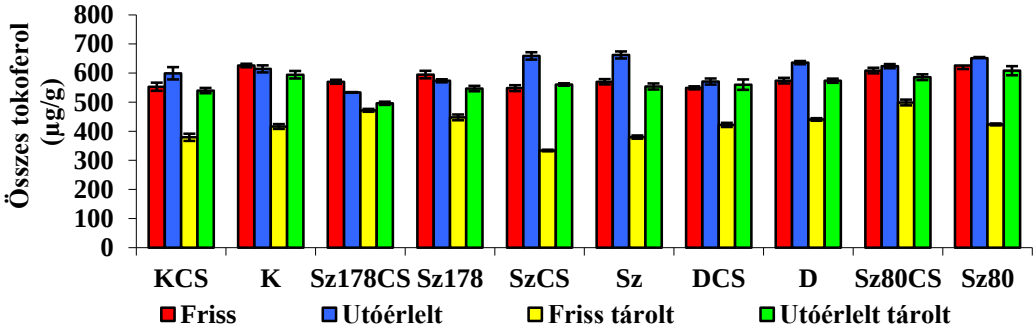

Figure 3. Total tocopherol-content of paprika powders

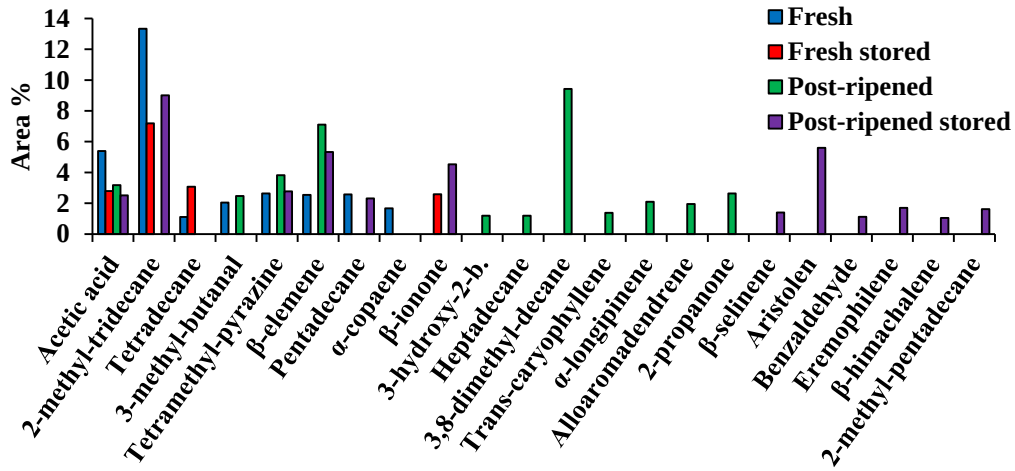

Figure 4. Aroma components in Szegedi 178 paprika powders (Sz178) measured with SPME GC-MS

CONCLUSIONS

According to our results Kaldóm paprika had the highest free capsanthin and vitamin C content but its aroma composition was the poorest. By contrast the highest level of aroma compounds was detected in Szegedi 178 pungent paprika. In case of Szikra pungent paprika the concentration of total carotenoid and vitamin C was the lowest. The Szegedi 80 paprika was outstanding in terms of total carotenoid and free capsanthin content. Among the 3 not pungent paprika (Kaldóm, Delikát, Szegedi 80) the Szegedi 80 was mostly outstanding as regards the concentration of bioactive components and among the 2 pungent paprika (Szegedi 178, Szikra) the Szegedi 178 was remarkable. The circumstances of post-ripening, processing and storage influence the bioactive components of spice paprika. Concerning the aroma composition, total carotenoids and total tocopherols content post-ripening is important.

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EXAMINATION OF PAPRIKA POWDERS OF DIFFERENT ORIGINS

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SUMMARY

In Hungary paprika cultivation expanded at the end of the 18th century, mostly in Szeged and Kalocsa areas. In our project, paprika powders from several countries were examined to find the most important differences between the characteristic properties of them. The measurements were carried out from Hungarian and foreign paprika powders (Serbian, Spanish, Chinese, Bulgarian, Peruvian). Bioactive components, microbiological and aroma properties of the samples were determined. The amount of carotenoids, tocopherols and ascorbic acid were determined by HPLC and flavouring properties by GC-MS. The highest concentration of carotenoids was found in samples from Peru and Spain, while the Serbian samples had the lowest values. Considering the tocopherol and ascorbic acid values, same tendency can be observed. The concentrations of total tocopherol and ascorbic acid were greater in Hungarian than in Chinese paprika. The microbiological purity of each sample was appropriate.

INTRODUCTION

In order to make the spices product chains safer, examinations were performed primarily for the quality control of paprika powders and determination of its origin. Measurements, concerning the statement of unique nutritional characteristics, were expanded to the determination of the concentration of bioactive components and aroma properties, as well. Bioactive components, microbiological and aroma properties were determined from Hungarian, Serbian, Spanish, Chinese, Bulgarian, and Peruvian paprika powders.

MATERIALS AND METHODS

Overall, 52 paprika samples were collected, taking care to include products of different origins and quality. The selected sample group contained Hungarian (22 from Szeged and Kalocsa) and foreign (5 Serbian, 7 Spanish, 14 Chinese, 2 Bulgarian and 2 Peruvian) paprika powders. Among the Hungarian paprika powders, there were samples with known origin and presumably mixed ones, too.

Half gram of ground spice paprika sample was extracted by adding 50 ml of a mixture of 2:1:1 1,2-dichloroethane-acetone-methanol, mixed for 15 min, and filtrated. After evaporation of solvent under vacuum, the residue was re-dissolved in 10 ml of mixture of 55:35:10 isopropanol-acetonitrile-methanol. A Waters Alliance HPLC (Model 2695 and Model 2996 Photodiode Array Detector, 470 nm) was used for the analysis of carotenoids. Data processing was performed by Empower software. Nucleosil C-18, 3 µm, 250 x 4.6 mm column with gradient elution (Daood, 2005) was used.

One g sample was mixed with 25 ml of 3% meta-phosphoric acid solution, shaken mechanically for 15 min at 20 °C, filtered, and cleaned up with 0.45 µm filter (Chromafil A-45/25, Cellulose mixed esters) before injection. Agilent Technologies 1200 Series high HPLC instrument (Quaternary Pump, Diode Array, Multiple Wavelength Detector SL, 265 nm) was used. The separation of vitamin C was performed on Nautilus Nucleosil C-18, 3 µm, 150 x 4.6 mm column with gradient elution.

From 0.5 g of ground paprika, tocopherols were extracted with 5 ml 30% methanolic KOH, 0.5 g ascorbic acid and 20 ml methanol (35 min under reflux). After cooling, the tocopherol fraction was extracted twice by 40 ml n-hexane. The hexane fractions were pooled

and washed with distilled water (3x), dried over anhydrous Na_2SO_4, evaporated, and re-dissolved in 5 ml of n-hexane. A Jasco HPLC (880-PU) instrument (Shimadzu C-R6A Integrator and RF-535 Fluorescence HPLC Detector, 295 nm and 320 nm) was used. Under normal-phased chromatographic conditions, the separation was performed on Nucleosil-100, 5 µm, 250 x 4.6 mm column with an isocratic elution of 99.6:0.4 n-hexane-ethanol.

One g of ground paprika was placed in capped headspace vial (40 ml volume), heated at 50°C for 30 min, then the SPME needle (100 µm PDMS coated fused silica fibre, Supelco) was exposed at 50°C for 30 min. Volatiles were desorbed at 250°C in the GC injection port and flushed into the GC column (Mazida, 2005). HP 5890/II gas chromatograph (Hewlett Packard) equipped with a 30 m x 0.25 mm x 0.25 µm RH-5ms+ capillary GC column and 5971 MS detector were used to analyse the volatile compounds of the red paprika powders. The detection was performed in the 35-350 mass range (Csóka, 2013). Compound recognition was based on mass spectra identification spectrum-library (Wiley275.L spectrum library) after individual background correction.

The analysis of mesophilic aerobic total count was performed according to the standard MSZ EN ISO 4833: plate pouring with Plate Count Agar (MERCK) and incubation at 30°C for 5 days. The analysis of mould and yeast counts were performed according to the standard MSZ ISO 7954: plate pouring with Chloramphenicol Glucose Agar (Biolab) and incubation at room temperature for 5 days.

RESULTS AND DISCUSSION

L-ascorbic acid is the most important water-soluble antioxidant in spice paprika products. The Hungarian samples had the highest level of vitamin C. Its value was 1353 µg/g (Fig. 1). Similar vitamin C content was measured from the Serbian samples. The concentration of vitamin C was low in the Spanish, Chinese, Bulgarian, and Peruvian paprika (160 µg/g), because these countries processed paprika in a different way.

The flesh and seeds of paprika contain considerable amounts of different homologues of tocopherol, the highly reactive components of vitamin E. The highest concentrations of total tocopherols were detected in Hungarian paprika powders (544 µg/g, Fig. 2). By contrast, the Bulgarian samples had only 399 µ/g, while the Serbian, Spanish, Chinese, and Peruvian samples contained about 470 µg/g. The α-tocopherol is the major tocopherol in the paprika fruit flesh, which is the biologically most active component of vitamin E. In the samples the average concentration of α-tocopherol was 403 µg/g. In the paprika seeds mainly γ-tocopherol can be found, which plays an important role in the stability of paprika products (Biacs, 1989). The value of γ-tocopherol is about 50 µg/g in each case of examined sample, which refers to the amount of paprika seeds in powders.

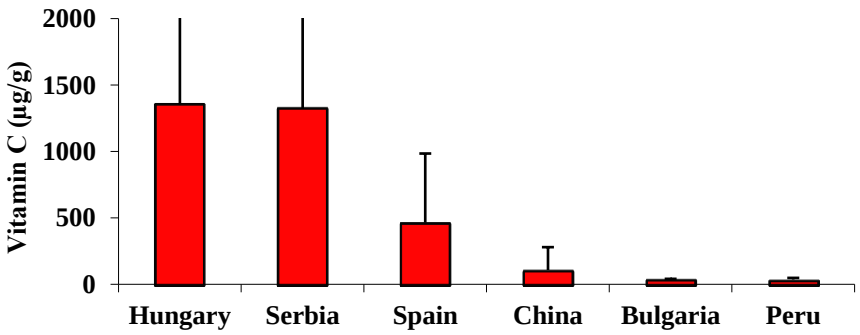

Figure 1.: Vitamin C content of paprika powders

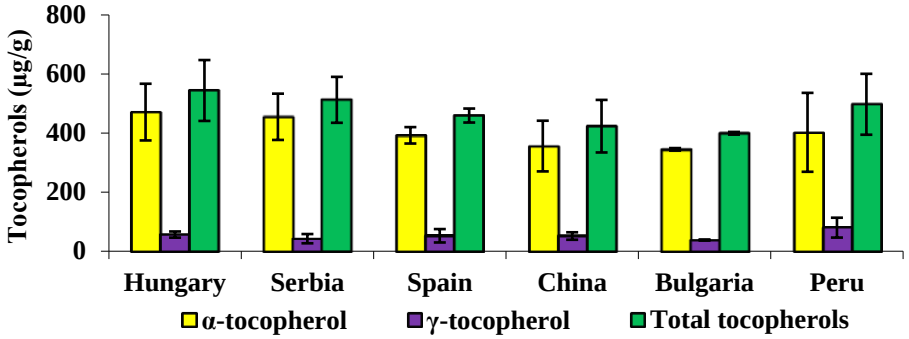

Figure 2.: Tocopherol content of paprika powders

Aroma components occurring in 3 or more paprika powders were β-elemene, trans-caryophyllene, α-muurolene, dihydroactinidiolide, 2,2,4,6,6-pentamethyl-heptane and β-ionone (Fig. 3). Acetic acid and neryl acetone were measured in each samples. The area% of neryl acetone was remarkable in all examined paprika powders. Only the Hungarian paprika powders contained 3-hydroxy-2-butanone, 6-methyl-5-hepten-2-one, and 2,3-butanediol. The Serbian samples contained α-terpinolene, I-phellandrene, and heptadecanoic acid. Linalool was detected in the Spanish paprika powders. In case of Chinese samples, 1,3-butanediol and δ3-carene were detected. 2-pentyl-furan, octadecanoic acid, methyl ester, and (+)-aromadendrene were only found in the Bulgarian samples. Only the Peruvian samples contained geranyl acetone (16.87 area%). In Hungarian and Spanish samples the area% of β-elemene was outstanding, 19.08% and 12.51%, respectively.

In the paprika powders the average number of Total Viable Count was $3.74x10^6$ cfu/g (Fig. 4). The average number of moulds was $7.22x10^3$ cfu/g, while of yeasts $1.42x10^3$ cfu/g. There was no significant difference between paprika powders regarding the microbiological load, all samples were appropriate.

CONCLUSIONS

In summary, the Spanish and Peruvian samples were outstanding in total carotenoids content. In Hungarian paprika powders the concentration of vitamin C and total tocopherols were high. Characteristic aroma components were detected to each paprika powder. The microbiological purity of each sample was appropriate. Based on our results, there was no clear correlation between the examined bioactive components and place of origin, so further studies are needed. In the future we are going to try to distinguish the paprika samples with the help of different statistical methods. The features of the terroir, the genotype, and the parameters of harvest, processing, and storage influence the bioactive components, aroma properties, and microbiological characteristics of paprika powders.

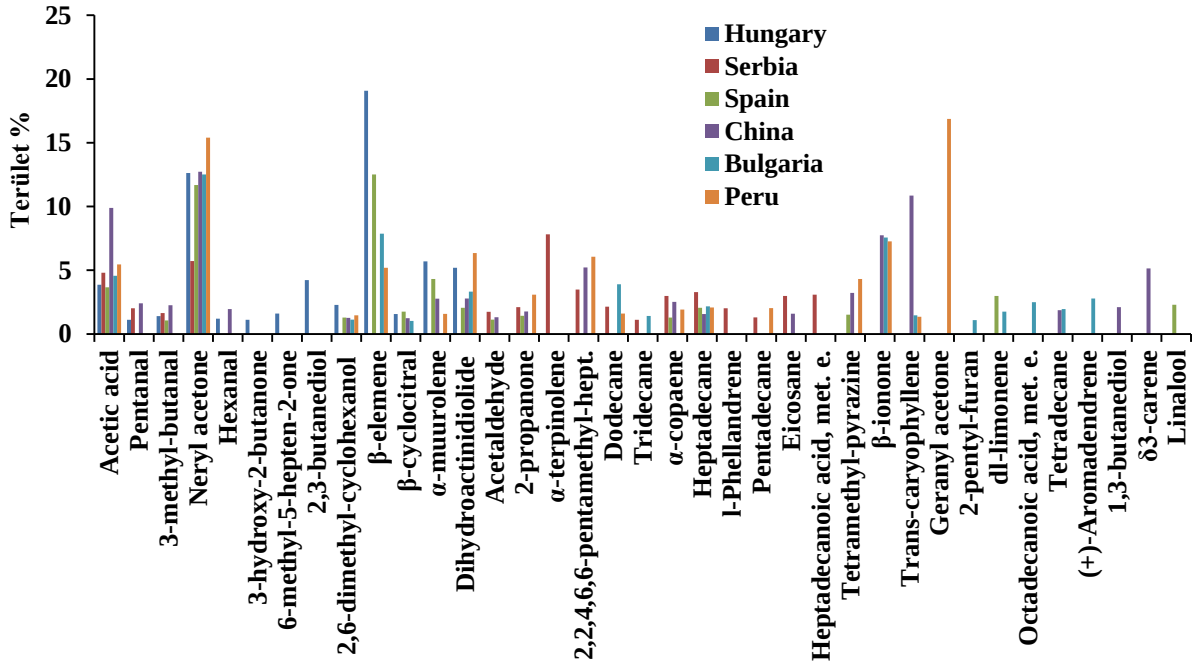

Figure 3.: Aroma components in paprika powders

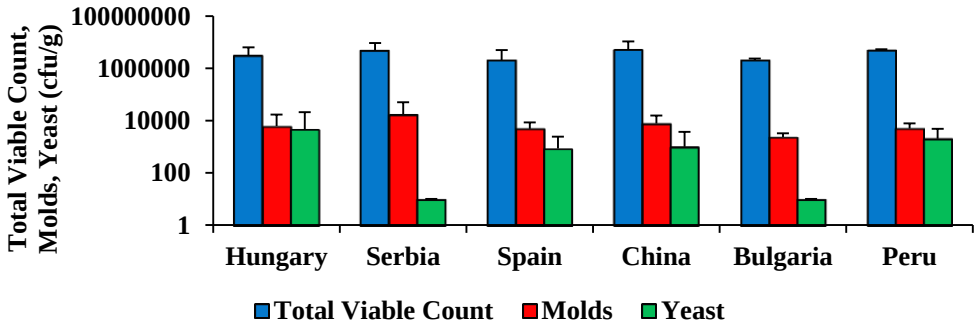

Figure 4.: Microbiological characteristics of paprika powders

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CHARACTERISATION OF BIOPRESERVATIVE - BACTERIOCIN

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SUMMARY

Bacteriocins are ribosomally synthesized proteinaceous compound. Different types of bacteriocins as an extracellular metabolite are synthesized by probiotics. Generally bacteriocins are synthesized by cluster of gene sequences. They are potent to tolerate temperature and pH shocks, and inhibit food pathogen by variety of mechanisms, including disruption of cell membrane of target cell, inhibit the DNA and/or RNA synthesis, and inhibit the protein translation. According to the chemical nature and structure, bacteriocins are classified as lantibiotics (Type I Bacteriocin), non-modified heat-stable bacteriocins (Type II Bacteriocin), large heat-labile bacteriocins (Type III Bacteriocin) and cyclic bacteriocins (Type IV Bacteriocin). Bacteriocin is popularly used as a preservative in dairy, cereal, meat and bakery products. In food products bacteriocins are used as free or immobilized condition. The following review emphasizes on the synthesis and regulation of Lantibiotics.

INTRODUCTION

All organisms produce antimicrobial peptides that represent part of the natural and innate immune system that protect them against invading organisms. In the microbial world, the antimicrobial peptides are an important part of the defense system of bacteria and they are referred to as bacteriocins. An important criterion of being a bacteriocin is that bacteriocins are ribosomaly synthesized, while multi-enzyme complexes make antibiotics. Also, most bacteriocins kill a narrow spectrum of bacteria, as compared to the traditional antibiotics (HansenJ.N, 1993). Moreover, most bacteriocins are more potent against their target bacteria while higher concentrations of traditional antibiotics are needed to kill the target bacteria.Both Gram-positive and Gram-negative bacteria produce bacteriocins. To say, bacteriocins from Gram-positive bacteria seem to possess a broader range of susceptible organisms and have a strong antimicrobial activity also against bacteria outside the genera of the producers (Galvez A et al., 1989). The Gram-positive bacteriocins are not directly active against Gram- negative bacteria, but it has been reported that when they undergo certain treatments, e.g., with EDTA or lysozyme treatment, they become susceptible to nisin (synthesized by Gram-positive bacteria). Furthermore, it has also been shown that Gram-positive bacteria can act synergistically with antimicrobial peptides from eukaryotes to kill Gram-negative bacteria (Konisky J, 1982). Since the last 20 years, bacteriocins from Gram-positive bacteria, in particular lactic acid bacteria (LAB) have been thoroughly investigated. Bacteriocins from Gram-positive are frequently found in LAB (e.g., lactococci, lactobacilli, pediococci) and they are generally regarded as safe for human consumption since they are found or used in food, for food safety and feed fermented products, and they are non-toxic to eukaryotic cells.

CLASSIFICATION OF BACTERIOCINS:

The antimicrobial peptides ribosomally produced by bacteria have been grouped into different classes based on the different criteria such as producer organisms, molecular sizes, physical properties, chemical structures, mode of actions etc., which have sometimes resulted in different names for the same compounds (e.g., thiolbiotics and lantibiotics; microcins and colicins, etc.). Bacteriocins from Gram-positive bacteria are presently divided into the four main classes;

Class I: Lantibiotics

Lantibiotics, are divided into type A lantibiotics and type B lantibiotics. Type A lantibiotics are elongated, cationic, pore forming peptides. Type B lantibiotics are compact, with globular structures, are enzyme inhibitors and are immunologically active. Lantibiotics (lanthionine-containing antibiotic peptides) are small (less than 5 kDa, with 19 to 38 amino acids) membrane- active peptides (Brotz H et al., 1997). Posttranslational peptide modification usually involves only the amino acids serine, threonine and cysteine, although lysine, aspartate and isoleucine residues may also be found in modified form (Diep DB et al., 1995).

Organization of Gene

Bacteriocins are ribosomally synthesized. The genes encoding bacteriocin production and immunity are usually organized in operon clusters. Bacteriocin gene clusters can be located on the chromosome. The lantibiotic biosynthesis operons generally contain genes coding for the prepeptide (LanA - the abbreviation lan refers to homologous genes of different lantibiotic gene clusters), enzymes responsible for modification reactions (LanB,C/LanM), processing proteases responsible for removal of the leader peptide (LanP), the ABC (ATP-binding cassette), superfamily transport proteins involved in peptide translocation (LanT), regulatory proteins (LanR, K), and proteins involved in producer self-protection (immunity) (LanI, FEG) (Aymerich T et al., 1996).

Biosynthesis pathway and regulation

Most bacteriocins are synthesized as a biologically inactive prepeptide carrying an N-terminal leader peptide that is attached to the C-terminal propeptide. For lantibiotics, the serine, threonine, and cysteine residues in their propeptide parts undergo extensive post-translational modification to form Lan/MeLan. The biosynthetic pathway of lantibiotics follows a general formation of prepeptide, modification reactions, proteolytic cleavagof the leader peptide, and the translocation of the modified prepeptide or mature propeptide across the cytoplasmic membrane (McAuliffe O et al., 2001). Cleavage of the leader peptide may take place prior to, during, or after export from the cell. Based on the biosynthetic pathway, 2 categories of genetic organization of lantibiotics, groups I and II, can be identified. (Sahl H, 1991).

The biosynthesis of lantibiotics and nonlantibiotics is usually regulated through well-known 2-component regulatory systems. These regulatory systems consist of 2 signal-producing proteins, a membrane-bound histidine protein kinase (HPK), and a cytoplasmic response regulator (RR). In this signal transduction pathway, HPK autophosphorylates the histidine residue in its intracellular domain responded by a certain concentration of bacteriocin in the environment. The phosphoryl group is subsequently transferred to the conserved aspartic acid residue on the RR receiver domain and the resulting intramolecular change triggers the response regulator to activate the transcription of the regulated genes. These regulated genes include the structural gene, the export genes, the immunity genes, and in some cases, the regulatory genes themselves (McAuliffe O et al., 2000).

Class II: Small heat-stable bacteriocins

Class II contains small heat-stable non-lanthionine peptides, and is divided into four groups: Class IIa consists of Listeria active peptides with an N-terminal consensus sequence. Class IIb

are two-peptide bacteriocins. Class IIc contains sec-dependent bacteriocins, and Class IId contains the small heat-stable non-lanthionine bacteriocins that do not belong to any of the three groups within Class II. These bacteriocins can be defined as small (less than 10 kDa), do not contain any unusual amino acids, are membrane-active and heat resistant up to temperatures of 100ºC, or autoclavable (Bhunia AK et al., 1991).

Class IIa: Listeria-active bacteriocins

Members of this group, also referred to as pediocin-like bacteriocins, are produced by a wide variety of lactic acid bacteria, and several have been biochemically characterized. Although the antimicrobial spectrum of these bacteriocins is different, they are all active against Listeria spp.(food-borne pathogen) and share a conserved amino acid sequence (McClerren AL et al., 2006).

Class IIb: Two-peptide complexes

The activity of these bacteriocins depends on the complementary activity of two peptides. Examples of plantaricins with this type of activity include plantaricin S a and b, plantaricin J and Kand plantaricin E and F. Some two-peptide bacteriocins need both peptides for activity, while one or both peptides of plantaricin S are active (Oh SJ et al., 2006). The combined effect of the two peptides of these bacteriocins is much greater than the total activity calculated from the individual effect of these peptides.

Class IIc: The sec-dependent bacteriocins

Some bacteriocins do not possess a double-glycine leader peptide, but are synthesized with a sec-type N-terminal leader sequence, leading to secretion and processing via the sec pathway (Martínez et al., 2000).

Class IId: Unclassified small heat-stable non-lanthionine bacteriocins

Bacteriocins that do not meet the criteria of the previous sections within the first three Class II bacteriocin classes are included in ClassIId.

Class III: Large heat-labile bacteriocins

Class III consists of large heat labile bacteriocins. These bacteriocins are more than 30 kDa in size. The bacteriocin helveticin J is representative of this group. The operon of the bacteriocin was cloned, and expressed in Lactobacillus acidophilus (Ivanova I et al., 2000). Class III bacteriocins were suggested to exclude since these include proteins with enzymatic activities that cause cell wall degradation and this group of antimicrobials should be referred to as bacteriolysins instead.

Class IV: Circular Bacteriocins

Due to the unique property as being circularized (head to tail), these bacteriocins have been proposed to be encompassed in a new class of bacteriocins, class IV. The circular antimicrobial bacteriocins should not be confused with the non-ribosomally synthesized antibiotics such as gramicidin S. These circular peptide-bacteriocins are covalently linked head to tail and bacteriocin AS-48 from E. faecalis is so far the most thoroughly studied one (Fimland G et al., 2002).

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BIOACTIVE PEPTIDES FROM SOYBEAN MEAL BY MEMBRANE INTEGRATED HYBRID BIOREACTOR

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SUMMARY

Under the proposed investigation bioactive peptide has been synthesized from soybean meal by an indigenous membrane integrated hybrid bioreactor (Externally associated membrane unit). Commercial grade Trypsin from bovine pancreases has been used for hydrolysis of soybean meal polypeptides in batch bioreactor and subsequently low molecular weight peptide has been purified from reaction mixture by membrane separation unit as permeate. For enzymatic hydrolysis reaction, optimum substarte concentration, pH of reaction medium, temperature have found to be 50 g.L^{-1}, 9 and 323 K respectively. Static turbulence promoter has been used to diminish the concentration polarization on the vicinity of membrane surface. Hydrodynamic studies of separation unit and separation characteristics of low molecular weight peptide and enzyme by filtration unit have been conducted. It has been observed that presence of static turbulence promoter has an immense effect on permeate flux and product purity even at low trans-membrane pressure (TMP.) Three stages discontinuous diafiltration with intermediate cleaning have been selected as optimum ones.

1. INTRODUCTION

The outstanding development in the field of biotechnology opens a new arena in the field of recycling and reuse of waste materials, as well as waste valorization. As agriculture is one of the main economical sources of tropical and sub-tropical countries, there is a wide scope to synthesis nutraceuticals from agricultural wastes (Anderson, 2002; World Bank Report, 2008). Soybean meal contain 40-45% (wt/wt) protein, a major byproduct of agricultural processing industry is generally used as fish, cattle and poultry feed. Therefore, without going for low cost uses, synthesis of low molecular weight peptide (protein hydrolysate) from soybean meal is considered as challenging step in the field of waste valorization. In the present investigation, low molecular weight peptide has been synthesized from soybean meal by an indigenous membrane integrated hybrid bioreactor (externally associated membrane separation unit). This is the first attempt to the best of our knowledge to synthesis and separation of low molecular weight peptide by membrane integrated hybrid bioreactor. Optimization of process parameters of hydrolysis reaction, and Separation characteristics of low molecular weight peptide and enzyme by filtration unit and hydrodynamic studies of separation unit have been performed.

2. EXPERIMENTAL

A membrane integrated hybrid bioreactor (batch bioreactor associated with membrane separation unit externally) has been used. For enzymatic hydrolysis of soybean meal, a 5 L jar fermentor (working volume 2 L) was used. The well instrumented batch bioreactor was equipped with a flat blade tubine type mechanical agitator and two probes for measuring pH and temperature during the reaction. Temperature of the reaction mixture was kept constant by thermostat through the jacket of the bioreactor. Batch mode enzymatic hydrolysis reaction under controlled pH and temperature has been performed. Soybean meal (protein concentration 40% (wt/wt) was used for experimental purposes. Free commercial grade

proteolytic enzyme, trypsine (3000 U. mg solid^{-1}) was used for hydrolysis reaction. Fixed amount of enzyme 0.035 g.L^{-1} was used for each hydrolysis reaction. To optimize the enzymatic hydrolysis reaction different operating parameters, such as initial substrate concentration, temperature and pH were varied ranging from 25-100 g.L^{-1}, 30-60^{o}C and 5.0-9.0 respectively. Reaction was conducted under programmed operating condition up to 6 hr. For each experiment, samples were withdrawn from the bioreactor through a capillary needle at every 1 hr interval. Samples were evacuated into sample tubes and were immediately placed in a water bath at 90^{o}C for 20 min to inactivate the enzymatic activity. After the enzymatic reaction low molecular weight peptides were purified by cross flow membrane. Single channel tubular ceramic membrane (Membralox T1-70, Pall Corporation, Germany) was used for filtration purpose. The tubular membrane has molecular weight cut off 5 kg. mol^{-1}. Stainless steel made twisted tapes static turbulence promoter was used inside the membrane tube to create turbulence. As concentration polarization is one of the major drawbacks of any pressure driven size-exclusion based membrane separation process, therefore to minimize the concentration polarization on the vicinity of membrane surface, different TMPs, ranging from 2–5 bar were used. In order to obtain more purified low molecular weight peptide four-stages of discontinuous diafiltration (DD) with intermediate cleaning was adopted. For experimental purpose, constant volume concentration factor (VCF 2) was used.

Total protein of sample was estimated by Kjeldahl and Lowry assay method (Barac et al., 2006; Lowry et al., 1951). Specific activities of protease in permeate and retentate were estimated by spectrophotometric method at 660 nm considering casein as a substrate (Guntelberg and Ottesen, 1954). Amino acids were estimated by amino acid analyzer (AAA 400, Ingos, Czech Republic). Molecular weight of peptide was estimated by gel electrophoresis (Bio-Rad mini Protein Tetra System, Bio-Rad, USA). Both SDS-page and native-page gel-electrophoresis were performed to estimate the molecular weight of peptide, according to the method described by Laemmli (Laemmli, 1970). Concentration of running gel and stacking gel were 20% and 6% respectively. Standard protein marker; molecular weight 250-2 kDa (Precision Plus Protein Dual Xtra) was used in experiment.

3. Results and Discussion

3.1 Optimization of hydrolysis process

3.1.1 Effect of substrate concentration

In Fig 1A, concentration of low molecular weight peptide in reaction broth has been plotted with time progress for different initial substrate concentration. From this figure, it has been observed that concentration of protein in reaction broth increases with time progress and after a certain time (t = 4 hr) it is saturated with time axis. In Fig 1B, the hydrolysis reaction rate has been plotted with initial substrate concentration. The figure depicts that reaction rate increases up to the substrate concentration 75 g. L^{-1} and after that it decreases.

3.1.2 Effect of temperature

In Fig. 1C, concentration of low molecular weight peptide has been plotted against hydrolysis time. From the figure it is observed that the concentration of low molecular weight peptide increases with increase of temperature up to 323 K. Where as, temperature more than 323 K, the concentration of low molecular weight peptide decreases may be due to denaturation of enzyme.

3.1.3 Effect of pH

To investigate the effect of pH on hydrolysis reaction, percentage of hydrolysis of large molecular weight peptide has been plotted against reaction pH (Fig. 1D,). It has been observed that with increase the reaction pH, percentage of hydrolysis increases and there is a maximum hydrolysis at pH 9. Where as, further increase of reaction pH the percentage of hydrolysis decreases.

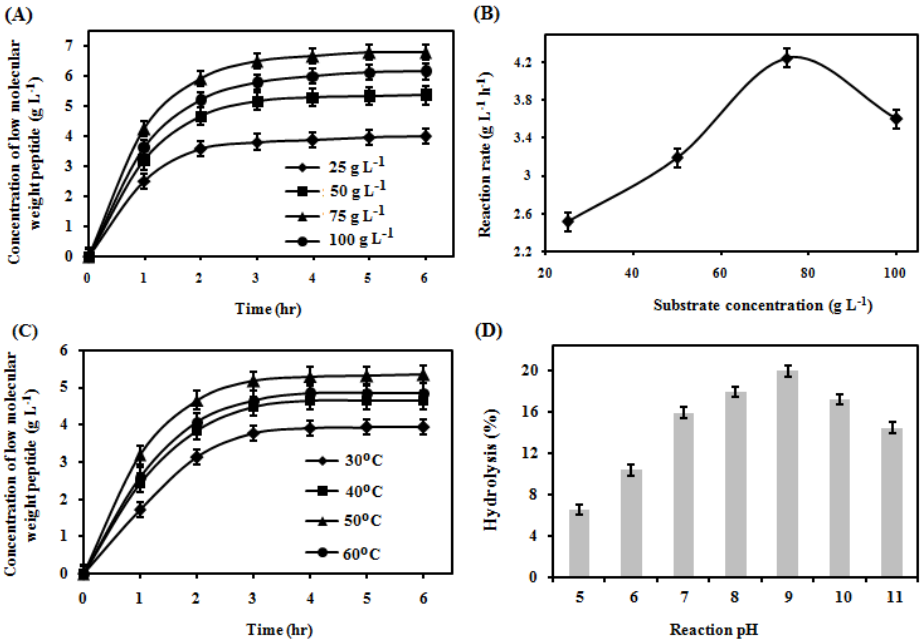

Fig 2. (A) Time history concentrations of low molecular weight peptides for different substrate concentrations, (B) Hydrolysis reaction rate for different substrate concentrations, (C) Time history concentrations of low molecular weight peptides for different reaction temperature, (D) Percentage of hydrolysis for different reaction pH at t = 2 hr.

3.4 Separation of low molecular weight peptide by membrane separation unit

After membrane compaction, series of water runs were taken to evaluate water permeability of membrane, which was found to be 0.03 $m.h^{-1}.bar^{-1}$. Time histories of permeate fluxes for different TMPs and DD stages have been studied. It has been observed that permeate flux declination is higher at low TMP and permeate flux declines rapidly, then gradually and eventually it becomes asymptotic with time axis, which is a typical time-history flux profile of any pressure driven separation process. In this case, permeate flux is found to increase with the increase in TMPs (2–5 bar) and at TMP 4 bar it becomes almost similar with next one. Therefore, effect of TMPs on permeation has been elucidated for 1st stage of DD process (Fig. 3A). It is notified that percentage decrease of permeate flux of each DD stage is reduced with increase of TMPs. Percentages of decrease of permeate flux for each DD stages under constant TMP (TMP = 4 bar) has been elucidated in Fig 3B. It is observed that under constant TMP, percentage decrease of permeate flux for each stages of DD process is decreased with increase of DD stages. Positive effects of static turbulent on permeation have been observed.

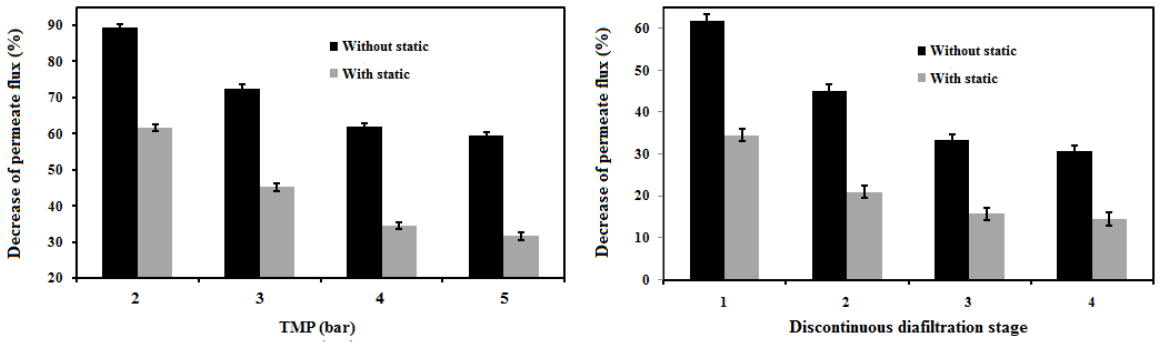

Fig 3. (A) Percentage decrease of permeate flux for 1st stage of DD process under different TMP, (B) Percentage decrease of permeate flux for different DD stages under constant TMP (TMP = 4 bar).

Protein concentration in permeate and rejection of enzyme in both permeate and retentate have been estimated to understand the separation characteristics of filtration process. It has been observed that percentage of rejection of enzyme and concentration of low molecular weight peptide in permeate increase with increase of TMP, up to 4 bar and has the similar value with 5 bar. At optimum condition 5 $g.L^{-1}$ low molecular weight peptide has been found at permeate. It has been found that the molecular weight of protein in permeate side is less than 5 $kg.mol^{-1}$.

Conclusions

In the present investigation, a membrane integrated hybrid bioreactor has been used to synthesis and separation of protein hydrolysate (low molecular weight peptide) from soybean meal. For enzyme hydrolysis reaction, the optimum substrate concentration, reaction temperature, pH have been found to be 50 $g.L^{-1}$, 9 and 323 K respectively. At optimum reaction condition 35% hydrolysis was done. For filtration process, optimum condition has been found as well (TMP = 4 bar along with 3 stages of DD process). At optimum filtration condition 5 $g.L^{-1}$ low molecular weight peptide has been found at permeate and the estimated molecular weight of protein in permeate side is less than 5 $kg.mol^{-1}$. It is expected that the proposed investigation will receive a great attention in the field of 'valorization of agricultural waste'.

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EXTENDING THE STORABILITY OF BANANA WITH CONTINUOUS OZONE TREATMENT

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SUMMARY
The aim of the experiment was to evaluate the potential of continuous ozone treatment for prolonging the storage life of banana. Ozone is created by a commercially available ozone generator. Decay incidence of banana was reduced by continuous ozone exposure during storage at 14°C, relative humidity 95%. Moreover, continuous ozone exposure at approximately 0.1 ppm inhibited brown spots on the surface of banana, markedly slowed down the postharvest ripening, however, increased weight loss throughout 16 days of storage compared to control fruits. No chilling injury was detected in ozonated air or in normal air treatment (control). Results showed that application of gaseous ozone during storage could delay the ripening, decrease decay incidence and maintain the appearance and quality of banana.

INTRODUCTION

Banana (*Musa x acuminate* L.) is one of the most popular fruits because of its flavor and nutritional value (Robinson, 1996). However, this fruit is vulnerable to pathogenic decays such as crown rot, anthracnose and brown spot on the peel surface that cause postharvest losses during transportation and storage (Basel et al., 2002).

Ozone is a powerful oxidizing and sanitizing agent (Palou et al., 2002). Ozone application in horticulture industry has been recommended (Skog and Chu, 2001). Fruits and vegetables treated with ozone have less decay and extend shelf-life. Moreover, ozone decomposes rapidly into harmless product, oxygen, when it is applied to food (Guzel-Seydim et al., 2004). However, studies dealing with continuous ozone in fruit and vegetable storage are still limited in number. Therefore, research about the potential of ozone in produce preservation is really required in order to widen this possible technique to increase storability.

The aim of this study was to evaluate the effect of continuous ozone on banana quality during storage at 14°C for both ethylene removal and antimicrobial purposes.

MATERIALS AND METHODS

Materials

Banana was bought from a wholesale fruit and vegetable distribution in Hungary, in May, 2015. Fruits were transported to the Faculty of Food Science, Corvinus University of Budapest, Hungary. Samples were selected for uniformity of size, shape and freedom from external damage.

Ozone was generated by an ozone generator (Neo.Tec XJ-100, China).

Methods

Experimental design

Samples were selected randomly into 2 boxes, each box containing 15 fruits. The weight of each piece was 180 ± 5g Banana was at mature-green stage 2 of ripening according to banana ripeness chart (Postharvest Technology Center, UC Davis).

Fruits were stored for 16 days at 14°C with continuous ozone concentration at approximately 0.1 ppm

Measurements were performed before storage (day 0) and in 4-d intervals during 16 days.

Measurement

- **Surface color.** Banana peel color was measured with a Minolta Chroma Meter CR-400 portable tristimulus colorimeter (Minolta Corporation, Osaka, Japan). CIE L^*, a^* and b^* color characteristics were determined at two opposite points on the surface of each fruit. Hue angle (H^o) value was calculated as arctangent (b/a).
- **Weight loss.** Samples were weighed at day 0 and at the end of each storage interval. The difference between initial and each storage period was considered as total weight loss during that interval and calculated as percentages on a fresh weight.
- **Decay percentage**. Decay was evaluated as fungal mycelia appeared on stem and calculated as the number of decayed samples divided by initial number of samples multiplied by 100.
- **ΔA index.** ΔA index is an index of the quantity of chlorophyll in a fruit. ΔA index was measured by a DA meter (Sintéleias.r.l., Italy) at two opposite points on the surface of each fruit.

Statistical analysis. All data were analyzed using analysis of variance (ANOVA) by SPSS version 11.0.1 (SPSS Inc, USA). The results were reported as means with standard deviations (95% confidence interval).

RESULTS AND DISCUSSION

Weight loss

The weight loss of samples increased throughout the storage period. In the first 4 days, there was no difference between control and treated samples. After that, treated fruits had higher weight loss than control and a significant difference was observed from the 8th day of storage (Fig. 1). This could be explained that continuous ozone exposure probably damaged fruit cuticle or epidermal tissue (Paulo et al., 2002). Another report also indicated that fruit cuticle in plums was injured at 0.1 ppm concentration of ozone (Crisosto et al., 1993). However, no visible injuries or shriveling on the fruit peel were detected during storage in case of treated samples.

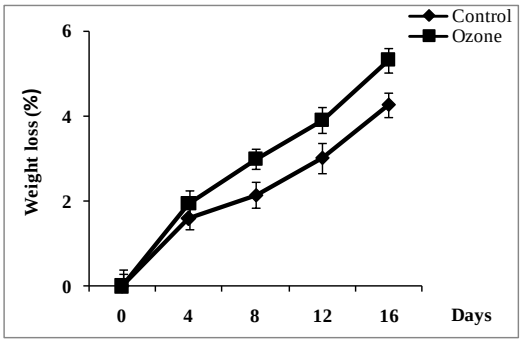

Figure 1.: Effect of continuous ozone on weight loss of banana during storage. Values are the mean ± SD

Surface color and ΔA index

The hue angle value and ΔA index declined during storage (Fig. 2). The decrease of chlorophyll content was closely related to ripening throughout the storage (Bron et al., 2004; Pongprasert et al., 2014). The color change from green to yellow is often the sign of ripening. The hue angle value and ΔA index showed a color shift from green to yellow after the first 4 days of storage in case of control, whereas treated fruits were still green. These results reflected a significant loss in chlorophyll content, because the control reached the advancing

ripeness and senescence stage. Ozone slowed the color change of banana peel throughout the storage. This could be explained by the effectiveness of ozone in ethylene removal so ethylene did not exert its action in ripening.

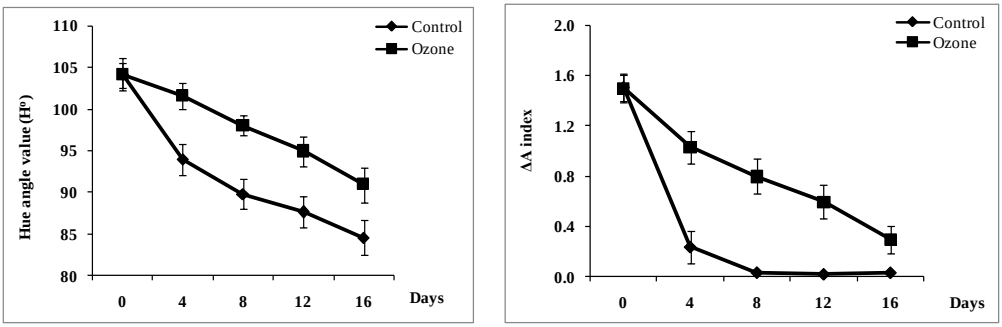

Figure 2.: Effect of continuous ozone on hue angle value and ΔA index of banana during storage. Values are the mean ± SD

Percentage of decay

Decay was an important source of postharvest loss. Microbial growth occurred on the 8th day of the storage period whereas no visible sign was detected for treated fruits. Mycelial development on banana was completely inhibited by continuous ozone at 0.1ppm. The reason of it could be that ozone inhibited growth of microbial pathogens on the fruit surface and in the chamber. The symptoms of anthracnose, crown rot appeared on control after 8 days storage at 14^{o}C and became severe at the end of storage (Table 1). Moreover, less brown spots were observed on treated fruits, compared to the control. The results suggested that continuous ozone was effective against fungal growth and maintained appearance quality for up to 16 days. This was in agreement with reports for peach, table grapes (Palou et al., 2002) and for date fruits (Habibi Najafi et al., 2009).

Table 1.: The percentage of decayed samples during storage

Sample	Decay (%)			
	4d	8d	12d	16d
Control	0	13.3a	33.3b	46.7c
Ozone	0	0	0	0

Means within each cultivar followed by the same letter were not statistically different at the 5% level

CONCLUSIONS

The results indicated that continuous ozone exposure at approximately 0.1 ppm could have a role in delaying the ripening and have antimicrobial effect during storage. The overall quality of treated fruits was better than that of the controls, and without negative effect of ozone. Continuous ozone treatment is a promising technique for increasing the storability of horticultural products, particularly in retail storage where the air is often infected.

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CHANGES IN FREE AMINO ACID AND BIOGENIC AMINE CONTENT OF SOUS VIDE TREATED MEATS DURING STORAGE

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SUMMARY

The aim of our work was to study the effect of sous vide (SV) technology on free amino acid (FAA) and biogenic amine (BA) formation in different kinds of meat (beef, pork and turkey) during storage. Total viable cell counts (TVC) were determined on plate count agar. FAA and BA analysis were carried out by an automatic amino acid analyser. The SV treatment reduced the level of TVC compared to the controls (C) but then TVC increased continuously during storage. The SV treatment increased the FAA content in turkey while in pork and in beef meat samples a decreasing tendency was observed compared to the controls during storage. BA content of meat samples increased during storage. The dominant BA profile in the SV samples was different and showed significantly lower levels than in control samples. Sous-vide treatment improved the microbial quality, and it was effective in reduction of biogenic amine content of meat samples.

INTRODUCTION

Sous vide technology (SV) is an advanced method of cooking in vacuumed plastic pouches at controlled temperatures. Sous vide treatment improves shelf-life and can enhance taste and nutrition of the food. Quality of meat is highly dependent on the free amino acid (FAA) content. FAAs are precursors of many compounds such as aroma and biogenic amines (BAs). Regarding meat product scientific literature on sous vide method mainly focused on microbial safety, but there are other potential hazards e.g. biogenic amine formation in meat (Vaudagna et al.,2002). High amounts of BAs may cause food related pseudo-allergic reactions in sensitive individuals, therefore several efforts have been made to reduce BA content of food.

The aim of this work was to study the effect of SV technology on FAA and BA formation in different kinds of meat (beef, pork and turkey) during storage. Meat samples were provided by Metro Meat Wholesaler's (Budapest, Hungary).

MATERIAL AND METHODS

Sous vide treatment

Samples (beef, pork, turkey) were cut into pieces transversally to the fibre direction and vacuum-packed in a 90µm PA/PE plastic poach with a vacuum packaging machine (MULTIVAC-C100, Multivac Sepp Haggenmüller GMBH&Co KG). Samples were cooked for 45 min at 60 °C in a Lauda E100 water bath (LAUDA DR.R. Wobser GMBH&Co. KG). After the treatment samples were put in the ice-water bath until the temperature reach at the 3-4 °C. Samples then were stored in a cooling cabinet at 6 °C for 4 weeks. Samples were taken every week.

Microbiological analysis

The aerobic colony counts in meat samples were determined as follows: 10 g of sample was placed in a stomacher bag (nylon mesh bag, pore size 1.0 mm) with 40 cm^3 of diluents

(1g peptone; 8.5 g NaCl in 1000 cm^3 distilled water). Five-time dilution meat homogenates were stomached for 2 minutes, and 10-fold dilution series were prepared. These were cultivated on Plate Count Agar (PCA, MERCK). PCA plates were incubated at 30 °C for 2-3 days. Samples were taken weekly for aerobic plate count determination during 4 weeks of storage.

Free amino acid analysis

Meat samples (2 g) were extracted with 10 cm^3 of 10% trichloroacetic acid for 1 h at room temperature, at 100 rpm using a Laboshake (Gerhardt, Germany). Samples were filtered through a 0.25 µm membrane filter (Nalgene, USA). Free amino acid analysis was performed with an AAA 400 Amino Acid Analyser (Ingos, Czech Republic) equipped with an IONEX OSTION LCP5020 ion-exchange resin (200×3.7 mm) column, using Li^+ buffers.

Biogenic amine analysis

The same extracts were used for the determination of biogenic amines as for the free amino acids. BA analysis was performed with the same Amino Acid Analyser equipped with an Ostion LG ANB ion-exchange resin (70×3.7 mm) column, using Na^+/K^+ buffers (Simon-Sarkadi et al., 2012).

RESULTS

Aerobic plate count

The development of the total viable count in control and sous vide treated samples stored at 6 °C over a period of 4 weeks is shown in *Figure 1*. The total viable count of control samples varied between 10^4-10^6 CFU/g depending on the types of meat and increased 1-2 log cycles during 4 weeks of storage.

The sous vide treatment reduced the level of total viable count by 3-4 log cycles compared to the controls and then the level increased continuously during storage.

Free amino acid content of meat

The sous vide treatment increased the FAA content in turkey (C: 3.8 mg/g; SV: 4.4 mg/g) while in pork (C: 4.4 mg/g; SV: 2.9 mg/g) and in beef (C: 2.4 mg/g; SV: 1.7 mg/g) meat samples a decreasing tendency was observed compared to the controls (C) during storage. 26 free amino acids were detected. The main amino acids in all samples were 1-methylhistidine (1mHis), Ala, Val, Leu, Gly, Thr and Ser. The sous vide treatment significantly decreased the Gaba concentration (C: 202 µg/g; SV: 3 µg/g), while the Asn (C: 25 µg/g; SV: 44 µg/g) and Gln (C: 79 µg/g; SV: 123 µg/g) content increased in all meat samples *(Figure 2)*.

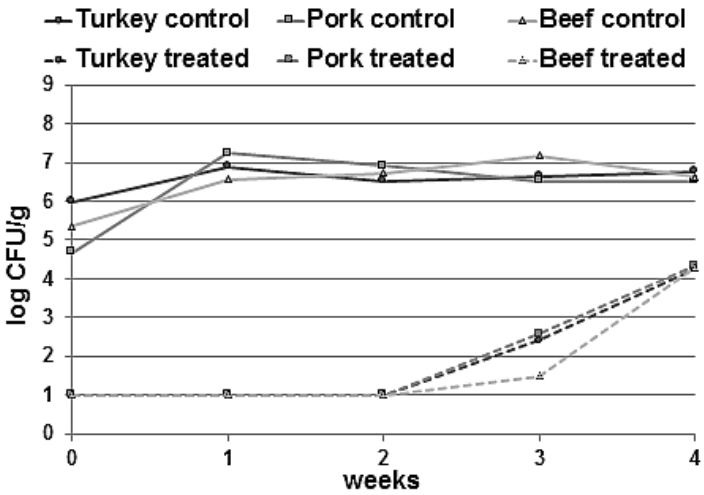

Figure 1: Aerobic plate counts of control and sous vide treated meat samples during 4 weeks of storage at 6 °C

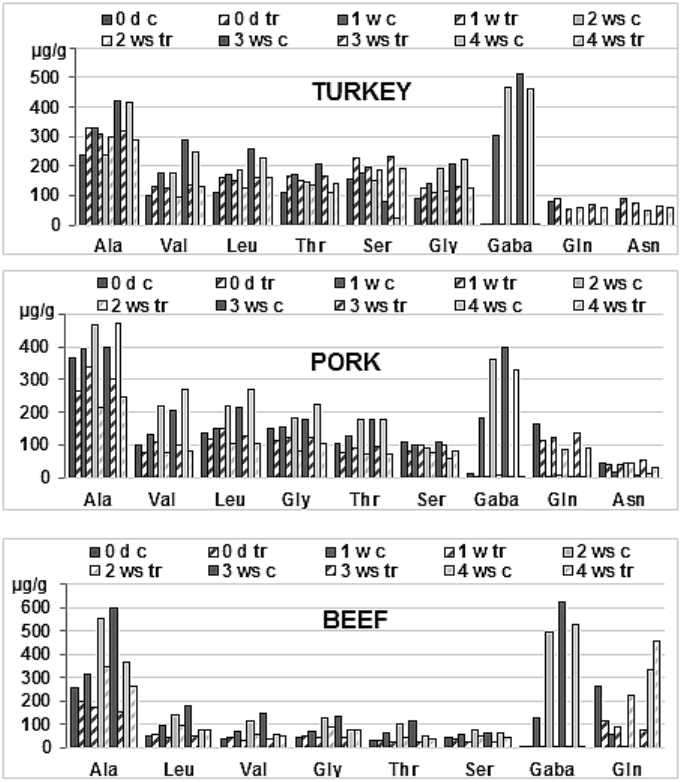

Figure 2: Changes of main free amino acid content in control and sous vide treated meat samples during 4 weeks of storage

Biogenic amine content of meat

Biogenic amine content of meat samples increased during the 4 weeks of storage. The total BA content ranged between 22-644 µg/g (turkey), 68-442 µg/g (pork) and 16-411 µg/g (beef) in control samples. In sous vide treated samples it ranged between 24-46 µg/g (turkey), 18-90 µg/g (pork) and 10-33 µg/g (beef) *(Figure 3)*. Sous vide technology reduced the level of biogenic amines by 90% in beef and turkey, and 80% in pork samples compared to that of untreated samples.

Cadaverine, tyramine and putrescine were the major amines in all control samples. Histamine concentration was also high in turkey meat (37-128 µg/g). SV treatment reduced the level of BAs by 80-90% in all meat samples compared to the controls. The dominant biogenic amines in the sous vide treated meat samples were different and showed significantly lower levels than in control samples. Cadaverine concentration was the highest in pork meat (12-56 µg/g) followed by turkey (1-3.8 µg/g) and beef (2-3.5 µg/g). Beside cadaverine histamine was also detected in beef (3.5 µg/g), putrescine in pork (1-8 µg/g) and turkey (1 µg/g) samples.

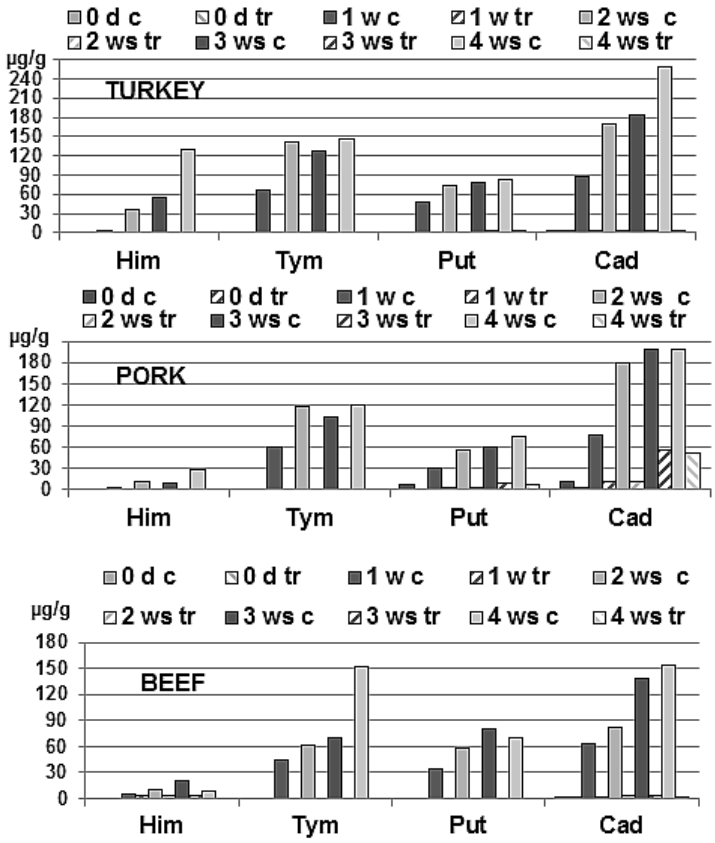

Figure 3: Changes of biogenic amine contents in control and sous vide treated meat samples during 4 weeks of storage

CONCLUSION

Sous vide treatment improved the microbial quality of meat samples, and was effective in the reduction of biogenic amine content of these products. Our result indicates that it is recommended to use sous vide treatment to obtain a healthier product regarding the biogenic amine content of meat.

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COMPARISON OF THE EFFECTS OF SLAUGHTERING TECHNOLOGIES AND ENVIRONMENTAL EFFECTS ON THE QUALITY OF CARP FISH

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SUMMARY

The aim of our experiment was to investigate whether quality differences of carp (Cyprinus carpio) fish due to environmental conditions can be accommodated by applying different cutting technologies. We modelled the environmental effect of summer, winter and autumn-spring months, that is, water temperatures between 4°C, 12°C and 24°C, then 4 different slaughtering methods were used on the three distinct populations. The quality parameters of processed carp fish were investigated from microbiological, colour, texture, pH, water retention and exsanguination aspects. Based on the results we concluded that the summer environment is the least favourable from the aspect of product safety and quality. The quality of fish deteriorated significantly as a result of the increased stress, whose duration could be as much as 30 to 40 seconds, during the technological step. Besides, we concluded that the so-called Ike-Jime technique resulted in the best fish quality.

INTRODUCTION

In the domestic fish processing industry practice the slaughtering technology of carp does not result in a raw material that is suitable for the production of a fresh product with a safely long shelf-life and a suitable quality that meets the market challenges despite the fact that carp (Cyprinus carpio L.) is the primary bred fish of our country. Its fish has an average water content of 78.6 %, protein content of 16.0 to 17.8g/100g and a fat content of 4.0 to 8.7 g/100g (Darázy,Aczél, 1987). Fish are subject to a huge stress during processing and slaughtering. Physical activity and stress have a highly disadvantageous effect on the exsanguination of fish (Mørkøre, Mazo T et al. 2008). In order to achieve a good quality product it is important that fish is processed immediately after being slaughtered and processing in the state of cadaveric rigidity is avoided. The energy level of fish after death depends on the nourishment of the fish and the amount of stress it receives during slaughtering, which can accelerate the onset of cadaveric rigidity and can have a negative effect on the fish texture as well (Mørkøre, Mazo T et al. 2008). The decrease in muscle temperature from the initial temperature to zero degree results in the removal of heat that can be freed to foster the decay of proteins that starts hours later, but in the living animal the quick decrease of body temperature also causes increased stress, especially if the initial temperature was high. An advantage of live chilling could be that pH does not decrease and the coagulation of blood is very long, which has a positive effect both on exsanguination and the remaining haemoglobin content of the fish (Skjervold, Fjæra et al. 2001). A general requirement for the stunning of fish is that fish are rendered unconscious until the moment of death to prevent any anxiety, suffering or pain. If stunning is inappropriate, the fish may be injured during wriggling, which fosters the increase in the number of mesophyll or psychrophyll microbes. During electric stunning fewer injuries occur and the fish are subject to a considerably shorter stress. Another recognised stress-free method is the traditional Japanese Ike Jime technique (Diggles, 2015), in which the medulla oblongata is destroyed in a moment with a sharp metal spike. As a result, reflex movements cease immediately and the muscles relax. The muscle contains several proteolytic systems that can cause the fish to soften after death. In marine fishing it is an established practice to pre-chill the fresh, filleted fish in flowing water of temperature 0 to 1 ºC until the core temperature reaches the storage temperature, then the fish are put into crates with ice layers.

The low temperature decreases enzyme activity, which not only slows the biochemical and physical changes in fish but also increases the generation time of microorganisms. As the storage temperature decreases, the lag phase before growth, during which the rate of growth is negligible, becomes longer. This way fish can be preserved considerably longer. Unfortunately this practice is completely absent from the domestic processing technology.

MATERIAL AND METHOD

Sample preparation

Before slaughtering the fish were kept alive at 3 different temperatures of 4°, 12° and 24°C for at least 24 hours. During the live chilling of fish we used a cooling rate of approximately 5°C/60 minutes. This was achieved by the continuous addition of ice flakes to the storage water, whose temperature and mixing was monitored at regular intervals. In order to investigate the set aims in a complex way the stunning and cooling methods were combined as follows:

Stunning:
1. electric shock for 1 minute (I= 0.3-0.6 mA, U= 40-50 V), then slaughtering by the incision of the infundibulum (*conus arteriosus*) from the branchial arch and the intersection of the tail artery and vein (*a. Caudalis, v. Caudalis*)
2. pre-shocking in icy water for 1 minute, then slaughtering with the Ike Jime method, that is, by destroying the medulla oblongata with a sharp object

Exsanguination:
1. 10 minutes in suspended position in air of temperature 12°C
2. 8 minutes in water bath of temperature 0°C containing 25% of ice, then 2 minutes in suspended position in air of temperature 12°C

Of the fish prepared this way, the skeletal muscles were detached and samples of mass 100 to 120g were cut from the fillets; the samples for the storage probe were created by packing into gas-tight PA/PE vacuum bags of width 60 microns, which were labelled based on the sample matrix; these were stored at a temperature of 0 to 2°C until the measurement.

Measurement

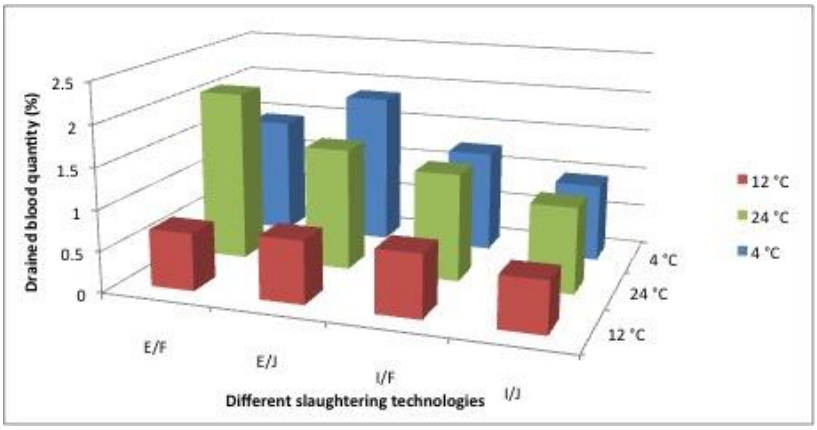
On the 0^{th} day, immediately after filleting we investigated the pH value, colour, microbiological state, texture and water retention of the samples. During storage sampling was performed on the 4^{th}, 6^{th} and 8^{th} days. pH was measured with a Testo 209 instrument, in each case 3 parallel measurements were made on the samples. As the pH value of fish is strongly influenced by its temperature, we worked at 3 different temperatures, so we slaughtered one more fish, whose fillets were used to investigate the auxiliary correlation between pH and temperature. During this measurement the pH value of the uncut fish fillet was measured continuously at 5 different points in the interval 2 to 25 °C. Juice drainage was investigated with a CAS MWP-300 scale to determine the amount of juice lost by the fish fillet as a function of time elapsed. The results are given as a percentage of the original mass of the fish fillet. Texture was measured with a Stable Micro System TA.XTplus texture measuring instrument. During the investigations penetration measurements were made with 10 parallel measurements on each fish fillet. For penetration we used the penetration needle probe (P/2N) supplied by the manufacturer, whose length is 5 cm, width is 2 mm and ends in the shape of a needle. According to the measurement program the probe head entered the fish to a depth of 15 mm at a speed of 2mm/s in each measurement.

The microbiological investigations were performed by determining the total aerobe mesophyll germ number.

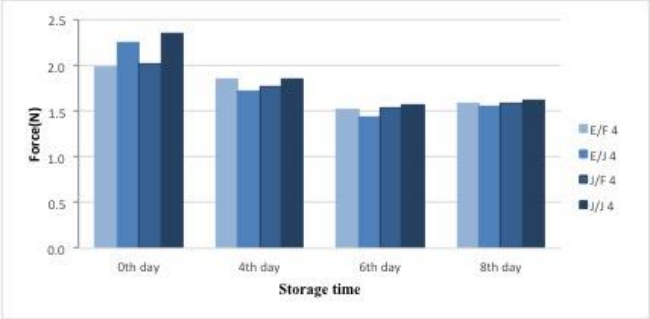
Total germ number: the total number of mesophyll aerobe and optionally anaerobe microorganisms living and capable of growth in a unit mass or volume of the investigated material. During the investigation a sample was taken from the material to be investigated under aseptic conditions, a set amount of sterile diluting solution was added to it and then it was homogenised. The sample prepared this way for investigation was diluted according to the increasing integral powers of ten and these dilutions were poured on a plate using TGE (Merck) agar. The inoculations were incubated for 48 hours at a temperature of 30°C under aerobe conditions and then evaluated. After determining the number of the positive cultures of the different dilutions the total aerobe germ number per 1g of the investigated sample was calculated (MSZ 3640/4-86). Water retention was determined with a press probe. The filter paper strip of size 5x10 cm, which was prepared for the investigation and stored in a desiccator, was laid on the object plate and a fish sample of 200 to 300 mg measured with analytical accuracy was laid on it. The sample was covered with another object plate and a 500-g weight was placed on it to provide the pressure required for pressing. The fish sample was pressed for 5 minutes. The fish juice absorbed by the filter paper was dried and the area of the spot was determined. The spot area per fish mass (mm^2/mg) characterises the water retention. The spot area was determined by measuring the mass. For this measurement the spot area was cut out from the filter paper and the remaining filter paper was measured on an analytical scale and related to the original mass of the filter paper to conclude the mass ratio.

RESULTS

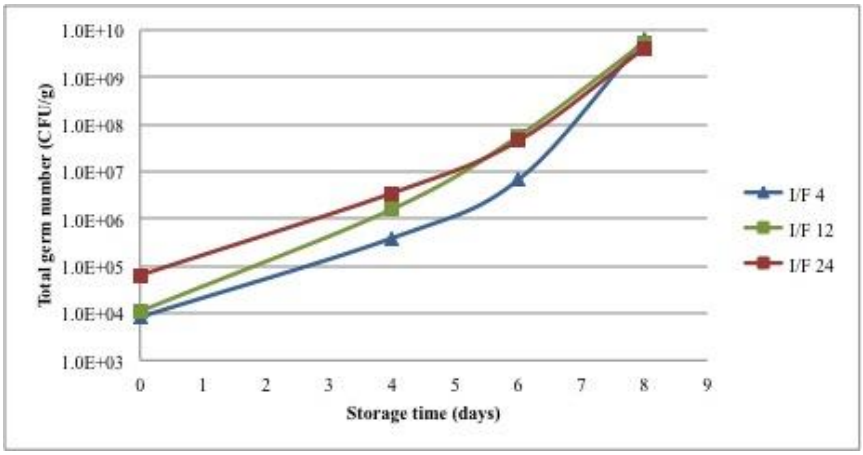
Based on the comparison of the combination of different stunning and slaughtering technologies, from the aspect of exsanguination in the case of fish of temperature 4º and 24ºC electric stunning combined with suspended exsanguination was the most effective (Chart 1). The same conclusion was reached for other parameters investigated on the day of sample preparation, such as water retention, texture investigation based on maximum force and penetration (2. chart). However, based on the investigation of the samples of the storage probe it can be stated that these parameters remained most stable when cold water shock, Ike Jime slaughter and exsanguination in icy water were combined. This means that this latter combination of technologies is recommended to achieve a stable product quality in the production of an ultra fresh product. During the measurements an important remark was made concerning the effect of the stress factor. When the experiments were carried out, the fish stored at 12°C could be held only at some distance from the site of stunning and slaughtering, so as much as 30 to 40 seconds passed between removing from the water and stunning. We owe it to the increased stress during this period that in the case of fish at a temperature of 12°C we got results that showed worse quality regarding each parameter. The total aerobe germ number measured on the epidermis of fish is closely related to the ambient temperature. In the case of fish held alive at 4°C and at 24°C the total measured germ number was higher by 1 magnitude in the latter case (3. chart), which in our opinion proves clearly that live chilling has a beneficial effect on the hygiene of processing, but we could not detect a significant difference between the effect of the two humane slaughtering technologies on fish quality in the measurement series of the storage probe. It is increased stress and ambient temperature that have a clearly measurable effect on fish quality.



1. chart: Drained blood quantity at the combined slaughtering t.



2. chart: Maximal strength during storage



3.chart: Total germ number during storage in case of Ike Jime technique at 4 °C fish

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PHOTOMETRIC MEASUREMENT OF ATP DECAY PRODUCTS TO DETERMINE THE EFFECT OF DIFFERENT CUTTING TECHNOLOGIES ON THE QUALITY OF CARP

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SUMMARY

We investigated the chilling of freshly cut fish bodies with icy water and its effect on fish quality as opposed to chilling with air, which is presently used in industry. We used the enzyme-catalysed transformation of ATP and its decay products into NADH and photometric measurement at 340 nm to measure the result of the two processes objectively. The K-index, which is calculated from the ratio of hypoxanthine, xanthine and inosine, is an objective indicator of the fish freshness. Due to chilling with icy water the IMP degradation decreased by 10% in the first hour, which affected the dynamics of the changes in ATP decay products during the 9-day shelf-life probe. This method for the post mortem chilling of fish slows the autolytic degradation of proteins considerably, so the shelf-life of the product can be extended by as much as 20%.

INTRODUCTION

In the domestic fish processing practice the practice of the cutting technology of carp does not result in a material that is suitable to produce a fresh product whose quality meets the market challenges and has a securely long shelf-life. The main product of domestic, semi-intensive polyculture fish-farming is the carp (*Cyprinus carpio*), which constitutes 70% of the total production. At the same time consumer habits have changed greatly in the past two decades and consumption, which was based on the purchase of live fish almost entirely, has shifted towards convenience products. The degradation of fish is the result of two different processes, degradation of microbiological origin and autolytic degradation (Huss, 1995). In the case of fresh raw fish autolytic degradation can be influenced solely by the optimisation of technological processes. In a previous experiment we established the technological steps that resulted in the best exsanguination, the best texture, but only the investigation of the biochemical processes occurring in fish can give an objective picture of the technology that slows protein degradation of enzymatic origin most effectively. The K and Ki indices are used for the objective evaluation of quality degradation through chemical methods, for these different limiting values are set by different countries and eating habits (Elira et al., 1989), but the available literature does not contain data for the carp species. In the deamination of adenosine derivatives ammonia is produced. With the splitting-off of ribose molecule adenosine--» inosine----»hypoxanthine transformation happens, this biochemical process is characteristic only of fish (Kosáry, 2013). NOVOCIB Precice® Freshness Assay kit was used for the photometric measurement of ATP decay products. In the processing of carp it is a problem that fish - especially from spring to autumn - enter the technological process from an environment whose temperature is 20°C or higher, so the chilling of the fish body below the desired temperature of 2°C may take 18 to 24 hours in practice. Our aim is the elaboration of a technological sequence that ensures the quick chilling of the product even in the warmer periods of the year, thereby slowing the autolytic decay processes. In this case fish will have a firmer texture at the end of the ripening process, which may be favourable from both technological and microbiological aspect. From microbiological aspect the looser texture a product has, the more prone it is to the spread of microorganisms, the matter can be accessed and penetrated more easily. Due to autolytic degradation the structure of tissue-forming

proteins disintegrates, they are reduced into free amino acids and other components, and these materials serve as a more easily accessible nutrient to microbes. Therefore, if autolytic degradation is slowed, the growth conditions for harmful microbes are deteriorated indirectly. So in the case of a fish with lower cutting temperature that has a firmer texture after the cutting, the microbiological shelf-life is more favourable, the conditions for the growth of microorganisms are worse. This is especially useful from the aspect of storage, because this way the shelf-life of the products can be extended considerably.

MATERIAL AND METHODS

Sample preparation

The fish of locally registered carp (*Cyprinus carpio*) species P34, which were used for the experiments, were obtained alive from a saline-soil fish farm near Akasztó, in the mass range 2.5 to 2.8 kg. The photometric measurement was performed on each sample on the same day, the sample series of the storage probe were prepared on the 10^{th}, 8^{th}, 6^{th}, 4^{th}, 2^{nd}, 1^{st} and 0^{th} days before the measurement. On the basis of the preliminary experiments 60 seconds of electric shock (40-50V, 30-60mA) was used to stun the fish, then after the incision of the infundibulum (*conus arteriosus*) from the branchial arch and the intersection of the tail artery and vein (*a. Caudalis, v. Caudalis*) suspended exsanguination was done for 180 seconds. The mean body temperature of the live animals was 18.25°C +/-1.25°C on the 7 sample preparation days. The samples were prepared using two different technological sequences, the first of these modelled the domestic industry practice. According to it 15 to 25 kg of fish is placed into plastic crates after slaughtering, then the bodies are covered with ice flakes of mass 10 to 15% of the body mass and air is used to decrease the core temperature below 2°C. The fish bodies in the second sample series were put into water at 0°C (containing 25% of ice) until the desired core temperature of 2°C was reached. After reaching the desired core temperature the fillets were detached from the fish bodies and cut into 120 to 150g pieces. The samples were stored in PP vacuum bags with the sample matrix codes marked on them in advance, at the temperature of melting ice. For sample preparation the pieces of the storage probe were homogenised separately using Philips HR 2160 type blender and 30 g of sample was measured into heat-resistant 50-mL FRASCO sample storage containers of PP using scale type CAS MWP-30. The 100 mL buffer solution, which was part of the PRECICE® Freshness Assay Kit, was diluted to 1000 mL using HPLC grade deionised water and 10 mL of it per 1 g of fish was put into the sample storage containers. The closed samples were heat-treated for 20 minutes in water of temperature 100°C, then cooled in icy water of temperature 0°C (containing 25% ice flake), this process caused the enzymatic reactions in the fish to stop and the ATP derivatives to enter the solution. The sample solutions were drawn into 50-mL PP plastic syringes and filtered through a JACFV02025100 glass fibre pre-filter attached to the syringe. The 3 different lyophilised enzyme mixes of the PRECISE® Freshness Assay Kit were dissolved in HPLC grade deionised water and the required cofactors were added according to the followings:

- 1 – 1 mL of diluted reaction buffer was added to cofactor 1 and cofactor 2 using a pipette, then the solutions were mingled by shaking for 30 seconds. After it, the contents of vials 1 and 2 were filled into a 15-mL test-tube using a pipette and an additional 10 mL of reaction buffer was added.
- Lyophilised enzyme mixes No1. and No2. were dissolved in 200 and 100 microlitres of HPLC grade deionised water, respectively.
- 12 mL of reaction buffer mixed with the two cofactors was available, 9 mL of it was filled into a test-tube using a pipette and Enzyme Mix No1. was added.
- 6 mL of the mixture was filled into another test-tube using a pipette and Enzyme Mix No2. and the contents of vial labelled cofactor 3 were added.

- 3 mL of the third mixture was filled into a fourth test-tube using a pipette and the contents of vial labelled Enzyme Mix No3. were added.

Measurement

During sample preparation the change in the post mortem core temperature of fish bodies was monitored with EBRO EBI 300 digital core thermometer. Enzymatic method was used to convert the ATP decay products, (IMP, Hx, Ino) into NADH, which absorbs light at 340 nanometres. THERMO microplate reader was used for the measurement. The preparations of the two sample series were filled into sample storage microplates using pipettes, 100 microlitres of each sample was filled into 4 sample storage microplates each, then 100 microlitres of the (blind) buffer, enzyme No.1., enzyme No.2., enzyme No.3. mixtures each were filled into one sample holder of each sample. The microplates were shaken at 1000 rpm for 2 minutes and then incubated for 30 minutes. IMP degraded into $NADH_2$ in each sample due to enzyme dehydrogenase marked "**enzyme mix 1**", while due to enzyme preparation marked "**enzyme mix2**" Hypoxanthine was converted into $NADH_2$ as well. Preparation marked "**enzyme mix3**" degraded all three investigated components, IMP, Hx and Inosine into $NADH_2$ (Godrich, 2014.). The spectrophotometer measured the light absorbance of the investigated samples at 340 nm, from the results the ratio of each ATP degradation form present in the sample could be calculated.

RESULTS

Based on the cooling curves (Chart 1) there is a remarkable difference between the changes in the temperature measured in fish bodies chilled with icy water and in the traditional way, with air. While the fish bodies chilled with icy water reached temperature below 4°C in 60 minutes and we registered the desirable core temperature below 2°C in 90 minutes, in the case of fish chilled with air even 18 hours was not enough to reach 2°C.

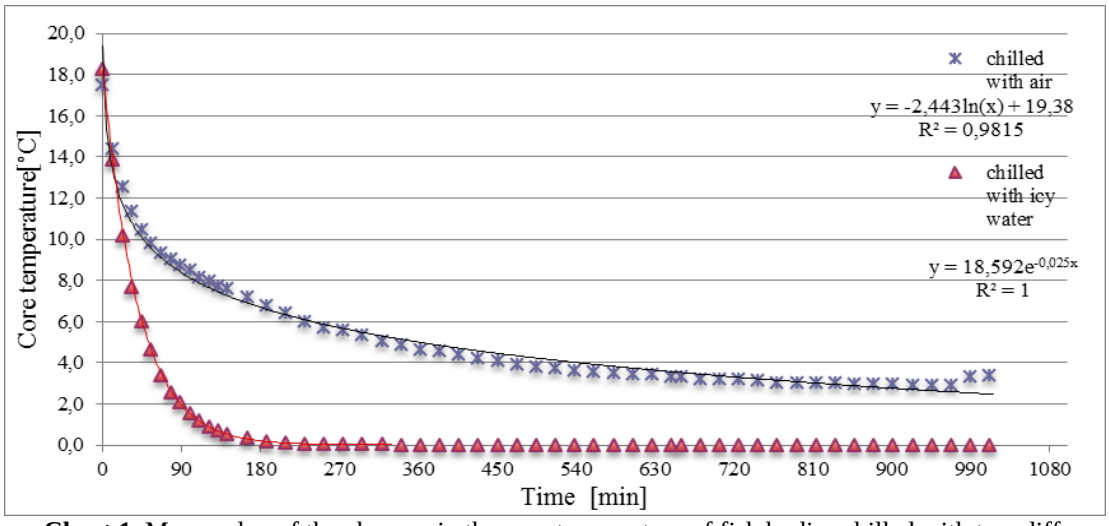

Chart 1. Mean value of the changes in the core temperature of fish bodies chilled with two different methods as function of time (C° / minute)

The initial slowing of the functioning of enzyme phosphatase, which is indicated by the lower K index value in the fish chilled with icy water (Chart 2), exerted a favourable effect throughout the storage. This is partly the result of the prolonged existence of the firmer texture due to the slower erosion of connective tissue proteins, and partly the smaller quantity of inosine and xanthine accumulating in the fish and causing the stale taint.

$$K = \frac{Hypoxantine + Inosine}{IMP + Hypoxanthine + Inosine}$$

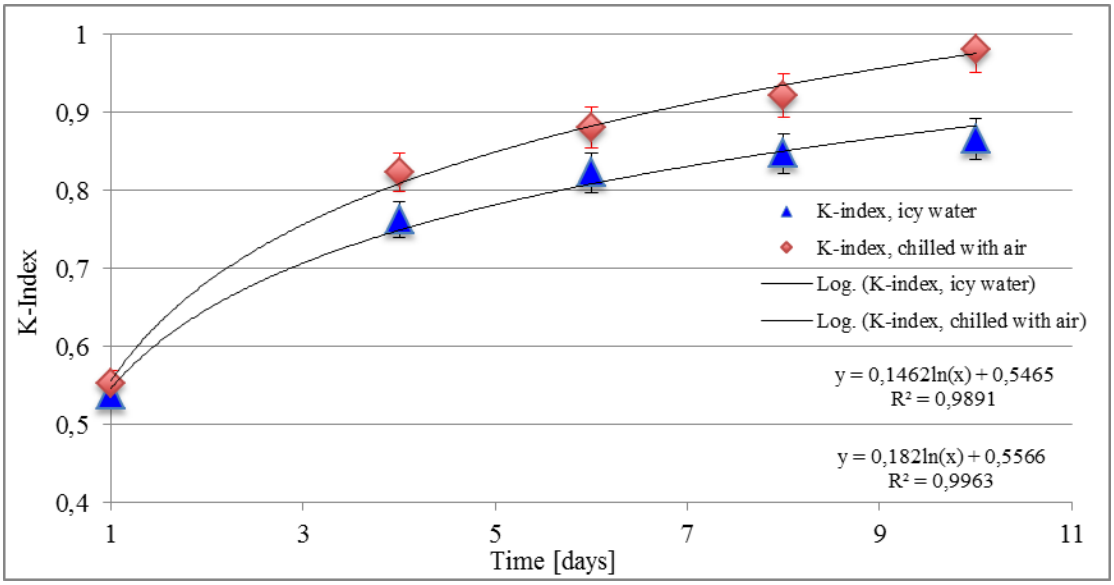

Chart 2. Changes in the K-index due to chilling with icy water and air as function of time (days)

We have established that the K index, which indicates the freshness of carp (*Cyprinus carpio*) has a higher initial value than of other sea fish species. The result supports the experimental fact that in general carp preserves its organoleptic properties, which give rise to a sense of consumability, for a shorter period during storage.

Based on our results we propose the insertion of immediate chilling with icy water after the slaughter into the processing technology in any case, because this method is proven to slow the autolytic degradation, which affects shelf-life, and therefore to increase the shelf-life of the products The NOVOCIB PRECICE® Freshness Assay Kit can be applied as an objective measurement method in the field of quality control as well. However, it is required to determine the K-index calibration curve of carp by comparing the results of other methods, such as TVB-N and organoleptic investigation.

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INSPECTION OF THE DISTRIBUTION OF SOME QUALITY PARAMETERS IN WATERMELON SLICES USING HYPERSPECTRAL IMAGING

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SUMMARY

The aim of this preliminary study was to test the ability of hyperspectral imaging to scan the differences of acidity and sugar content in watermelon slices.
Intact fruits were cut in half, slices were divided radial into three parts according to its natural structure, on these three parts in multiple points pH value and Brix degree were measured. Hyperspectral images of slices were also acquired, on the images the same regions were selected to analyse average spectra.
Spectral data were preprocessed using moving average and standard normal variate transformation. Thereafter principal component regression and partial least squares regression was applied in order to find relationship between the measured quality parameters and the average spectra of the selected regions.

INTRODUCTION

Watermelon is a widely consumed, popular fruit because its sweet taste and high water content. The enjoyable taste of this fruit is mainly affected by its acid and sugar content. Commonly used methods to measure these quality attributes are the measurements of pH value and soluble solids content (SSC).
In the last decade the number of applications of new methods on the field of food quality inspection is increasing. These include mechanical, electrochemical as well as elecromagnetic technologies, like near-infrared spectroscopy and imaging techniques. (Abbott, 1999)

In a prewious rewiev advanced nondestructive techniques of watermelon quality assessment have been compared. VIS/NIR spectroscopy was found to be an important method in the field of internal quality inspection due to its applicability. (Sun, Huang, Xu, & Ying, 2010)

Internal colour-related parameters in the pulp of intact melons can be assessed using NIRS technology. (Sánchez, Torres, De la Haba, & Pérez-Marín, 2014)

A prototype to determine on-line and non-destructively SSC value of watermelon with visible and near infrared diffuse transmittance technique was developed using spectra in the wavelength range of 687–920 nm. (Jie, Xie, Rao, & Ying, 2014)

Hyperspectral imaging is a relatively new method in food science. It has the advantages of spectroscopy and imaging providing informations about spatial differences of the chemical composition in the examined object. Several applications in terms of fruit and vegetable quality inspection has been reviewed. (Lorente et al., 2011; Nicolaï et al., 2007)

In previous study melons harvested at different ripeness stages were examined using hyperspectral imaging in the wavelength range of 400-1000 nm and a distribution map of sugar content was created. The greatest (however inverse) correlation between sugar content and spectral intensity was find at 676 nm. (Sugiyama, Tsuta, & Sun, 2010)

MATERIAL AND METHODS

Intact fruits were cut in half, and two slices were cut out of two samples. Slices were divided radial into three parts according to its natural structure, on these three parts in multiple points pH value and SSC (Brix degree) were measured.
Hyperspectral images of slices were also acquired, on the images the same regions were selected with CuBrowser Matlab algorithm (Firtha&Éder, 2012) to analyse average spectra.

Hyperspectral images were recorded using a pushbroom HSI instrument (Headwall Photonics: Specim spectrograph, Xeneth InGaAs 14 bit sensor having 256*320 resolution) within the wavelength range of 900-1700 nm with 5 nm spectral resolution. Before the measurement a two point spectral calibration was carried out. On the selected regions of interest (Fig.2.) average spectra were saved with CuBrowser. Spectra were processed with a three point averaging to remove spikes then standard normal variate transformation was carried out.

Data of the two samples were processed separately using RStudio version 0.99.473 software (pls package). To analyse spectral data principal component regression (PCR) and partial least squares regression (PLSR) were applied.

PLSR and PCR are both methods to model a response variable when there are a large number of predictor variables, and those predictors are highly correlated or even collinear (such as spectral data). (http://www.mathworks.com)

RESULTS AND DISCUSSION

Assessment of pH value

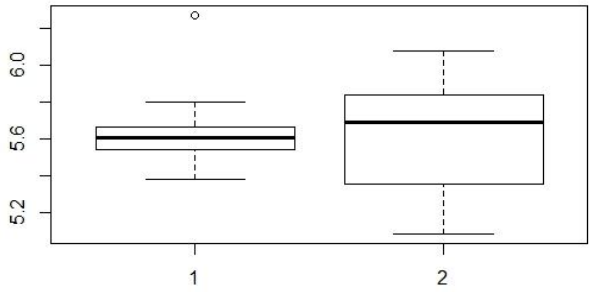
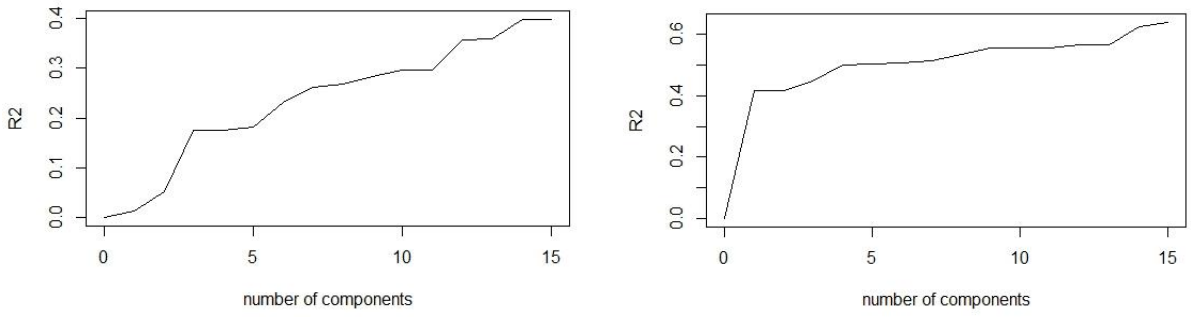


Figure 1.: Boxplot of the pH value of the samples

There are minimal differences in pH value of the first sample (Fig.1.). Amongst these data there is an outlier, this value was measured at the edge region, but it is not far from the maximum value of the second sample. The values of second sample show greater differences.



a., b.,

Figure 2.:. Determination coefficient (R^2) of fitted PCR model on the pH value data of first (a.,) and second (b.,) sample

The value of R^2 at the data of the first sample reaches the value of 0.4 using 15 components, the value of the RMSEP is around 0.1 (Fig.2.), however the variance of the data is also small and the outlier can also decrease the efficiency of the fitting. The results of the second sample show better fitting, the R^2 is around 0.6 and the RMSEP is 0.15 using 15 components (Fig.3.).

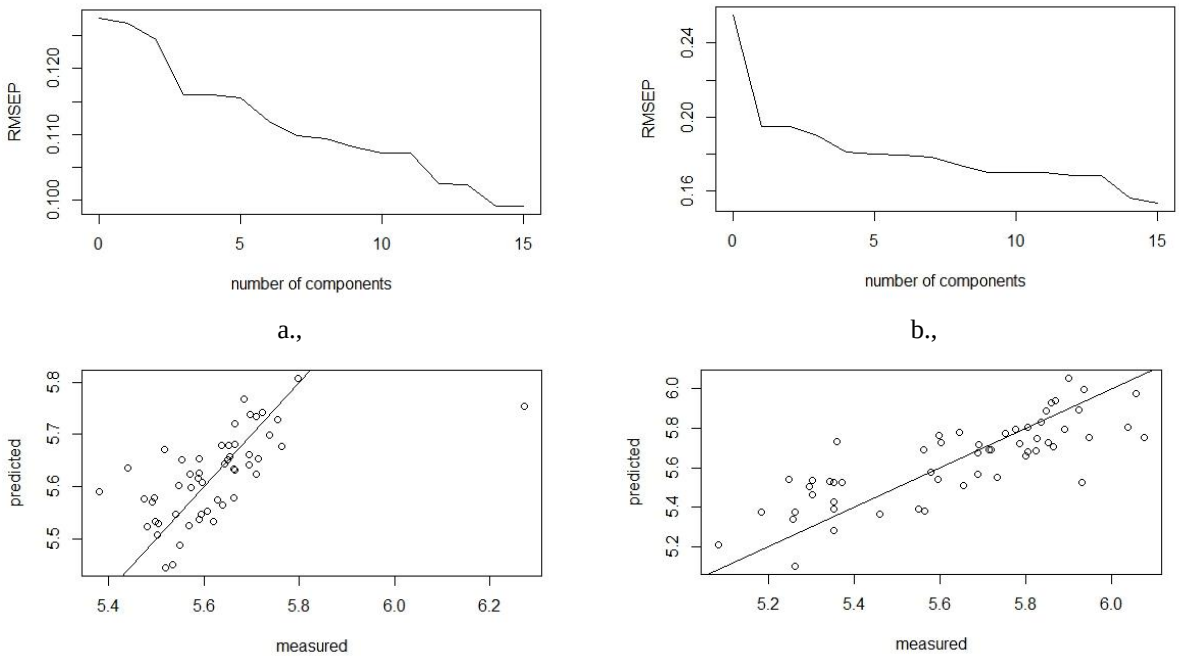


Figure 3.: Root mean square error of prediction values of first (a.,) and second (b.,) sample and prediction plot (with 15 components) on the data of first (c.,) and second (d.,) sample of fitted PCR model on the pH value

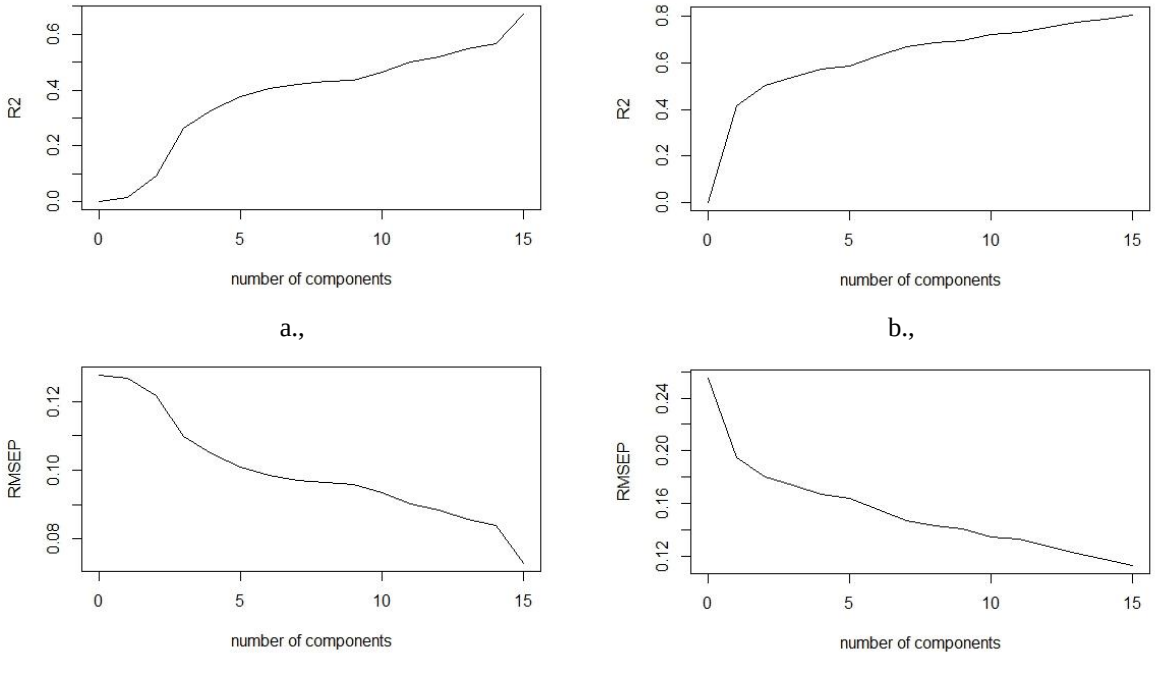
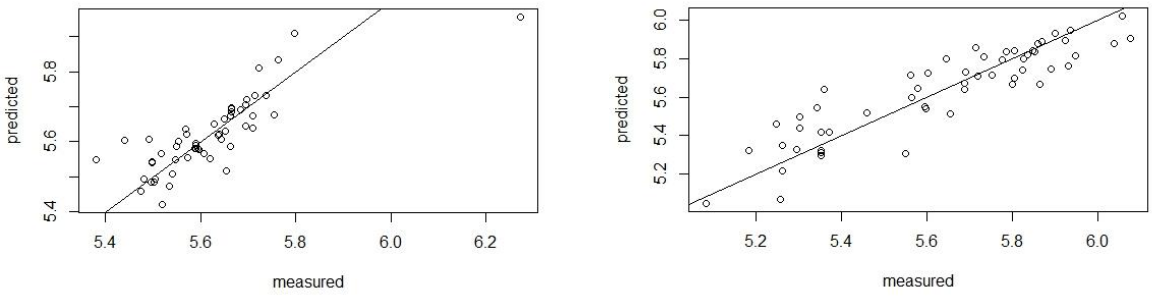


Figure 4.: Determination coefficient (R^2) by first (a.,) and second (b.,) sample and root mean square error of prediction values by first (c.,) and second (d.,) sample of fitted PLSR model on the data of pH value

Using PLSR method better model performance was reached (Fig.5.): the value of R^2 was above 0.6 at the first sample and around 0.8 at the second sample, although the RMSEP was slightly greater than using PCR (Fig.4.).



a., b.,

Figure 5.: Prediction plot of fitted PLSR model with 15 components on the pH value data of first (a.,) and second (b.,) sample

3.2 Assessment of Brix value

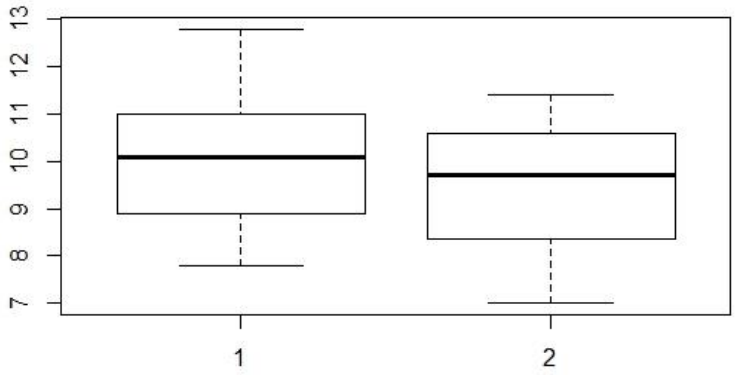
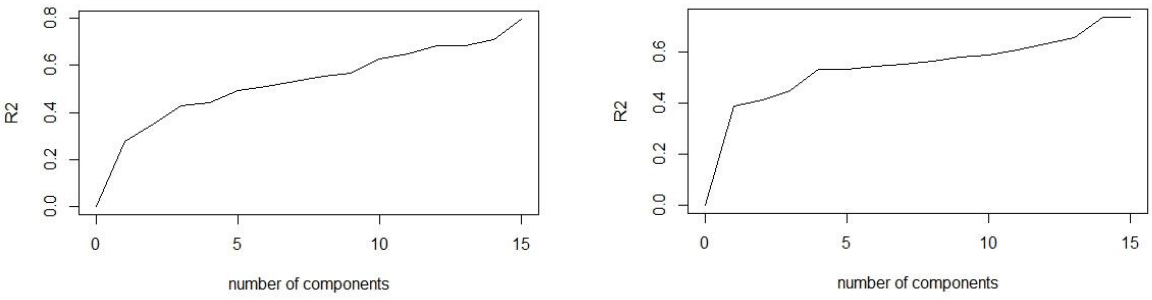


Figure 6.: Boxplot of the Brix value of the samples

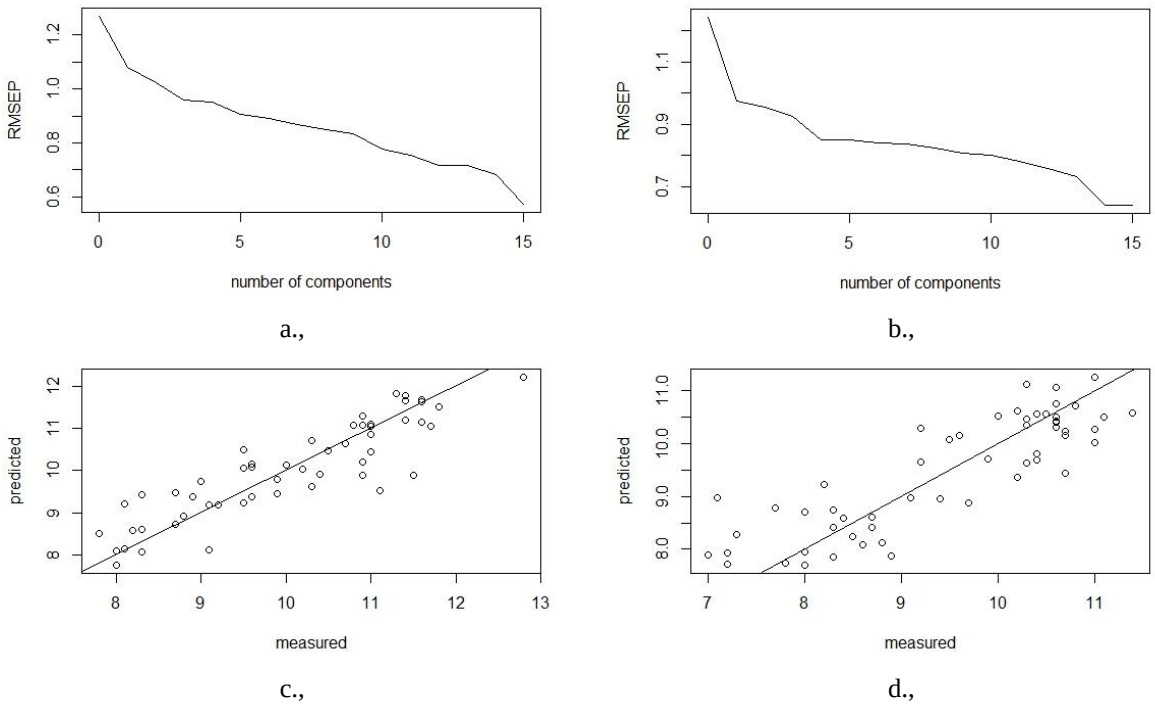
The distribution of brix value by the two samples does not differ to a high degree (Fig.6.).

The fitting of PCR model at the Brix degree was more successful, than at pH value. The R^2 was 0.8 by the first and 0.7 by the second sample, while the RMSEP was 0.57 by the first and 0.64 by the second sample, using 15 components (Fig.7., Fig.8.).



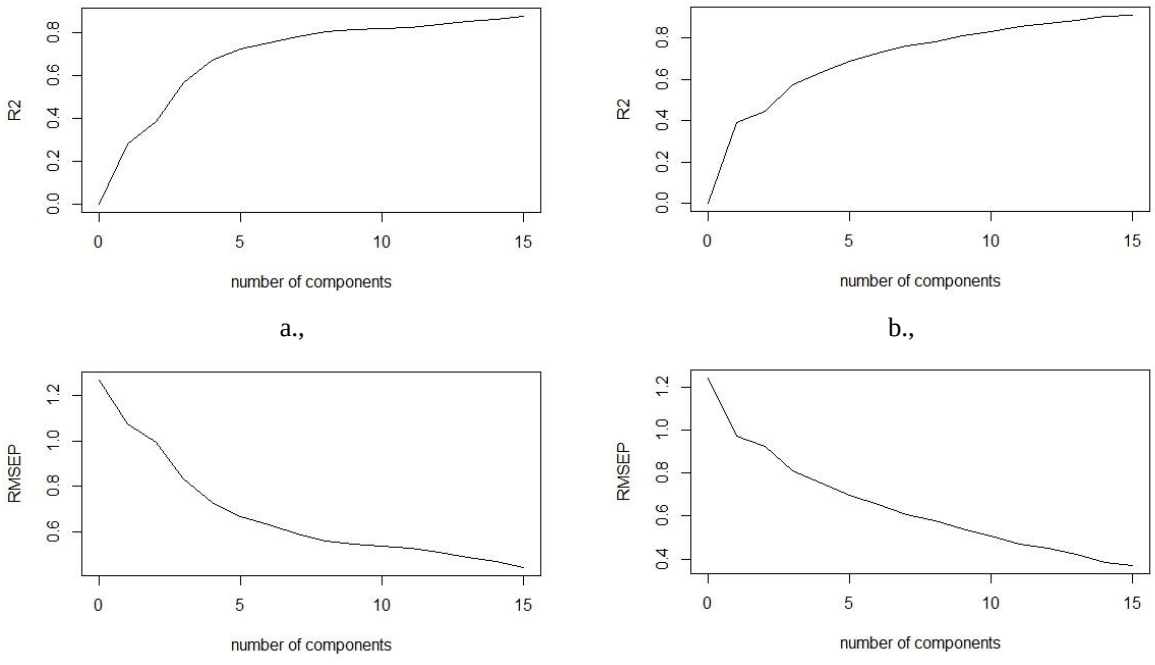
a., b.,

Figure 7.: Determination coefficient (R^2) of fitted PCR model on the Brix value data of first (a.,) and second (b.,) sample



a., b., c., d.,

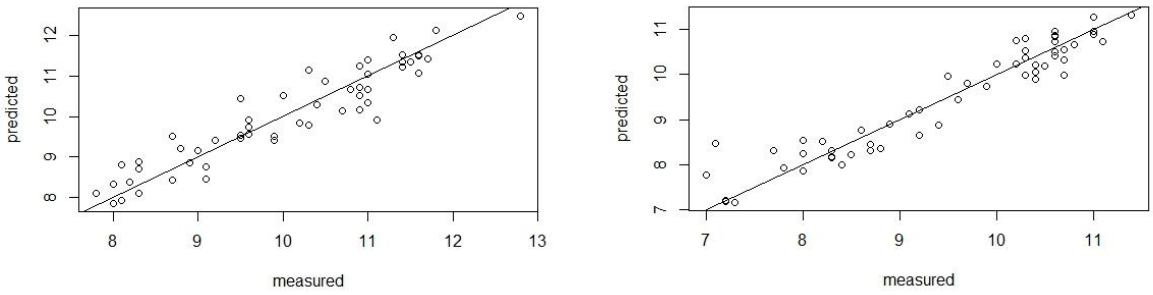
Figure 8.: Root mean square error of prediction values by first (a.,) and second (b.,) sample and prediction plot of fitted PCR model with 15 components on the Brix value data of first (c.,) and second (d.,) sample



a., b., c., d.,

Figure 9.: Determination coefficient (R^2) by first (a.,) and second (b.,) sample and root mean square error of prediction values of fitted PLSR model on the Brix value data of first (c.,) and second (d.,) sample

Tha analysis of Brix data was also more successful with PLSR model than using PCR (Fig.10.). The R^2 was 0.88 at the first sample and 0.91 at the second sample using 15 components, while using 5 components its value was 0.73 at the first and 0.69 at the second sample. The RMSEP was 0.44 Bx° at the first and 0.37 Bx° at the second sample (Fig.9.) using 15 components.



a., b.,

Figure 10.: Prediction plot of fitted PLSR model with 15 components on the Brix value data of first (a.,) and second (b.,) sample

CONCLUSIONS

The prediction of acidity by the first sample was difficult, the data were very close to each other and there were an outlier (according to the boxplot), however its value was not much greater than the maximum value of the second sample.

PLSR model performed in both cases better, than PCR model.

Quality attributes could be predicted more accurately by means of having more data points in a wider range of the predicted value. The objective of this study was to monitor the differences in one sample using hyperspectral imaging, in this case the way to collect data in a wider range can be the measurement at different ripening stages.

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EVALUATION OF RHEOLOGICAL PROPERTIES OF XYLO-OLIGOSACCHARIDES

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SUMMARY
Aim of study was to investigate rheological properties of xylo-oligosaccharides (XOS) a prebiotic dietary fiber currently being under authorization in EU and in Hungary. Three types of XOS was used in experiments: two powders (70% - 95%) and a liquid syrup (70%). Elementary tests showed that all types of XOS had good water solubility and did not affect significantly colour or turbidity of solutions at low concentrations. In the temperature range between 4-90°C XOS proved to have higher viscosity than fructo-oligosaccharides (FOS) widely used prebiotics in food industry. Results indicated that XOS a new prebiotic fiber could be appropriate for increasing nutritional value of food products. XOS showed no detrimental effect on the appearance of food products and improved their texture predicting its widespread utilization in food industry.

INTRODUCTION

The plant by-products (bagasse, corn cob) are rich in lignocellulose including xylan which can be enzimatically hydrolysed to xylo-oligosaccharides (XOS). XOS are non-digestible oligosaccharides that are proven to have prebiotic effect. In the European Union XOS is under authorization as novel food ingredient.

Aim of research was to investigate the rheological behavior of XOS in aqueous medium and in presence of texture modifiers using different rheological methods. (Nabarlatz, D et al., 2007; Vázquez, M. J. et al., 2000)

MATERIALS AND METHODS

Three types of XOS: 95P (powder, contain 95% XOS), 70L (syrup, contain 70% XOS) 70P (powder, contain 70% XOS) and as control fructo-oligosaccharide FOS (syrup, contain 85% FOS) and commercially available sucrose were used in the experiment.

In order to evaluate potential synergent effect with texture modifiers oscillation rheological tests were performed using commercially available thickening agents: pectin (PE), xanthan (XA) and locust bean gum flour (LB).(Table 1.)

Table 1.: Summary of measurement methods and the composition of samples

Measurement	Rheological method	Samples	Control	XOS Concentration (w/w%)
Type effect	rotation	3 XOS	FOS Sucrose	70
Concentration effect	rotation	3 XOS	FOS	0,5;5;50;70
Temperature effect	rotation	3 XOS	FOS	50
Synergent effect	oscillation	3 XOS + XA 3 XOS + LB 3 XOS + LB+XA 3 XOS + PE	XA (2%) LB (2%) LB + XA (1+1%) PE (2%)	2 2 2 2

The measurement was carried out with Physica MCR 51, Anton-Paar rheometer. For the rotational measurement concentric cylinder measuring system (CC27+ C-PTD) was used. The flow curve (shear stress in function of the shear rate) of the samples was recorded at 20°C, the

shear rate increased between 400-1200 1/sec and 1200-400 1/sec. During the test of temperature dependence in 4-90°C interval the shear rate was constant: 1000 1/s and concentration of samples was 50 w/w%.

Oscillation tests were performed with a plate and plate (P-PTD200 and PP50/S) measuring system using 1 mm gap size. The amplitude sweep method was performed at 4°C, at constant angular frequency (10 rad s^{-1}), increasing strain from 0.01 to 200%. Storage (G', Pa) and loss modulus (G", Pa) were recorded in function of shear stress (τ_0, Pa). Results were recorded and analysed using Rheoplus software ver 3.2.

Based on Herschel-Bulkley model fitted to the rotational measurement results apparent viscosity (K Pa.s), flow index (n) and yield stress (τ, Pa) were determined. During the evaluation of oscillation measurement data of the samples Crossover and initial G' and G" values were calculated. (Mezger, 2006)

The statistical analysis was performed using R.software environment, version 3.2.2. Because of not normally distribution of the data Kruskall-Wallis test was used to evaluate effect of certain factors (type, concentration etc.) Wilcoxon rank test was used for comparisons of control sample to XOS containing samples in pairs, at 95% confidence level. Examining the differences between the XOS types Student's t test was used with Excel software.

RESULTS AND DISCUSSION

Type effect

XOS 95P and 70P showed a flow curve hysteresis (thixotropic behavior), while 70L, FOS and sucrose did not. Based on the Herschel-Bulkley model fitted, the flow index (n) were around 1, i.e. the samples are considered Newtonian fluids. The apparent viscosity (K) values based on analysis of variance showed a significant difference ($p = 8.19 * 10^{-19}$), indicating that type of the non-digestible oligosaccharides affected the rheological properties. Compared the types to each other, the Student's t-test also established that there is a significant difference between the various types.

Table 2. Herschel-Bulkley parameters of different oligosaccharide solutions

Samples	K (Pa.s)	n	R^2
FOS	0,085±0,005	0,999	0,999
70L	0,013±0,003	1,100	0,995
95P	0,78±0,004	0,985	0,998
70P	2,51±0,009	0,980	0,999
Sucrose	0,72±0,020	0,987	0,999

Based on the measurements and the calculated values the 95P and 70P samples showed higher viscosity than the FOS and sucrose control samples. The 70L had the lowest value. The viscosity of the sample 95P was significantly higher than the viscosity of the 70% XOS samples suggesting that the rheological properties are influenced by the XOS concentration. The quantity of XOS was the same in 70L and 70P, indicating that the supplementary materials –such as maltodextrin- also affect the viscosity of the products. Xylo-oligosaccharides proved to have better texture modifier potential than fructo-oligosaccharides and the 95% XOS had the same values as sucrose.

Concentration effect

The apparent viscosity of the solution of three XOS products and FOS in function of the concentration showed similar changes. Viscosity values slightly increased until 50% concentration and then sharply increased until 70%. Based on the results XOS 70P proved to have the strongest texture modifier effect among the samples tested. (Fig. 1.)

The results of these measurements could be utilized in the production technology development, because viscosity of XOS types may show up to order of magnitude differences. Therefore in case of XOS application in food or planning the material transport this aspect must be taken into account.

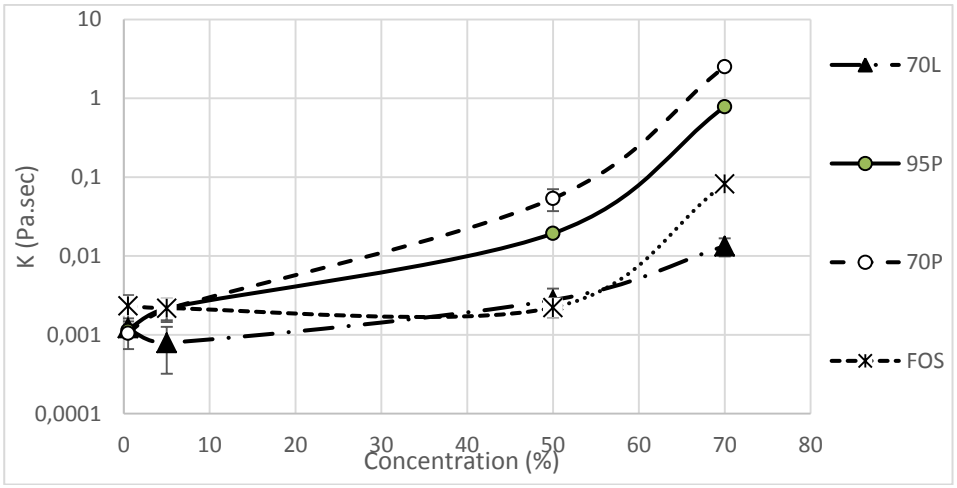

Figure 1.: Changes of apparent viscosity of XOS and FOS aqueous solutions in function of concentration

Temperature effect

According to Park et al. (2001) the viscosity of XOS is more stable against temperature than that of sucrose. At the beginning of the measurement, XOS 95P had the highest shear stress value and FOS the lowest. The viscosity of FOS and of XOS 70L slightly decreased until 50°C and then was constant until the end of the measurement. The viscosity of XOS 70L and 95P increased between 4-10°C. Further increase of the temperature up to 30°C resulted marked decrease of viscosity. Above 30°C both of curves showed continuous decrease. Results indicated that though viscosity of XOS samples was higher than that of FOS the latter was more resistant to temperature increase. (Fig. 2.)

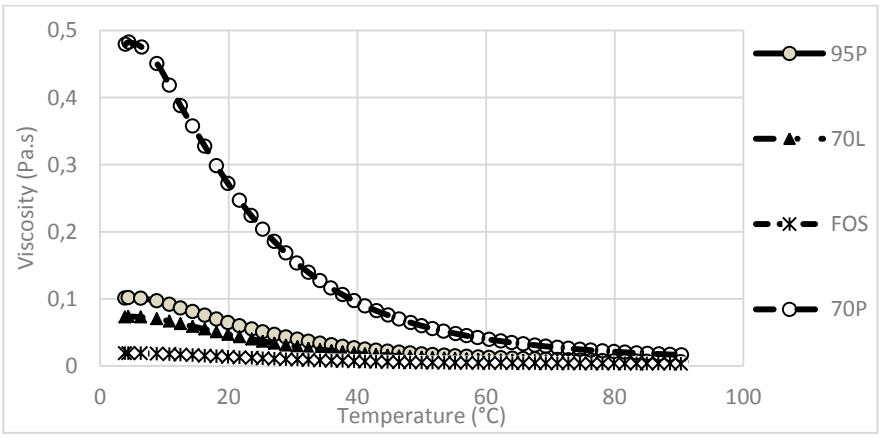

Figure 2.: Changes of viscosity of XOS and FOS aqueous solutions in function of temperature

Whether these results is positive or negative for XOS depends on the application: at refrigerator or at room temperature the XOS increase the viscosity of a foodstuff more than FOS, but at higher temperatures (for example in ready-to-eat meals or custards) there is no significant difference between the texture modifier effect of two kinds of oligosaccharide.

Synergent effect

Two parameters of the oscillation rheogram of gels produced by using four different carbohydrate-based texture modifiers were evaluated. The crossover, which refers to the stability of the sample against high deformation force, and G_0'/G_0'' ratio, which indicates the stability of the sample in rest. The mean of the measured results, the standard deviation and the significant difference are shown in Table 3.

Table 3.: The two calculated average values of the oscillation's parameters

	Xanthan		Locust bean gum		Xanthan+ Locust bean gum		Pectin	
	G'/G"	Cro	G'/G"	Cro	G'/G"	Cro	G'/G"	Cro
0	2,41±0,2	445±8,7	1,18±0,04	295±20,1	4,08±0,2	574,±0,01	4,27±0,5	444±12*
95P	2,53±0,1	451±8,2	1,23±0,06	285±5,1	4,51±0,5	511±12	5,33±0,6	613±17*
70L	2,38±0,1	457±5,8	1,20±0,03	272±0,9	4,08±0,8	515±9,7	5,83±0,9*	1208±34*
70P	2,16±0,1	458±5,8*	1,20±0,06	278±8,1	4,35±0,1	461±38	4,89±0,3	671±19*

* Significant difference when compared to the control (student's t-test, 95% confidence level)

In case of samples made with LB and LB+XA the XOS addition did not significantly change either positively or negatively the sample stability and initial hardness. Stability of xanthan gel was slightly increased by addition of 70P. XOS addition increased both the initial and flow stability of the pectin gels suggesting a synergent effect between XOS and pectin.

It can be concluded that the XOS addition did not influenced negatively the texture modifier potential of gelling agents widely used in food industry and in case of pectin it had a positive effect on that.

CONCLUSIONS

Based on the rheological measurement results XOS 95P and XOS 70P have higher viscosity than fructo-oligosaccharide a prebiotic widely used in food industry. Furthermore, in the case of the concentrated solution that two samples have better values. At low (4-10 ° C) and at high (60-95°C) temperatures XOS products showed better texture modifier potential than FOS. In gels produced with carbohydrate-based texture modifiers XOS did not affect negatively the gelling properties. Based on this study there is no technological limitation of XOS application in food products.

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EFFECT OF EXTRUSION VARIABLES ON HARDNESS OF BEAN-BASED EXTRUDATES

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SUMMARY

A blend of semolina bean (50%), einkorn wheat (40%), and buckwheat (10%) was extruded in a laboratory single screw extruder (Brabender 20 DN, Germany) with screw diameter 19 mm and die diameter 3 mm. Effects of moisture content, barrel temperature, screw speed, and screw compression ratio on hardness of bean-based extrudates were studied. Response surface methodology with combinations of moisture content (16, 19, 22, 25, 28%), barrel temperature (120, 140, 160, 180, 200ºC), screw speed (120, 140, 160, 180, 200 rpm), and screw compression ratio (1:1, 2:1, 3:1, 4:1, 5:1) was applied. Feed screw speed was fixed at 50 rpm. Feed zone temperature and metering zone temperature were kept constant at 100 and 140ºC, respectively.
The hardness of the extrudates was measured with a TA.XT Plus Texture Analyser, Stable Micro Systems. The textural profiles of the extrudates showed that screw compression ratio had the highest effect on the hardness.

INTRODUCTION

Mixtures of cereals and legumes are a good option not only as commercially valued product, but also as nutritious formulations. Grain legumes are important sources of food proteins (Berrios, 2006). In many regions of the world, legume seeds are the unique protein supply in the diet. From the nutritional viewpoint, all legume storage proteins are relatively low in sulphur-containing amino acids, methionine, cysteine and tryptophan, but the amounts of another essential amino acid, lysine, are much greater than in cereal grains (Duranti, 2006). Therefore, with respect to lysine and sulphur amino acid contents, legume and cereal proteins are nutritionally complementary (Lazou & Krokida, 2010).

In recent years one can observe wide interests in healthy food, namely dietetic, gluten-free, high-cellulose products or those fortified with new “natural” additives of high biological value (like buckwheat, einkorn wheat, etc.) (Ekielski et al., 2007).

Extrusion cooking is a modern high-temperature short-time (HTST) processing technology. It offers several advantages over other types of cooking processes, such as faster processing times and significant reduction in energy consumed, which consequently results in lower prices for the final products. The products of extrusion are of major importance in the food and feed industries today. Application of extrusion to legume flours is a relatively new area of investigation with the exception of soy bean (Lazou et al., 2007).

Despite increased use of extrusion processing, extrusion is still a complicated process that has yet to be mastered. Small variations in processing conditions affect process variables as well as product quality (Ding et al., 2006).

There are many research methods capable of presenting relevant data about the structure of the products. If force is used on food material, it is in the form of compression, shear or cutting, or a combination of shear and compression (Brnčić et al., 2006; Simitchiev, 2013).

The objective of this study was to determine the effect of process parameters (moisture content, barrel temperature, screw speed, and screw compression ratio) on the hardness of bean-based extrudates.

MATERIAL AND METHODS

Raw materials and preparation

The raw materials einkorn wheat and buckwheat are provided and delivered by village of Lomets, municipality of Troyan, Bulgaria. The bean is variety "Bivolare" and it is grown in the Rhodope Mountains, Bulgaria.

Bean seeds, einkorn wheat, and buckwheat were ground using a hammer mill and passed through standard sieves to be obtained homogenized meals. The bean meal, einkorn wheat meal, and buckwheat meal were blended at a ratio of 50:40:10 (w/w/w). Samples of prepared composite meal were mixed with distilled water to be obtained various moisture contents (Table 1). The wet materials were placed and kept in sealed plastic bags for 12 h in a refrigerator at 5ºC. The samples were tempered for 2 h at room temperature prior to extrusion.

Table 1.: Independent variable values and corresponding levels

Independent variables	***Corresponding levels***				
	- 2	**- 1**	**0**	**+ 1**	**+ 2**
Feed Moisture Content (W), % - X_1	16	19	22	25	28
Final Cooking Zone Temperature (T),°C – X_2	120	140	160	180	200
Screw Speed (N), min^{-1} – X_3	120	140	160	180	200
Screw Compression Ratio (*K*) – X_4	1:1	2:1	3:1	4:1	5:1

Extrusion

The samples were extruded in a laboratory single screw extruder (Brabender 20 DN, Germany). The compression ratio of the screw was 1:1, 2:1, 3:1, 4:1, 5:1 according to the experimental design (Table 1). The extruder barrel (476.5 mm in length and 20 mm in diameter) contained three sections and independently controlled die assembly electric heaters. The screw speed was 120, 140, 160, 180, 200 rpm. Feed zone temperature and metering zone temperature were kept constant at 100 and 140ºC, respectively. The temperature of the extruder die was 120, 140, 160, 180, 200ºC. The feed screw speed was fixed at 50 rpm and the die diameter was 3 mm.

Hardness

Hardness of the extrudates was measured with a TA.XT Plus Texture Analyser (Stable Micro Systems Ltd., England) using a 50 kg load cell and a 2-bladed Kramer shear cell. The test settings were as follows: Pre-test speed 2 mm/s, Test speed 1 mm/s, Post-test speed 10 mm/s.

A force-distance curve was recorded and analyzed by Texture Exponent 32 to calculate the peak force. The highest value of force was taken as a measurement for hardness.

Twenty randomly selected samples of each extrudate were measured and an average taken.

Experimental design and data analysis

The effect of extrusion variables on hardness of bean-based extrudates was investigated using response surface methodology. A regression model is the following:

$$y = b_0 + \sum_{i=1}^{n} b_i.x_i + \sum_{i=1}^{n} b_{ii}.x_i^2 + \sum_{i=1}^{n} \sum_{j=1}^{n} b_{ij}.x_i.x_j \qquad \text{i)}$$

where b_0, b_i, b_{ii} and b_{ij} are constant coefficients.

RESULTS AND DISCUSSION

The average hardness values of the extruded samples are given in Table 2.

The results of the statistical analysis of variance (ANOVA) for the hardness show that 5 effects have P-values less than 0.05, indicating that they are significantly different from zero at the 95.0% confidence level (Table 3). The R-squared statistic is 0.90; the standard error of the estimate - 11.86, the mean absolute error - 6.47.

Table 2.: Hardness of extrudates

№	*F, N*	*№*	*F, N*	*№*	*F, N*
1	80	**10**	96	**19**	91
2	84	**11**	130	**20**	103
3	71	**12**	112	**21**	112
4	96	**13**	101	**22**	78
5	69	**14**	79	**23**	51
6	77	**15**	131	**24**	104
7	59	**16**	89	**25**	78
8	57	**17**	147	**26**	77
9	89	**18**	145	**27**	76

Table 3.: Regression coefficients and analysis of variance for hardness of extrudates

	Regression coefficients	*Sum of squares*	*F - values*	*P – values*
Constant	621,567			
A: W	-47,412	80,667	0,57	0,4636
B: T	-0,921	368,167	2,62	0,1317
C: N	0,673	1120,670	7,96	0,0154*
D: K	-4,042	4816,670	34,23	0,0001*
AA	1,637	4628,230	32,89	0,0001*
AB	-0,035	72,250	0,51	0,4873
AC	-0,079	361,000	2,57	0,1352
AD	-2,292	756,250	5,37	0,0389*
BB	0,006	131,120	0,93	0,3534
BC	-0,008	156,250	1,11	0,3127
BD	0,388	961,000	6,83	0,0227*
CC	0,005	83,565	0,59	0,4558
CD	0,131	110,250	0,78	0,3934
DD	-2,396	122,454	0,87	0,3693

* Significant at $P < 0.05$

Each of the estimated effects and interactions are shown in the standardized diagram (Fig. 1). The linear effect due to the screw compression ratio had mostly influence on the hardness followed by quadratic effect of the feed moisture content and linear effect of the screw speed.

The effect of extrusion conditions on hardness of extrudate are shown in Fig. 2.

Screw compression ratio was found to have the most significant effect on extrudate hardness which corresponds with the results established from Toshkov (2011).

Feed moisture was found to have a significant effect on hardness. It is well known that the decrease of moisture content in extrusion tends to increase specific mechanical energy, and consequently to favor macromolecular degradation of starch though dextrinization. The resulting melt then gives more fragile structures leading to low resistant cell walls and more structural fractures. In contrary, lentil flour (protein source) resulted in more rigid structures

and elevated values of elastic modulus. Similar results have also been reported for other protein based extrudates (Lazou & Krokida, 2010).

Screw speed was also found to have a significant effect on hardness. An increase in screw speed resulted in an extrudate with lower hardness. It is possible that an increase in screw speed may be expected to lower the melt viscosity of the material in the extruder, resulting in a less dense, softer extrudate. The results correspond with these established from Fletcher et al. (1985) and Ding et al. (2006).

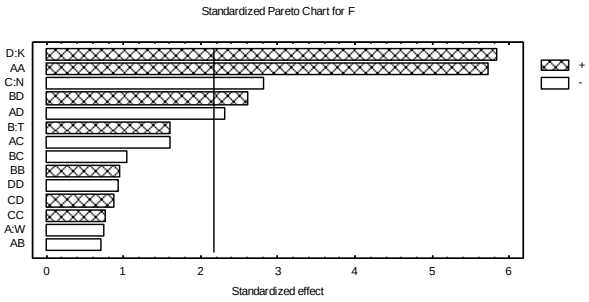

Figure 1.: Estimated effects of regression model coefficients on the hardness

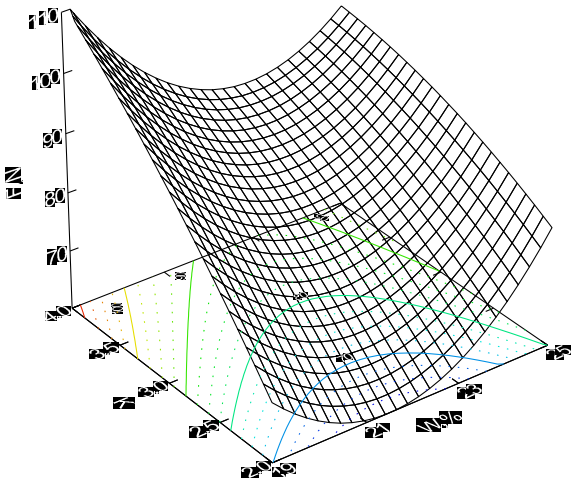

Figure 2.: Effect of feed moisture and screw compression ratio on the hardness

CONCLUSIONS

The effect of extrusion variables on the hardness of bean-based extrudates was studied. The linear effect due to the screw compression ratio had mostly influence on the hardness. The hardness values of extrudates vary from 51 to 147 N.

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FUNGAL CONTAMINANTS OF IRRADIATED FOODS MADE FOR IMMUNO-COMPROMISED PATIENTS

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SUMMARY

Immuno-compromised (IC) patients could be prone to more serious infections than healthy people, therefore low-microbial diets (LMDs) are recommended in order to reduce the risk of infections. Although fresh fruits and dairy products are very important sources of nutrients, IC patients have to avoid them because of the presence of microbes. One of the possibilities to introduce these foods to the patents' diet is their decontamination by ionising radiation.

Cottage cheese and raspberry fruit samples were gamma irradiated by low irradiation doses (1, 2 and 3 kGy). The applied treatments could efficiently reduce the number of fungi, although yeasts and moulds could be isolated from the samples and identified by molecular methods. Among these isolates opportunistic pathogen species like Candida inconspicua and Candida lusitaniae have occurred, which draw the attention to safety problems of these food products.

INTRODUCTION

Food irradiation could enhance the microbial safety of food products as well as extend their shelf life without significantly altering the nutritional properties (Farkas, 2006, Farkas and Mohácsi-Farkas, 2011). These facts have particular importance in case of immuno-compromised (IC) patients, whose natural defence responses against the pathogens could be very limited, therefore low-microbial diets (LMDs) or sterilized foods are recommended to consume in order to reduce the risk of infections (Vicenski et al, 2012; http://demeterhaz.hu). Although fresh fruits and dairy products are important sources of nutrients, IC patients have to avoid them because of the presence of microbes, therefore in development of foods for IC patients the microbial control is crucial (IAEA, 2009).

It has been shown that ionizing irradiation is a suitable method to control food-borne pathogens in foods for immuno-compromised patients (Lee et al., 2012, Park et al., 2015). For food product development it has to be taken into account that radiation sensitivity of the microorganisms could be different and is influenced by inheriting properties such as chemical and physical structure of the cells, recovery abilities, etc. (Farkas, 2006). Although many research focused on the microbial safety of the irradiated foods, only limited numbers of the publications have been concerned with fungal contaminants. The aim of the present study was the identification of fungal survivors of gamma-irradiated freeze-dried raspberries and cottage cheese and determination whether they could be food safety hazards of these products.

MATERIALS AND METHODS

Irradiation treatment and isolation of fungal strains

The food irradiation treatments were performed on frozen (-18°C) cottage cheese (250 g) and raspberry (50 g; homogenised) samples by ^{60}Co irradiator with doses of 1, 2 and 3 kGy at AGROSTER Co. Ltd, Budapest. The irradiated food samples were purchased from the supermarket.

The irradiated and non-irradiated control samples were homogenised, diluted and plated onto Rose Bengal Chloramphenicol medium (**RBC**) (Merck). The plates were incubated at 25ºC and after 3 days the colony forming units were determined.

The yeast and filamentous fungal colonies with different morphological properties were chosen for isolation and pure cultures were obtained by streaking onto **YEPD** agar plates (0.25% (w/v) yeast extract, 0.5% (w/v) peptone, 1% (w/v) dextrose and 1.5% (w/v) agar). Yeast strains were maintained on **YEPD** while filamentous fungal isolates were kept on Complete Medium (**CM**: 20 ml 5x Czapek solution; 10 µl Vogel microelement solution; 0.3% (w/v) yeast extract, 0.3% (w/v) peptone, 0.3% (w/v) soya peptone, 2% (w/v) dextrose and 1.2% (w/v) agar; final volume was adjusted to 100 ml with distilled water).

Characterisation of isolates by traditional microbiological methods

In case of yeast isolates the cell and colony morphologies, hyphae or pseudohyphae formation, urease activity and growing capability at different temperatures were determined on the basis of standard protocols (Kurtzman et al., 2011). The isolates were also plated onto **WL** nutrient agar (Atlas, 1995) and incubated at 25ºC for 5 days. After incubation the isolates were characterised by their colony morphology, colour and acid production.

In case of filamentous fungal strains the colony morphology and colour, structure and organisation of the conidiophores, the shape of conidia, the growing activity at 5°C, 15°C, 25°C and 30°C temperatures were examined. For morphological studies the fungal strains were grown on **MEA** medium (2% malt extract, 2% glucose, 0.1% peptone, 2% agar) at 25°C. For determination of growth rate the isolates were inoculated in the middle of **CM** agar and incubated until the Petri dish was completely covered by mycelia. The growing activity of the strains was determined by measuring the colony diameter at every second day. This experiment was done in two replicates.

Molecular ribotyping and identification of fungal strains

The D1-D2 domain of 26S rDNA was amplified for **ARDRA** by using the primer pair NL-1 and NL-4 (Kurtzman and Robnett, 1998). The PCR reaction mixture (30 µl) contained 10 pmol of each primer and 100 ng of template DNA. The cycling conditions were as follows: initial denaturation (5 min at 95°C), 35 cycles of denaturation (95°C for 30 sec), annealing (51°C for 30 sec), extension (72°C for 1 min), and final extension (5 min at 72°C). The amplicons were digested by *Hae*III, *Rsa*I and *Scr*FI restriction enzymes and the obtained fragments were separated using 1,5 % agarose gel. Based on the results of morphological, physiological tests and ARDRA the isolates were grouped and a prominent member of each group was chosen for molecular identification.

Molecular identification was carried out by sequence analysis of the hypervariable D1-D2 domain of the large subunit (LSU). The resulting sequences were aligned with sequences in the molecular databank (www.ncbi.nlm.nih.gov) and identified by BLAST.

RESULTS AND DISCUSSION

The low dose gamma-irradiation treatment could reduce efficiently the fungal population of the examined food products. In case of cottage cheese the initial yeast population was 10^6 CFU/g. The 1 and 2 kGy irradiation treatments resulted in a 1 and 2 orders of magnitude reduction, respectively. Instead of significant cell death, the number of surviving fungi was higher than 50 CFU/g, which limit was recommended for IC patients' diet by researchers of the FAO/IAEA Co-ordinated Research Project. These results indicate that higher irradiation doses should be applied for increasing the microbial safety of cottage cheese for IC patients. The initial yeast and mould counts of the frozen raspberry were about $5*10^2$ CFU/g.

After gamma-irradiation using 1, 2 and 3 kGy the fungal numbers reduced 0.5, 1 and 1.5 orders of magnitude, respectively. In case of treatments with 2 and 3 kGy doses the final cell numbers were lower than 50 CFU/g, therefore these treatments sufficiently decreased the number of fungal biota below the safety limit.

For evaluation the microbial safety of the non-irradiated and irradiated samples the appearing fungal colonies with different properties were isolated. Altogether 23 yeast strains were collected from cottage cheese samples, while 12 yeast and 10 filamentous fungal strains were isolated from raspberry samples (Table 1).

Table 1. Results of isolation of fungal strains from gamma-irradiated food samples

Treatment	**Yeasts / Cottage cheese**	**Yeasts / Raspberry**	**Filamentous fungi / Raspberry**	∑
Control	9	6	2	**17**
1 kGy	6	2	3	**11**
2 kGy	8	2	4	**14**
3 kGy	-	2	1	**3**
∑	**23**	**12**	**10**	**45**

The isolates were characterised by traditional and molecular methods. Based on the results the strains were grouped. The 23 cottage cheese isolates formed 10 groups, while yeasts originated from raspberry belonged to three different groups. The filamentous fungal strains could be classified to five groups. One representative of each group was chosen for sequence analysis of the D1-D2 domain of 26S rDNA. For *species*-level identification only scores showed *>99% identity* with a reference *sequence were assumed*. The final identification was performed on the basis of morphological, physiological and molecular properties of the strains.

In case of the cottage cheese 8 species were identified, and among them opportunistic pathogenic species have occurred (Table 2.). Beside the frequently isolated *Kluyverymyces marxianus* and *Pichia fermentans* clinically important yeasts like *Candida inconspicua, Clavispora lusitaniae (*anamorf *Candida lusitaniae)* and *Malassezia globosa* were also identified. *C. inconspicua* has occurred only in the non-irradiated (control) cottage cheese samples, but the other opportunistic pathogen *C. lusitaniae* could survive the 2 kGy gamma-irradiation. Some isolates were identified as *Trichosporon* spp., however has to be mentioned that opportunistic pathogen species such as *T. asahii, T. asteroides, T. cutaneum, T. inkin, T. mucoides* and *T. ovoides* belong to this genus (Colombo et al, 2011).

Table 2. The identified species from cottage cheese samples

Treatment	**Control**	**1 kGy**	**2 kGy**
Identified specieses	*Pichia fermentans* *Kluyveromyces marxianus* ***Candida inconspicua*** ***Clavispora lusitaniae***	*Pichia fermentans* *Candida zeylanoides* ***Trichosporon* sp.** *Hanseniaspora uvarum*	*Hanseniaspora uvarum* ***Clavispora lusitaniae*** *Malassezia globosa*

The species diversity of the raspberry was lower than that of the cottage cheese. The dominant yeast species was *Hanseniaspora uvarum,* while the most frequently isolated filamentous fungi were species of *Cladosporium* and *Aspergillus* genera. From the samples irradiated by 2 and 3 kGy *Cryptococcus wieringae, A. glaucus* and *C. cladosporoides* were

derived. The species isolated from the raspberry samples do not have direct health risk for patients.

CONCLUSIONS

Low dose irradiation treatment is effective for reduction of the fungal community of fruits and dairy products, however in case of cottage cheese higher doses is recommended to reduce the microbial cell number under the required limits. The identified opportunistic pathogen yeast species like *Candida inconspicua* and *Candida lusitaniae* draw the attention to safety problems associated with cottage cheese. These findings emphasise, that more extended studies are needed for developing foods for IC patients. The studies have to concern the following questions: how the initial microbiota influences the microbial safety of the irradiated food products, which fungal species can contaminate the irradiated foods, what is the sufficient irradiation dose for elimination the opportunistic fungal pathogens in a particular food stuff.

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OPERATIONAL EXPERIENCES OF A WATERWORKS USING BIOLOGICAL AMMONIUM REMOVAL

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SUMMARY

Well water for drinking purposes generally needs certain water treatment processes before distributing into the water supply system. Besides iron, manganese and arsenic ions the produced well water often contains ammonium which has to be decreased according to the Hungarian Government Regulation and European Union Directives. The conventional chemical technique is the breakpoint chlorination which can generate several undesirable compounds such as trihalomethanes (THM), adsorbable organic halogens (AOX). Because of the stricter regulations and the environmental aspects more and more water treatment experts suggest biological ammonium removal systems to municipalities as a sustainable drinking water treatment for well water. In the case of biological ammonium removal systems the ultrafiltration is an outstanding opportunity to provide microbiologically safe treated water. In Hungary only a few of these waterworks have been established. The recent study provides details of the experiences gained at the starting operation period of such waterworks.

INTRODUCTION

Ammonium presence in groundwater results in having undesirable effects in the water for potable supply (Kollár and Ribári 1991). Generally, the waters originating from deep confined aquifers do not contain anthropogenic contaminants, however, due to the geological environment, the concentration of certain components (e.g., iron, manganese, ammonium, arsenic, natural organic matter) exceeds the maximum allowable concentration values, therefore the application of water treatment technology is needed (Takó 2001). In Hungary, Government Regulation 201/2001. (X. 25.) limits the content of ammonium ion in the treated well water for drinking water supply. The concentration limit value for ammonium is 0.50 mgl^{-1}. Conventionally, four techniques can be taken into consideration for decreasing the content of ammonium of well waters.

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MATERIALS AND METHODS

Water treatment plant containing ultrafiltration for ammonia removal

Figure 1. shows a water treatment facility for ammonium removal from well water. The processes of the technology are the following: aeration, multimedia filter, ultrafiltration, water storage tank.

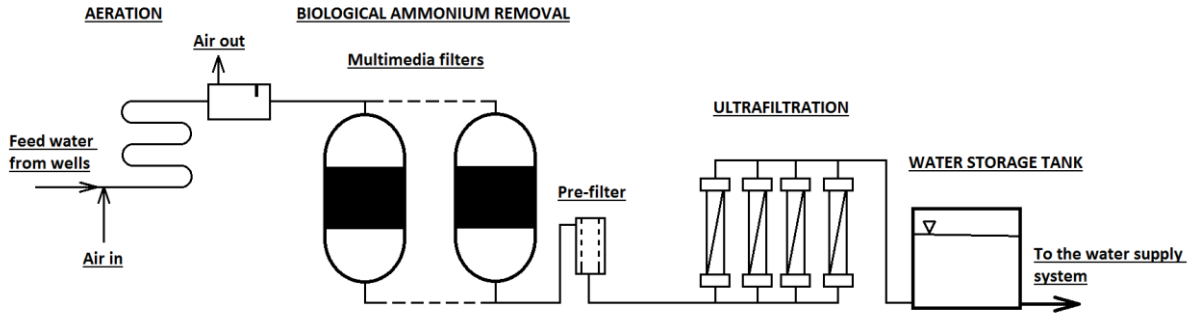

Figure 1.: Biological ammonium removal and subsequent ultrafiltration

As a first step, the raw water is saturated with air using compressor. After a sufficient contact time, residual air is removed by a mechanical degassing element. Then aerated water enters into multimedia send filter units. These units act as mechanical filters to retain undesirable solid particulates. In some cases iron and manganese removal takes place here.

Other main function of the multimedia filters is to provide necessary area for biofilm formation and growth. The microorganism cells adhere onto the surface of this media particles and start to metabolize ammonium for their homeostasis. As a result, ammonium content of the water is decreased drastically and nitrate ion is formed. Government Regulation 201/2001.
(X. 25.) limits the nitrate concentration value maximum 50 mgl^{-1} which is a relatively high concentration region. Since the formed nitrate ion concentration is much less than this specified value, usually there is no need for an additional nitrate removal process.

A subsequent physical barrier for retaining bacteria is indispensable and ultrafiltration with the pore size of 0.01-0.04 μm is an ideal solution for this purpose. A safety mechanical filter with the pore size of 200-300 μm has to be applied to preserve the ultrafiltration unit from mechanical damage. Finally, treated water is stored in a water tank and disinfectant such as sodium hypochlorite is added to avoid contamination and quality degradation.

RESULTS AND DISCUSSION

Background of ammonium removal from well water

Chemically driven processes are the breakpoint chlorination and a stripping procedure of ammonia gas by adjusting pH of the raw well water. In the case of breakpoint chlorination the process has two goals: ammonium removal and disinfection. A chlorine based oxidative agent, usually sodium hypochlorite solution or chlorine gas is dosed to the raw water and oxidizes the nitrogen atoms bounded in ammonium ions. As chlorine reacts with ammonium, chloramines are formed. Chloramines have less disinfection power and can cause unpleasant odor, thus, it is mandatory to oxidize further to finally form nitrogen gas, or they have to be removed by using granulated activated carbon (GAC) columns. After removing ammonium adding additional chlorine results in the appearance of free chlorine molecules. Besides chloramines, during chlorination toxic trihalomethanes (THMs) and adsorbable organic halogens (AOXs) can be formed, although, the concentration of these toxic compounds strongly depends on the organic content of the raw water and can also be removed by using GAC.

The main advantage of ammonium removal by using breakpoint chlorination technique is the relatively low capital costs, however, along with increasing ammonium level of the raw water increasing chemical costs are arisen.

Removing ammonia by gas stripping is based on the dissociation of ammonium ion in water. Ammonia nitrogen exists in aqueous solution as either ammonium ion or ammonia. The ammonium ion is largely predominant at neutral or slightly basic pH: the ratio of the ammonium to the ammonia concentration is equal to 100:1 at a pH of 7.4 and a temperature of 20°C. This equilibrium is the basis of the ammonia-stripping process, in which the pH of the water is raised to 10-11 and the $NH_{3(aq)}$ formed is removed from the solution by water/gas exchange. The buffer capacity of Hungarian waters are relatively high, thus, high amount of base has to be dosed into raw water in order to reach a certain pH value. After removing ammonia, the pH value of the treated water has to be neutralized and it needs an additional chemical dosage (Takács 2012). Therefore, ammonia stripping for drinking water treatment is a relatively costly method.

Adsorption, as a physico-chemical process, is also a competitive process to remove ammonium from raw water. Zeolite has been used for decades to decrease the concentrations

of ammonium in municipal effluents (Mercer et al. 1970) and, more recently, in freshwater (Burgess et al. 2004). Clinoptilolite is one of the natural zeolites used to remove the cations and ammonium ions from aqueous solutions. Rahmani et al. pointed out in their study that the results obtained from the cation selectivity of zeolite present high selectivity of the Clinoptilolite toward K^+ and NH_4^+ while much lower selectivity towards Na^+, Ca^{2+} and Mg^{2+}. Therefore, it can be stated that ammonium ions can be removed efficiently by zeolites, however, this technique is not popular yet because the sufficient subsequent regeneration procedure of the zeolite is rather expensive, and the competitive ion load of water also increases the costs.

Biological ammonia removal is more environmentally friendly and more cost effective than chemical and/or physico-chemical methods. The nitrifying microorganisms belong to the chemoautotroph bacteria. They use the inorganic carbon dioxide as carbon source and ammonium is used as energy source. The nitrification process consists of two steps. As a first step the "ammonia oxidizing bacteria" (*Nitrosomonas,* in addition *Nitrosospira, Nitrosococcus, Nitrosolobus* and *Nitrosovibrio*) oxidizes the ammonium content of the water to nitrite. In the second step the nitrite-oxidizing bacteria (*Nitrobacter,* in addition *Nitrospira, Nitrospina* and *Nitrococcus*) oxidizes the nitrite to nitrate. As a result of the previous reactions the released energy is used for the life processes of the nitrifying microorganisms. The reaction requires dissolved oxygen and appropriate temperature. A minimum 2 mgl^{-1} of dissolved oxygen concentration is needed to operate nitrifying "biofilters" properly (Takó 2001). Usually, sand filters are used to filter out the microorganisms. Applying ultrafiltration for removing microorganisms is a recent hybrid technology

Operational experiences of a waterworks using biological ammonium removal

Start-up operating phase of the water treatment system is a crucial period. In the case of biological ammonium removal technology, a continuous water stream needs to be provided to the facility for the effective growth of the microflora. As a first step, ammonia oxidizing bacterial cells start to grow and oxidize the ammonium to nitrite, however, nitrite-oxidizing bacterial cells require more time for having a metabolism. If the waterworks produces treated water directly to the drinking water supply system, the level of nitrite has to be monitored continuously. Furthermore the amount of nitrite has to be eliminated by chemical oxidation.

Chemical oxidation of nitrite can be performed by using breakpoint chlorination method. However, it is important to note that if a considerable amount of natural organic matter can be found in the raw well water, THMs and AOXs can be formulated by the chlorination, thus, it is recommended to avoid chlorine dosage or additional GAC treatment has to be applied.

Breakpoint chlorination process is necessary to carried out until both of the ammonia oxidizing bacterial cells and nitrite-oxidizing bacterial cells are metabolizing continuously. It is easy to recognize because the free chlorine concentration is increasing. If the biological ammonium removal operates properly, more than 90-95% of ammonium can be eliminated from the raw well water and can be convert to nitrate. Furthermore, nitrite concentration of the treated water has to be close to zero, or zero. It is important to note that the technology is strongly depends on the temperature and the dissolved oxygen concentration, thus, these parameters always have to be set properly.

CONCLUSIONS

Biological ammonium removal is a conventional technology, however, other techniques are also existed in the water treatment sector for the same purpose. Chemical and physico-chemical processes requires high amount of chemical dosage which can lead environmental risk and ecosystem hazard. Eliminating ammonium load of a well water can be realized by

using biological metabolic routes, and the physical barrier for removing bacteria can be ultrafiltration. Such waterworks can produce high quality of drinking water and in some cases shows great opportunity to replace chemically driven ammonium removal processes.

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EFFECT OF DIFFERENT TEMPERATURE-TIME COMBINATIONS ON COLOUR FEATURES OF SOUS-VIDE COOKED BEEF ROUND

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SUMMARY

Sous-vide cooking (low temperature-long time treatment of vacuum packaged meats) has emerged as a popular technique during the last two decades in catering services and food processing, to provide ready-to-eat foods of superior sensory quality with a longer shelf-life. It has a tenderizing effect, while the myofibrillar based toughening is still not very intense. Nevertheless due to the protein denaturation, colour change also occurs. In our study the meat slices were cooked sous-vide applying different combinations of temperature (52, 60, and 68°C) and time (9, 12, 15, 18, 21 and 24 h). Two heating methods were applied: water bath and steam cooker. As a consequence of heating, lightness (L*) and yellowness (b*) values largely increased in most cases. As the cooking temperature increased the more reduction in the redness values were measured. We could also observe that main changes in the colour parameters occured in the first quarter of the heat treatment.

INTRODUCTION

The sous vide system appears as an interesting alternative to produce ready to eat beef products (García-Segovia et al., 2007 and Vaudagna et al., 2002). Sous-vide processing is based on the application of a controlled cooking-pasteurization process to raw material (or raw material with intermediate foods) vacuum packaged in a heat-stable pouch or container (Hauben, 1999). After the treatment, the product is rapidly cooled at temperatures around 0–3 C, and under this condition it could be stored for 3–5 weeks before reheating and consumption (Nyati, 2000). Heat treatment at low temperatures between 50°C and 60°C for prolonged times (LTLT) increases tenderness of beef. The LTLT treatment method is increasing in popularity in the catering industry and among chefs due to the possibility of increased consistency along the muscle as well as appealing texture and colour of the meat (Christensen et al., 2012 and Mortensen et al., 2012).

Texture and colour are the main drivers of liking in LTLT treated meat, while the flavour has been evaluated as neutral (Christensen et al., 2012). Only few studies (Hansen et al., 1995; Vaudagna et al., 2002) have evaluated the sensory eating quality of LTLT treated meat at temperatures below 60 °C, and the effect on sensory attributes other than tenderness and juiciness, e.g. flavour and colour has not been thoroughly investigated

The colour change of meat caused by heating is used as an indicator of doneness. The myoglobin protein is the primary heme pigment responsible for meat colour, but there are other species contributing to colour changes during the cooking of meat (deoxymyoglobin, oxymioglobin, sulfmyoglobin, metmyoglobin …). However, myoglobin is stable up to about 60 °C, but denatures and precipitates or coprecipitates with other proteins at higher temperatures. Not only the heating temperature but also the length of time at that temperature affects the denaturation of myoglobin (Bernofsky, Fox, & Schweigert, 1959)

The ability to improve palatability, with the intent to increase consumer acceptance, while simultaneously maintaining product colour for purchasing appeal is an important concept. Additionally, differing beef muscle types are inherently different in terms of palatability (Rhee, Wheeler, Shackelford, & Koohmaraie, 2004), and in terms of colour stability (McKenna et al., 2005).

The aim of the present study was to evaluate the colour characteristics of LTLT treated Semitendinosus from beef heated at 52, 60 and 68 °C for prolonged heating times up to 24 h.

MATERIAL AND METHODS

Sample preparation

Fresh beef semitendinosus was obtained from SPAR meat plant of Bicske and transported to the Corvinus University of Budapest under chilled circumstances. 2 pieces of semitendinosus were boned, which came from the carcasses of the same cattle. The sample preparation was carried out at the university. All external fat and membranous, connective tissue parts were removed. Muscle was cut, 250-300g sample pieces which had a rectangular shape were formed. The samples were vacuum packed into plastic pouches by Multivac C100 packaging machine. Sous-vide treatment was performed with steam in a Lainox VE 051P type cooking unit and in water bath (Labor Műszeripari Művek LP507/1) for 9, 12, 15, 18, 21, 24 hours at 52, 60 and 68 °C. Treatment was followed by cooling down the samples in iced water below 3°C, subsequently measurements were made within 1 hour.

Colour measurements

Color was recorded using a Minolta CR 400 colorimeter. The instrument was calibrated against ceramic reference prior to use. 10 random readings at different locations per sample, 5-5 per side were taken and averaged. CIELAB system, L* (lightness), a* (redness), and b* (yellowness) were measured. The individual differences in L*, a* and b* values of each cooking treatments in respect of the color of the raw samples were evaluated using ΔE according to Eq. (1) (CIE, 1978).

$$\Delta E^*_{ab} = \sqrt{\left(\Delta L^*\right)^2 + \left(\Delta a^*\right)^2 + \left(\Delta b^*\right)^2} \quad (1)$$

RESULTS AND DISCUSSION

CIELAB colour analysis suggested that 9 hour long sous-vide treated steaks were generally lighter (higher L*) and more yellow (higher b*), whereas a* (red colour) decreased as temperature and cooking time increases. However, lightness (L*) of samples immersed into water do not change further if we increase the treatment time from 9 hour up to 24 hour (Fig.1). It likewise can be said in case of samples exposed to steam, results are not shown.

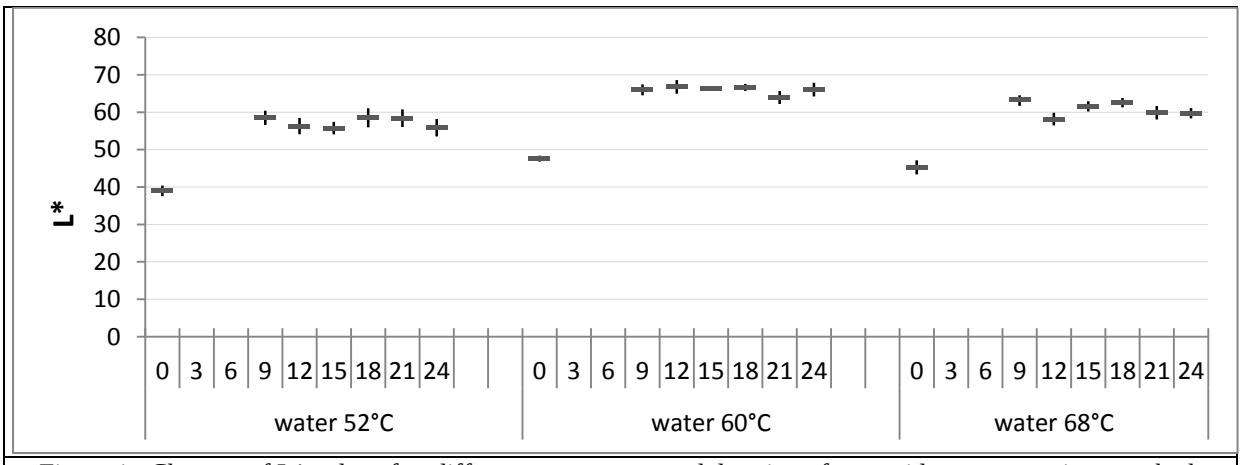

Figure 1.: Changes of L* value after different temperature and duration of sous-vide treatments in water bath

The a* values were not changed substantially after 52°C sous-vide treatment (both in water bath or steam exposure). However after 60 and 68°C treatments, the a* values decreased, the samples lost from their red colour (Fig.2). As the temperature was higher and treatment times were longer, we could observe the higher decrease in the red chromacity (a*). The a* value can have a function to indicate the "doneness" of the beef samples.

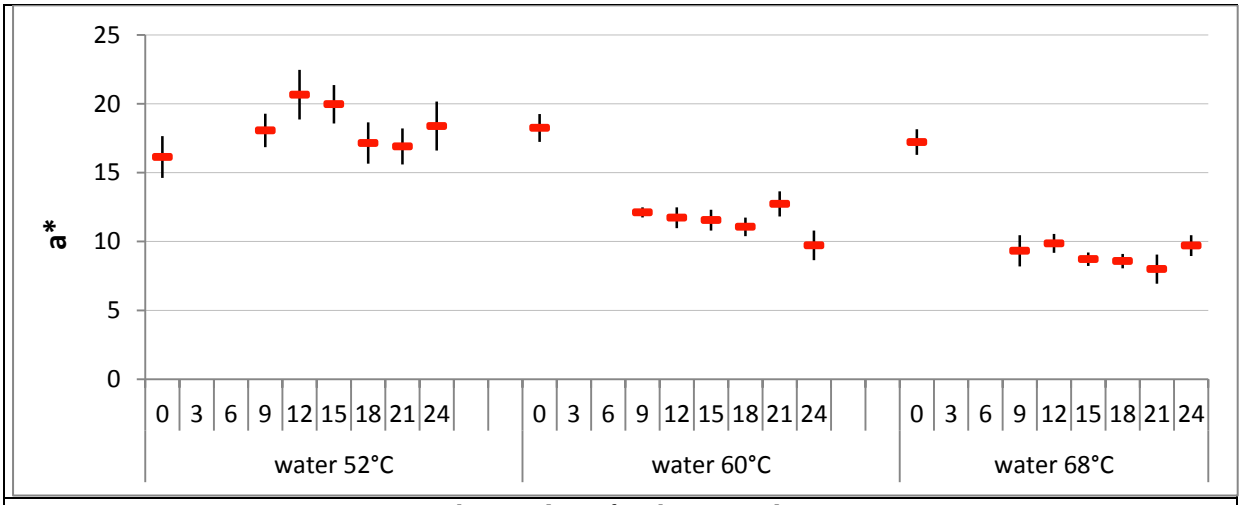

Figure 2.: The a* values after the sous-vide treatments

Figure 3 shows that the b * values increased slightly after 9-hour treatment compared to untreated samples, however, did not increase further after that extended treatment times, similarly in the case of L *. According to these result values, the samples colour got lighter and more yellow after treatments. If we compare the application of water bath and steam exposure, basically we cannot observe differences in colour parameters.

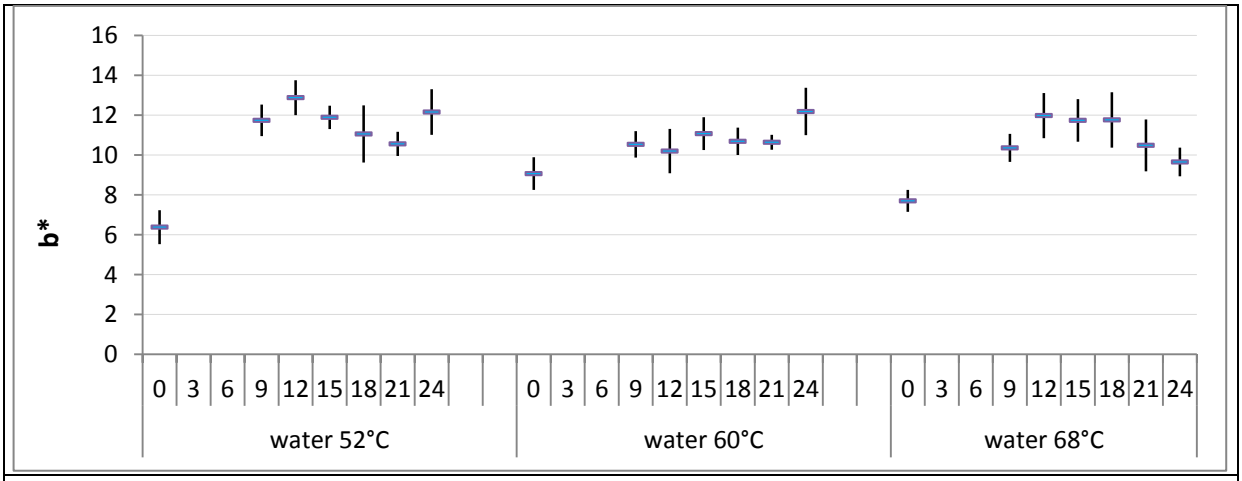

Figure 3.: The b* value changes due to sous-vide treatments

The ΔE results reflect well how much the colour properties changed to the human eye. Compared to the control sample all treated samples exceeded the large category due to sous-vide treatment (Table 1.). If we compare the result by 3-hour stepwise, each result to the previous one with shorter treatment time, the ΔE values will be mostly in the noticeable category, so between treated samples compared to each other step by step, we can find only noticeable changes in the colour.

Table 1. Changes of the ΔE value after sous-vide treatments						
ΔE^*_{ab}	52°C		60°C		68°C	
	Steam	Water	Steam	Water	Steam	Water
0-9	15,72	13,87	19,22	21,52	19,04	19,85
0-12	17,73	12,65	17,63	22,32	17,27	15,45
0-15	19,83	11,59	17,88	22,06	19,16	18,83
0-18	18,70	13,66	21,77	22,41	17,52	19,74
0-21	20,23	13,44	19,11	19,43	18,01	17,48
0-24	16,57	11,53	18,48	22,53	20,08	16,41
9-12	2,48	3,60	3,46	0,93	4,99	5,40
12-15	2,15	1,33	2,04	0,99	3,37	3,61
15-18	1,72	4,05	4,19	0,69	2,32	0,98
18-21	1,95	0,57	2,80	3,14	2,21	3,03
21-24	3,81	3,37	0,93	3,97	2,91	1,90

CONCLUSIONS

The sous-vide treatments at low temperatures for long time have changed the colour of beef samples, they became lighter less red and yellower. Basically no difference observable between the application of water bath or steam exposure on the beef sample The ΔE values show large differences compare to control sample, however the longer treatment durations do not affect remarkably the further colour. The a* values after 60 and 68°C treatment may correspond to the "doneness" of the beef samples – they are loosing the red hint. At the 52°C samples are keeping their redness values. Our plans are to carry out further testing, expanded with sensory and texture analysing.

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EFFECT OF HIGH HYDROSTATIC PRESSURE (HHP) PROCESSING ON PHYSICOCHEMICAL PROPERTIES AND SHELF LIFE OF FISH SALAD WITH MAYONNAISE

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SUMMARY

The aim of the present study was to evaluate the effect high hydrostatic pressure (HHP) processing (450, 600MPa for 300s) on microbial quality as well as physico-chemical properties of fish salad with mayonnaise during 26 days of storage at 5 and 10°C. The salad contained diced smoked trout fish, mayonnaise, and different kind of spices. These freshly made salads usually have only a couple of days of shelf life. The HHP treatmen t basically did not affect the physical and organoleptic characteristics of the fish salad with mayonnaise. On both storage temperature, the HHP treated samples showed good microbial quality for 3 weeks.

INTRODUCTION

High hydrostatic pressure (HHP) processing is a non-thermal technology used to enhance safety of food products maintaining product quality. (Considine, Kelly, Fitzgerald, Hill, & Sleator, 2008: Rendueles et al., 2011). It is also effective in preserving the organoleptic attributes of many foods.

In present study we researched how we can enhance the shelf life of fish salad with mayonnaise meanwhile avoid basic quality changes of the salad. It can have a function as ready food, or dressing for further vegetable salads. Sea foods are highly perishable, contain a wide variety of microorganisms and usually spoil faster than other muscle food. The chances of outbreaks to occur are even greater, when high risk ingredients, such as raw eggs, are used to prepare a specific meal or food product (Howard, O'Bryan, Crandall, & Ricke, 2012). We have to be aware of the high risk of cross-contaminations. Among egg products, homemade mayonnaise salad (HMS) prepared with raw eggs has been identified as a major food involved in salmonellosis outbreaks (Fica, A., et al., 2012, Norton, S., et al., 2012. So usually these fresh salads if they are vacuum packaged and cold storage applied, have a shelf life of up to only 3-5 days.

Application of high hydrostatic pressure (HHP) processing might provide a solution for preservation of fish salad with mayonnaise. For example, HPP at 300 MPa or higher for a few minutes significantly reduces the initial load and/or growth rate of spoilage microorganisms and enzymatic activity in many fish products stored under chilled conditions (Erkan et al., 2010, Kamalakanth et al., 2011). High pressure also can inhibit the formation of putrefactive compounds and maintains the hardness of fish muscles, resulting in higher sensory quality compared to untreated muscle over storage time.

MATERIAL AND METHODS

Sample preparation

The sample preparation was carried out by the plant of PLP Seafood Hungary Ltd. on Budaörs, the plant provided the raw materials. Ingredients of the fish salad were: home-made mayonnaise (egg yolk, Dijon mustard, salt, cayenne pepper, lemon juice and sunflower oil), smoked trout meat and mixture of spices from the plant. During the preparation of the samples smoked trout flesh was torned/ diced into small pieces and mixed with mayonnaise

and spices. Then the fish salad was portioned to 100g samples, vacuum packed into foil pouches, and delivered to the university. During the transport cold storage at 2°C was assured.

High hydrostatic pressure treatment

The pressure treatments were performed in a model Resato 100-2000 FPU high-pressure equipment at the Corvinus University of Budapest. The samples were pressure-treated at 450 and 600 MPa for 5 min at 15°C. The pressure transmission medium was PG fluid, provided by the producer. The pressure come-up time was 100 MPa/min and the pressure release was around 5 seconds. The temperature increase due to adiabatic heating was approximately 2.5°C per 100 MPa.

Measurements and storage conditions

After pressure treatment all samples, including untreated controls were stored at 5 and 10°C for up to 26 days. Samples were analysed in triplicate after 0, 7, 14, 21, 26 days of storage. The pH of samples was determined by immersing a pH electrode (Testo 209) into the fish salad. Colour was measured at 5 different locations along the pouch using a Konica Minolta CR-400 colorimeter. L* (brightness), a* (+a, red; -a, green) and b* (+b, yellow; -b, blue) values were measured at 5 different locations per sample and averaged. The individual differences in L*, a* and b* values of each cooking treatments in respect of the color of the raw samples were evaluated using ΔE according to Eq. (1) (CIE, 1978).

$$\Delta E^*_{ab} = \sqrt{\left(\Delta L^*\right)^2 + \left(\Delta a^*\right)^2 + \left(\Delta b^*\right)^2} \quad (1)$$

Samples were periodically analysed for aerobic total plate count in each treatment. Samples were taken aseptically, stomached for 2 min and 10-fold serial dilutions of the samples were made. the appropriate dilutions was plated on Nutrient count agar. Plates were incubated for 48 h at 37 °C, and by colony counter the colonies were counted.
Sensory analysis was performed by a 10-member panel. The panel consisted of 10 researchers and technicians at the Corvinus University of Budapest (50% male/females, age range between 25 and 57 years). The panel regularly performs sensory analysis, has some experience with evaluation of fish products. All samples were given a three digit number and served randomly. The evaluated attributes were intensity of colour, aroma and taste, beside the textural parameters (hardness of fish, viscosity of mayonnaise) and mosaicity. They had to compare the HHP treated samples always to the controll sample on a 11 point scale,where -5 corresponded to „dislike very much” and +5 corresponded to „like very much”, 0 to „there is no any difference”.

RESULTS AND DISCUSSION

pH value

Evolution of pH is presented in Table 1. Samples pH was not affected considerably by HHP treatment regardless of the condition of the control salad. Initial pH ranged between 5.6-5,8 for fish salads. During fermentation the pH decreased to around 4.6 for the salami samples. The pH values of both 450 and 600 MPa pressure-treated samples stored at 5 °C fluctuated in the same range between around 5,7-5,8 throughout the storage. In contrast, the control samples pH began after 7 days a continuous, significant decrease, until approximately a pH of 5.3, at which seemed to have stabilized. Larger, but similar fluctuations and changes occurred in case of 10°C storage.

Table 1. Changes of the pH values during chilled storage						
Days of storage	Control		450 MPa		600 MPa	
	5°C	10°C	5°C	10°C	5°C	10°C
0	5,71±0,02	5,62±0,02	5,65±0,02	5,59±0,03	5,78±0,02	5,88±0,04
7	5,83±0,03	5,68±0,06	5,84±0,03	5,49±0,03	5,69±0,06	5,69±0,06
14	5,61±0,03	5,13±0,03	5,82±0,03	5,55±0,06	5,79±0,02	5,98±0,04
21	5,32±0,05	5,08±0,06	5,72±0,02	5,73±0,09	5,76±0,04	5,77±0,06
26	5,29±0,03	5,2±0,03	5,75±0,02	5,64±0,03	5,83±0,13	5,92±0,03

Colour changes

The color values in each case fluctuated within a relatively narrow range compared to the initial values, respectively, no systematic color changes were detectable between the control, 450 and 600 MPa pressure-treated samples (Fig. 1). It can be said, that by the effect of the pressure treatment noticeable colour changes have not been occurred in either case of colour values.

The ΔE^*_{ab} colour difference value, which includes both the values of L*, a* and b* and makes distinct categories for the human eye, showed, that during the storage the effects of the pressure treatment appeared up just in the noticeable category.

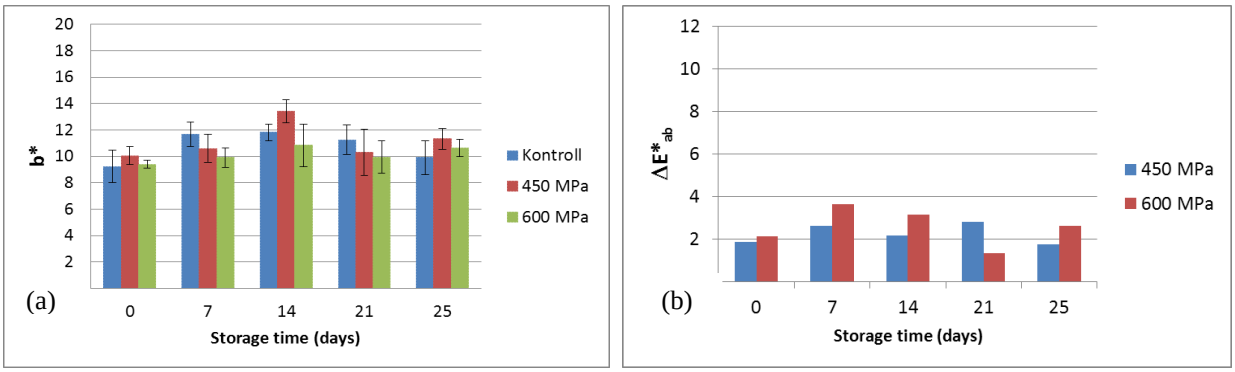

Figure 1.: Changes of colour properties (a) and ΔE^*_{ab} values (b) due to high pressure treatment during storage

Sensory analysis

We made testing only for 21 days, consider about the microbiological spoilage. 0. day results show, that pressure treatment basically did not changed any of the sensory attributes. Also, based upon the last day test results, we were unable to discover larger deviations during storage in the essential properties between the pressure treated and control samples (Fig. 2).

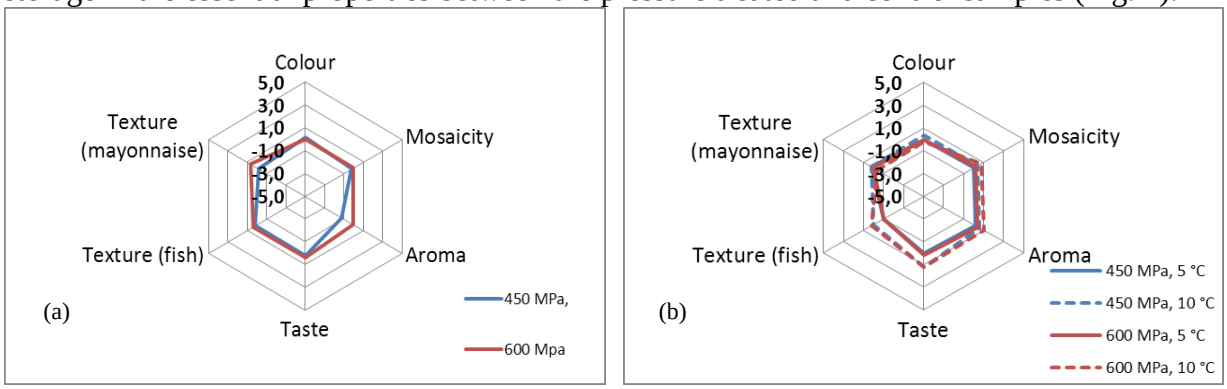

Figure 2.: Results from sensory analysis for pressure treated samples on 0. day (a) and 21. day of storage (b)

Microbiological results

Microbiological investigations showed that the pressure treatment of 450 MPa was capable to reduce 1 $\log^{-1}$ the initial number of bacteria in the samples, while 600 MPa treatment 2 $\log^{-1}$ (Fig 3.). Samples stored at 10°C approached 10^7 CFU/g of total bacteria number already on the 7. day of storage, these samples had sour odour and showed deterioration during tests.
Overall, the favorable microbiological quality due to the pressure treatment is clearly visible through the storage in the case of 600 MPa treated samples stored at 10°C exhibited about 1 $\log^{-1}$ microbial state compared to 450 MPa treated samples, so with 600 MPa pressure treatment favorable results could be achieved.

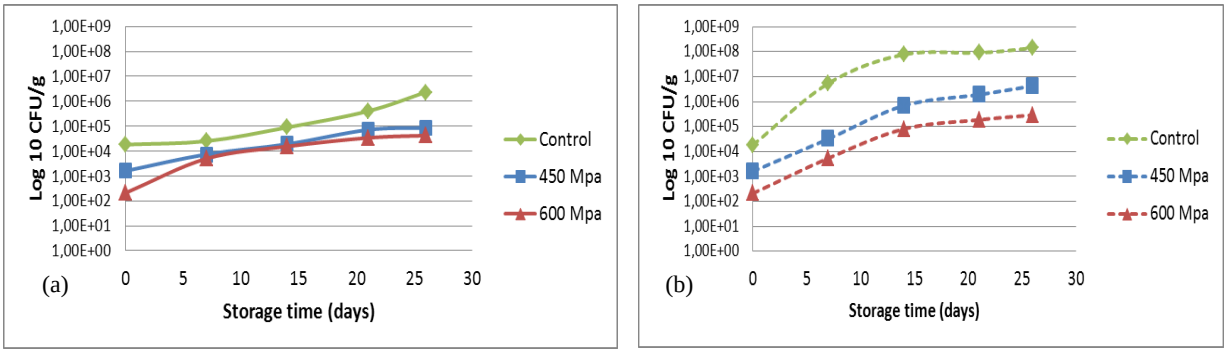

Figure 3.: Changes in total plate count during 5°C (a) and 10°C (b) of storage

CONCLUSIONS

Overall, it can be concluded, that the high hydrostatic pressure treatment basically did not affect the physical and organoleptic characteristics of the fish salad with mayonnaise. However, due to pressure treatment microbiological condition were significantly beneficial. During storage the physical, organoleptic and microbiological status of the samples treated by high hydrostatic pressure changed lesser extent than in the case of untreated samples. The HHP treated samples stored at 5 and 10°C showed appropriate quality for 3 weeks.

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APPLICATION OF ELECTRONIC TONGUE TO COMPARE BEER MIXES

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SUMMARY

In this work, the application of an electronic tongue (ET) based on a specific ion-selective sensor array for discrimination of different Hungarian alcoholic and non-alcoholic beer mixes with lemon flavor is presented. The tested beer samples were produced in five different Hungarian brewery. The electronic tongue equipped with the specific sensor array containing seven IFSET sensors was used to analyze the beer samples. The steady state of ET sensor signals was used as variable for the statistical evaluation. The obtained ET responses were evaluated using different pattern recognition methods. Principal component analysis (PCA), provided some initial patterns to exclude the outliers. Linear discriminant analysis (LDA) used to build models to separate beer samples based on the brand and the alcohol content. In both case the corresponding LDA-classification matrix confirmed that all samples or brands were correctly classified by the model i.e. both the recognition and prediction abilities were found 100%, in the calibration and the validation (3-fold CV), respectively.

INTRODUCTION

Nowadays quality assessment is an important topic in food quality control. Therefore, there is an increasing need for rapid instruments which can measure the quality or some quality parameters of different food products. Results of many previous studies showed electronic tongue (ET) has high potential in the area of food science, therefore, itcan be a useful tool in this field. The ET concept emerged first in the beginning of the nineties (Kovács et al., 2009).The principle of the concept is to measure with cross-sensitive and partially selective sensors (Legin et al., 1999). ET is a system that usually consists of an array of non-specific chemical sensors combined with appropriate data acquisition systems and chemometric tools. During sample assessment ET sensor array produces an unresolved analytical signal, which correlates with the chemical composition of the sample.

Comprehensive information of quality of food products and its composition has became increasingly important for consumer choice. Geographical origin, agricultural practices and chemical composition, together with sensory qualities play a vital role in the purchase decision of consumers. These factors are even more important if we are talking about beers. Beer-mixes are alcoholic soft drinks mixed from alcoholic or non-alcoholic beer and non-alcoholic fruit juices. The taste and scent is very important as the consumers do not buy these products for its alcohol content, but for its flavor. Although alcohol content still does count, interesting thing to know if alcohol content have an effect on the tastes of the beer-mixes or not. If there is no difference that means someone who cannot drink alcohol for some reason can still enjoy a flavored beer-mix without having bad effect on themselves.

Therefore the objective of this work is to develop an evaluation protocol applying ET and to find the differences between the beer mixes from different Hungarian brewery according to the different alcohol content.

MATERIALS AND METHODS

Materials

Different beer mix samples with lemon flavor were evaluated in two different experiments. The beer samples are large-scale beer-mixes with lemon flavor, from the four largest brewery of Hungary. There was two beer-mixes with lemon flavor from each brand, one of them had approx. 2.0 V/V% of alcohol, the other had 0.0 V/V% alcohol in each case. During the selection of the brands the aim was to have a homogeneous variety of beer mixes representative to the Hungarian large-scale market of lemon flavored beer-mixes.

The lemon flavor was chosen because this flavor had widest variety at the probative period with five different brands.

Electronic tongue measurement

Alpha ASTREE II (Alpha M.O.S., Toulouse, France) potentiometric electronic tongue equipped with a specific ISFET sensor array coated with different organic membranes developed for liquid food analysis was used to measure the samples.

The ET consists of an autosampler, containing 16 slots for samples. During the assessment ISFET based potentiometric sensors were used. All measurements were performed with seven sensors. The sensors were preconditioned and calibrated before the tests according to the manufacturers' recommendation. For conditioning 0.01M HCl solution was used (recommended by the manufacturer). The conditioning was performed according to AlphaSoft (Standard analysis). The calibration of the sensors were performed with the mix of the tested beer samples containing all the 10beer in the same percentage. The detailed description of the instrument was introduced in many previous publications (Kovács et al. 2010).

Every sample was measured in nine replications for 120 seconds. The samples were measured two times in two different concentrations: 100% beer, 50%-50% beer and distilled water.

Statistical analysis

Various multivariate statistical evaluation methods were used for the data evaluation.

Principal component analysis (PCA) was used to detect patterns and to visualize the results of the electronic tongue tests. PCA is frequently applied as a tool to exploratory data analysis. Without losing useful information it is able to reduce multidimensional data sets to lower dimensions for analysis and to discover outliers.

Linear discriminant analysis (LDA) as a supervised method was applied to evaluate the capability of discrimination of the current system. It is maximizing the distances between classes by transformation of variables. Cross-validation (CV) was applied to confirm the LDA models. The 2/3 of the data was the training set, the other 1/3 was the test set in three replicates.

All analysis were carried out using the software R-studio 3.0 (Boston, USA) and Statistica 9.0 softwere (StatSoft, Inc., Tulsa, Oklahoma, USA).

RESULTS AND DISCUSSIONS

Preliminary data evaluation was performed with PCA to detect outliers. Further evaluations were carried out using the results of the best discriminating sensors selected by Stepwise Discriminant Analysis. Based on discrimination ability six sensors were selected.

Classification by Linear Discriminant Analysis

The results of LDA performed on ET results showed significant differences between the measured samples. Figure1 shows the score plot of the LDA results for the undiluted samples where the discrimination was performed based on the types. The corresponding LDA-classification matrix confirmed that all samples were correctly classified by the model i.e. both the recognition and prediction abilities were found 100%, in the calibration and the validation (3-fold CV), respectively.

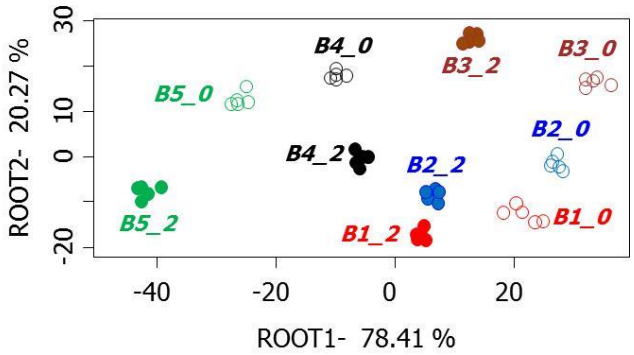

Figure 1. LDA of the electronic tongue measurements performed with the undiluted beer mixes (10 classes by the different types, n=50)

The LDA plot of the diluted beer mixes is shown in Figure2. The LDA model recognises 100% of the samples; and predicts correctly 94% of B1_2 samples, misclassifying 6% as belonging to B5_2.

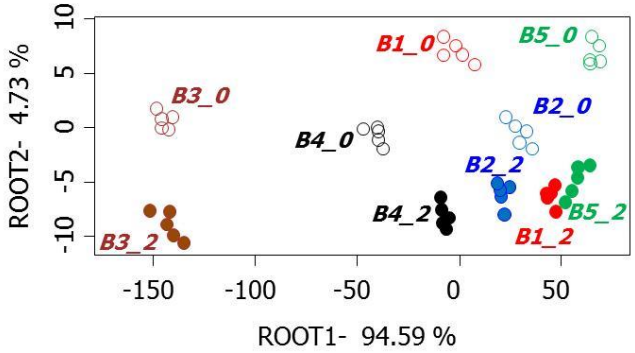

Figure 2.LDA of the electronic tongue measurements performed with the diluted beer mixes (10 classes by the different types, n=50)

Discrimination of beer mixes according to the brands was also performed the score plot of LDA model shown in Figure3. The brand groups were clearly discriminated along Root1. The corresponding LDA-classification matrix confirmed that all brands were correctly classified by the model i.e. both the recognition and prediction abilities were found 100%, in the calibration and the validation (3-fold CV), respectively.

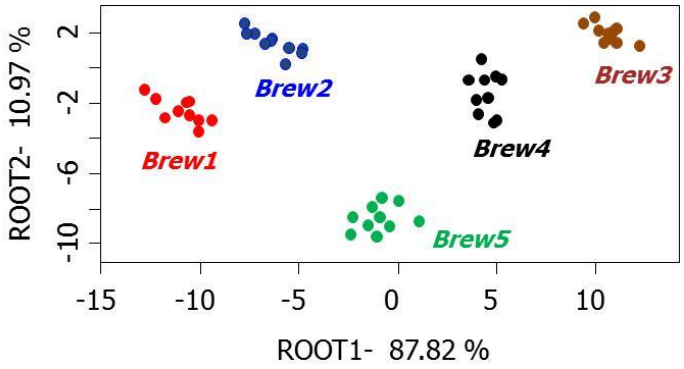

Figure 3. LDA plot of ET measurement to discriminate undiluted beer mixes according to the brand

The LDA plot in Figure4 shows also good discrimination ability, when 50% diluted beer mixes were analyzed. The LDA model recognises 100% of the brands; and predicts correctly 95.18% of Brew5 samples, misclassifying 4.82% as belonging to Brew1.

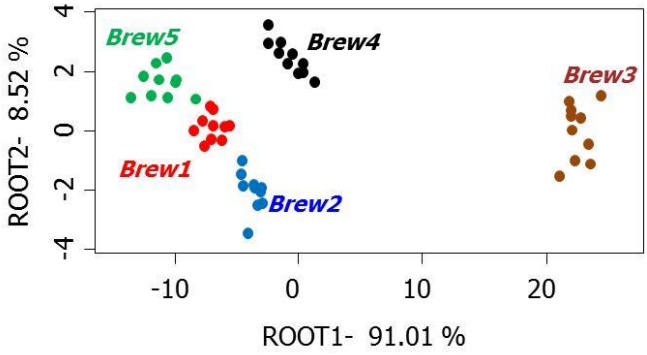

Figure 4. LDA plot of ET measurement to discriminate undiluted beer mixes according to the brand

CONCLUSIONS

The electronic tongue equipped with the specific sensor array is suitable to analyze the beer mixes and give a good opportunity to discriminate the brands. Linear discriminant analysis performed on the electronic tongue results showed good classification of the samples when undiluted beer mixes were analyzed. In this case the corresponding LDA-classification matrix confirmed that all samples or brands were correctly classified by the model i.e. both the recognition and prediction abilities were found 100%, in the calibration and the validation (3-fold CV), respectively. The results showed the ET is able to classify the undiluted lemon flavored beer mixes with low- or without alcohol content according to the brands or types.

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COLOUR STABILITY OF ELDERBERRY CONCENTRATES IN NATURAL YOGHURT

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SUMMARY

Elderberry concentrate is widely used for colouring of foods with low pH level such as soft drinks, water ice cream, sauce, dairy products, confectionery, table jellies and conserves because of high pigment content. In our study, elderberry juice concentrates of Samocco and Haschberg varieties were used to colour natural yoghurt to study their colour parameters and stability during storage. It was shown that pigments of Samocco were more stable during storage because total anthocyanin concentration decreased only by 12.1% in 6-weeks, while its reduction in yoghurt coloured with Haschberg concentrate was 39.3%. It can be concluded with regard to cyanidin-based anthocyanins that conjugates with more complex saccharide moieties are more robust during storage. Namely cyanidin-3-O-glucoside-5-O-sambubioside was the most stable pigment in natural yoghurts. The most informative red-green value (a) continuously decreased during the 6-week storage but there was no visible difference compared to the initial values.*

INTRODUCTION

Food colourants from natural sources are becoming more preferred as alternatives of synthetic food agents to adjust food colour. The only permitted natural food colorants of animal origin are carminic acid and carmines often referred to as cochineal but allergy-like reactions, IgE-mediated reactions linked to cochineal have been reported (Greenhawt & Baldwin, 2009). Therefore, plants are considered as primary sources of natural food colorants, yielding anthocyanins, carotenoids, and chlorophylls as major representatives (Scotter, 2001). Anthocyanins can be a good choice for red colouring of foods with low pH such as soft drinks, conserves, dairy products, water ice cream, table jellies, sauce, and confectionery because anthocyanins are more stable under acidic conditions (Cooper- Driver, 2001; Bakowska-Barczak, 2005).

Elderberry is one of the most promising alternatives to synthetic red food colourants due to its high anthocyanin concentration (Jakobek *et al*., 2007; Veberic *et al*., 2009). Elderberry pigments are almost exclusively cyanidin glycosides, from which cyanidin-3-*O*-glucoside and cyanidin-3-*O*-sambubioside are the major ones (Dawidovicz *et al*., 2006). In addition, cyanidin-3-*O*-sambubioside-5-*O*-glucoside and cyanidin-3,5-*O*-diglucoside were detected as minor compounds (Hong & Wrolstad, 1990; Veberic *et al*., 2009). The stability of anthocyanins is strongly influenced by many factors such as heat treatment, pH of the medium and the presence of complexing agents, enzymes, flavonoids, proteins and metallic ions (Bakowska et al., 2003).

The aim of this study was to evaluate colouring potentials and colour stabilities of concentrated elderberry juices prepared from two different varieties in a commercially available strawberry yoghurt during a 6-week-long storage experiment.

MATERIAL AND METHODS

Elderberry concentrates

Two varieties of elderberry fruits were harvested at the optimal maturity stage from Nagyvenyim (46°57'N, 18°51'E) in Hungary in 2013 and were processed into concentrate form (ca. 60 °Brix) according to the industrial practice.

Production of yoghurt sample

Test samples were prepared in the laboratory of Sole-Mizo diary company (Szeged) applying the recipe and ingredients routinely used in their commercially available strawberry yoghurt. In addition, elderberry juice concentrates were added during manufacturing for colouring according to the following experimental setup: yoghurt with uncoloured strawberry puree (No. 0.); yoghurt with carmine-coloured strawberry puree (No. 1.); yoghurt with uncoloured strawberry puree coloured by Haschberg (0.5 m/m%) concentrate (No. 2.); yoghurt with uncoloured strawberry puree coloured by Samocco (0.4 m/m%) concentrate (No.3.) The required quantity of elderberry concentrates were determined based on preliminary colour measurement (data are not shown) and visual judgement to reach the colour of commercially available strawberry yoghurt. Each sample was made in 3 replicates and was stored at 5°C for 6 week the absence of light.

Quantification of anthocyanins

Sample preparation of yoghurt products was carried out according to the method of Nagy *et al*. (2009) with slight modifications.

Tentative identification intact anthocyanin components was carried out by an HPLC-DAD-qTOFMS system (Agilent 1200 HPLC and Agilent 6530 qTOFMS) whereas absorbance at 520 nm measured with DAD was used for quantification (Szalóki-Dorkó et al., 2015). External calibration was performed using cyanidin-3-*O*-glucoside reference standard (Extrasynthese, Genay, France). Concentrations of all anthocyanin compounds were assessed from peak areas and are given as µg cyanidin-3-*O*-glucoside equivalent (CGE) per 1 g of yoghurt sample. Total anthocyanins (TA) were expressed as sum of individual anthocyanin concentrations.

Colour measurement

Changes in colour was monitoring in the CIELab system and among others, a*(red/green coordinate), was measured using Konica Minolta CR 410 digital device.

RESULTS AND DISCUSSION

Figure 1. shows anthocyanin concentrations of Haschberg-coloured yoghurt during the 6-weeks storage. Pelargonidin-3-*O*-glucoside (Prg) is the most abundant pigment component of strawberry puree which was present in all samples. Prg concentration decreased continuously during the storage, average reduction was 30% in case of the investigated yoghurts after 6 weeks.

Total anthocyanin concentration of Haschberg-coloured sample decreased from 6.01 µg CGE/g to 3.81 µg CGE/g (Figure 2.) after 6 weeks, which was 39.7 % pigment reduction during storage (Figure 3.). Degradation rate of TA was greater between 3 and 4 weeks than in

the other sections. Regarding the individual anthocyanin molecules from elderberry, cyanidin-3-*O*-sambubioside-5-*O*-glucoside (Cy3G) was the most stable pigment in yoghurt samples, however cyanidin-3-*O*-glucoside (CyG) content reduced by 50.4 %. This means that storage time mostly affected CyG. The second most stable anthocyanin was cyanidin-3-*O*-sambubioside (Cy2G) in elderberry concentrates, its concentration decreased by 35.3 % at the end of experiment.

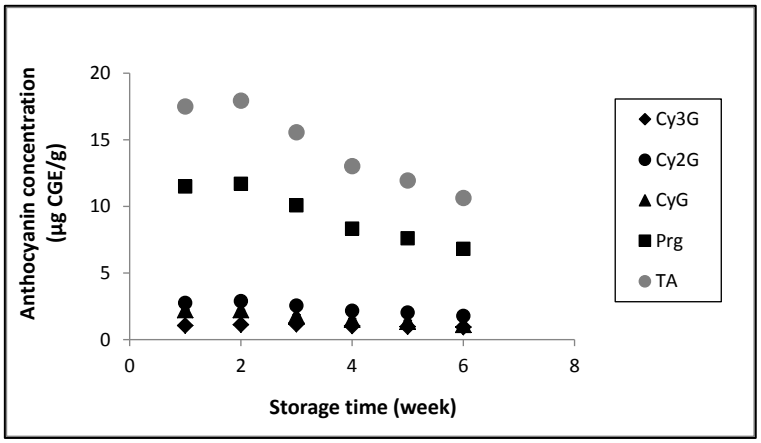

Figure 1.: Change in anthocyanin concentration of Haschberg coloured yoghurt during storage

Anthocyanins in Samocco-coloured yoghurts were degraded during the storage as well, TA decreased from 16.7 µg CGE/g to 11.6 µg CGE/g. This reduction was 12 % which is lower than was observed in Haschberg-coloured yoghurt, despite of the fact that Samocco concentrate was used at 0.1 m/m% lower concentration to colour samples.

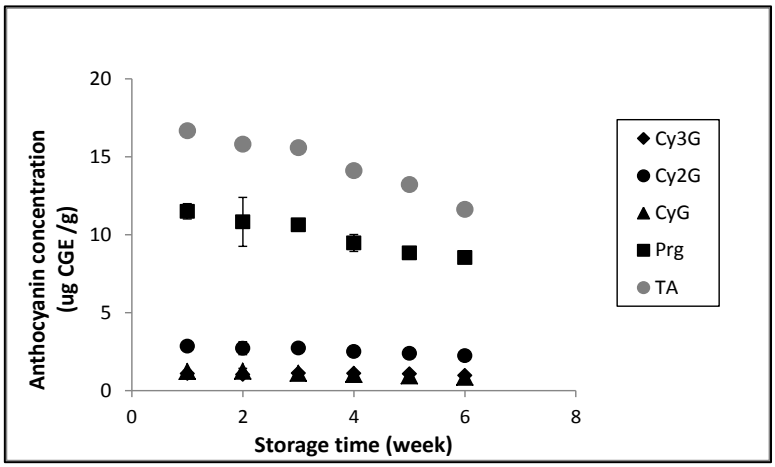

Figure 2.: Change in anthocyanin concentration of Samocco coloured yoghurt during storage

Anthocyanins of Samocco were more stable during storage than in Haschberg. Cy2G was present in the highest concentration among elderberry anthocyanins in yoghurt, which is a typical characteristic of Samocco variety, while CyG and Cy3G were detected almost equal quantity. Considering the robustness of these molecules during storage, concentration of Cy3G reduced by 11.3 %, in case of Cy2G the reduction was 21.6 %. CyG was the most unstable component, its amount decreased by 30.2 % after 6-week long storage. It suggests that the stability of cyanidin-compounds in elderberry concentrate seems to be positively correlated to the complexity/number of sugar derivatives linked to the cyanidin aglycone.

Due to the experimental setup, a* values of Haschberg-coloured (No. 2.) and Samocco-coloured (No. 3.) yoghurts were almost equal to the one with uncoloured strawberry puree plus carmine (No. 1.) in the initial time point, however, during storage minimal changes were

observed (Figure 3.). The a* values decreased continuously during the experiment and after 6 weeks storage, yoghurts coloured with elderberry concentrates showed almost the same red-green intensity. 5% reduction was observed in both cases after storage.

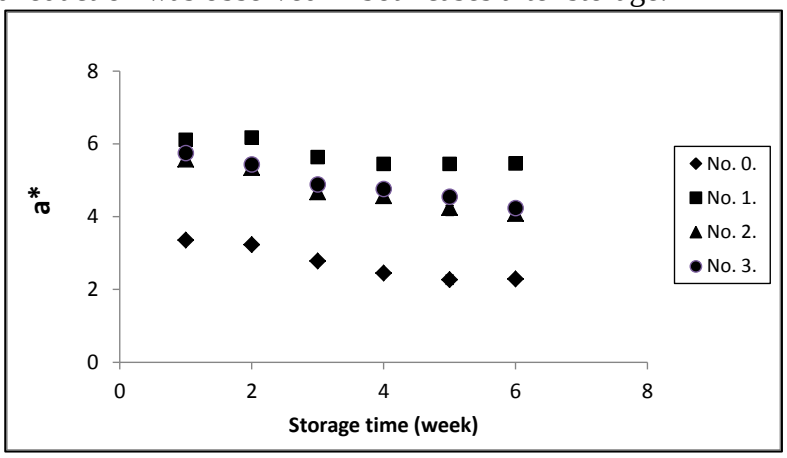

Figure 3. Change of red-green value (a*) during storage

The a* value of yoghurt coloured with carmine proved to be the most stable among all samples; it preserved red colour intensity better than the others, however the difference was not visible.

CONCLUSIONS

Elderberry concentrate is a good alternative to replace carmine from animal source in strawberry yoghurt product. Anthocyanins from elderberry are sufficiently stable during storage for several weeks without visible colour change. Among the two investigated varieties, Samocco concentrate provided the same colour intensity at lower concentration than Haschberg. Furthermore, yoghurt coloured with Samocco concentrate preserved more of its anthocyanins during storage. Regarding the individual pigment molecules, stability of cyanidin-compounds in elderberry concentrate seems to be positively correlated to the complexity/number of sugar derivatives linked to the cyanidin aglycone. That means cyanidin-3-*O*-sambubioside-5-*O*-glucoside is the most stable pigment during storage, whereas cyanidin-3-*O*-glucoside is the most unstable one.

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COMPARISON OF PEF-TREATED AND HEAT-TREATED ELDERBERRY JUICE PROPERTIES DURING STORAGE

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SUMMARY

The effects of pulsed electric field (PEF, 28 kV/cm, 300 µs) and heat treatment (HT, 90°C, 30 s) were evaluated on physical and chemical properties of elderberry juice during 42-days storage at 4°C. Conductivity, pH values, non-enzymatic browning index of treated juices practically did not change. There was no visible colour difference in the treated juices during the experiment. Regarding the microbiological stability of the samples, the values were <1 CFU/mL in case of both preservation technologies during the whole experiment. Immediately after PEF treatment, total polyphenol and total anthocyanin values were higher (2% and 17%), while heat treatment caused degradation (5% and 10%) compared to the control juice. Furthermore, total pigments in PEF-treated samples were more stable than heat-treated pigments during the storage. Total antioxidant capacity values decreased by 23% in case of heat-treated juices and by 4% in PEF-treated samples at the end of experiment.

INTRODUCTION

Thermal treatment is typically used for juice pasteurization with a pH equal or less than 4.5 for a few seconds at temperatures between 60 and 100 °C. Heat processing (HP) inactivates spoiling microorganisms efficiently, but taste, colour, flavour, and nutritional quality of foods, such as polyphenolic and vitamin content, may also degrade (Qin et al., 1995).

Pulsed electric field (PEF) technology is a non-thermal minimal food processing, which successfully destroys vegetative microorganisms in fruit juices without any significant change in their nutritional and sensory properties (Min & Zhang, 2003; Yeom et al., 2000). Generally, PEF technology works by very short time high voltage pulses producing pulsed electric field between two electrodes. The applied electric field damages the cell membrane and causes significant change of membrane permeability that leads to the inactivation of microorganisms.

There are a lot of studies on effects of PEF treated fruit juice that investigate microbial stability and several physical, chemical, and organoleptic properties of treated sample. However, effects of PEF on elderberry juice - which is one of the most promising natural alternatives of synthetic food colourants in the food industry - are less researched area. The aim of this work was to investigate some quality parameters of elderberry juice after PEF treatment and during 42-days storage compared to the traditional thermally processed juice.

MATERIALS AND METHODS

Elderberry juice

Haschberg cultivar of elderberries was grown in Nagyvenyim region (Hungary) and harvested in 2014 season. After removing stems, raw fruit were crushed and squeezed by a manual press until no further juice could be obtained. The juice was filtered to gain pure fluid without pulps. After filtration, the juice was diluted to reach the conductivity value of ca. 2 mS/cm to provide appropriate operation of PEF equipment.

PEF and heat treatments

The PEF treatment was carried out in a continuous flow laboratory scale system (OSU 4B, Ohio State University, Ohio, USA). The treatment system consists of six co-linear electrode pairs in series, each one with two stainless steel electrodes separated by a gap of 0.29 cm. The original flow system of the machine was changed from syringes to bottles, the flow rate was 0.63 mL/s. The experimental settings of PEF treatment is shown in Table 1.

Table 1. Experimental settings of PEF treatment

Electric field strength	Treatment time	Pulse duration	Time delay	Pulse frequency	Max. temperature	Energy input
28 kV/cm	300 µs	2 µs	10 µs	1429 1/s	45 °C	168 J/mL

The heat treatment was carried out in a water bath (Memmert, Schwabach, Germany) at 90 °C for 30 s. Control was evaluated as untreated sample and compared to the treated juices in the initial section (0^{th} point on the graphs). Samples were stored in each case at 4 °C by absence of the light after treatments for 42 days.

Measurements

Mesophilic aerobic total count was determined on PCA (Plate Count Agar – Merck) incubated at 30 °C for 3-5 days, according to the standard MSZ EN ISO 4833. Total polyphenol content was determined based on the method of standard MSZ 9474-80 with Folin-Ciocalteu reagent, and results were expressed in gallic acid equivalent (GAE). Total anthocyanin concentration was measured in cyanidin-3-glucoside equivalent (CGE) according to pH differential method (Lee et al., 2005). Determination of antioxidant capacity (FRAP) was made according to the method of Benzie and Strain (1996). Colour was monitored by digital chroma meter CR 400 (Konica Minolta, Japan), pH values were measured by digital equipment (Testo 206, Lenkirch, Germany). Conductivity was determined by Voltcraft LWT-03 ATC digital instrument (Conrad Electronic, Munich, Germany). Non-enzymatic browning index (NEBI) was measured according to the method of Cohen et al. (1998).

RESULTS AND DISCUSSION

The Colony Forming Unit value of the control sample was $2.0x10^3$ cfu/mL, and immediately after treatments the microbial load decreased to 0 cfu/mL. During the storage all treated samples were microbiologically stable (<1 cfu/mL).

The changes in total phenolic compounds after processing with PEF and heat treatments and during the storage are shown in Figure 1. After the treatments, the values increased minimally in case of PEF, however, initial concentration of HP-treated samples decreased by 4% compared to the control sample. The storage time had the same effects on PEF- and heat-treated elderberry juices.The highest values were detected after 14 days of storage (1112.5 and 1096.4 mg GAE/L) and the lowest data were measured at the end of experiment (1046.4 and 1026.8 mg GAE/L).

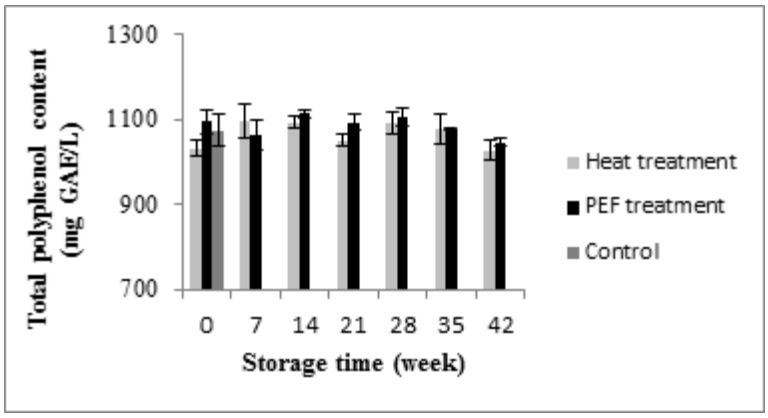

Figure 1. Total polyphenol content of elderberry juice during storage

Generally, the samples that were treated by the non-thermal technology had higher polyphenol contents, although the differences were not significant ($P<0.05$) compared to the thermal pasteurization.

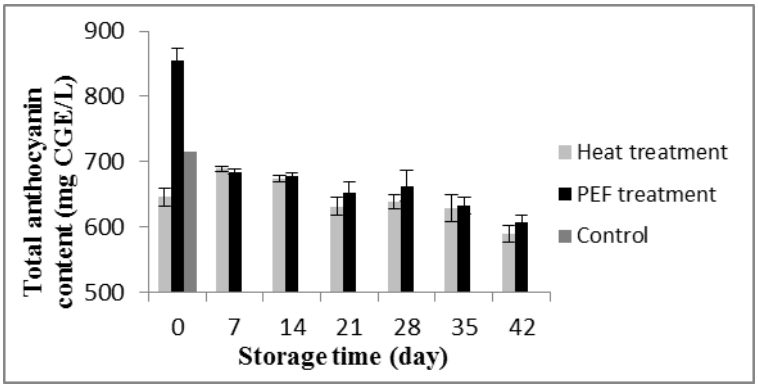

Figure 2. Total anthocyanin content of elderberry juices during storage

The total anthocyanin content (TA) of treated samples changed during the 42-day storage (Figure 2). Immediately after the treatments, TA significantly increased ($P<0.05$) in case of PEF-treated juices (17 %), while samples after HP showed lower values by 10 %.

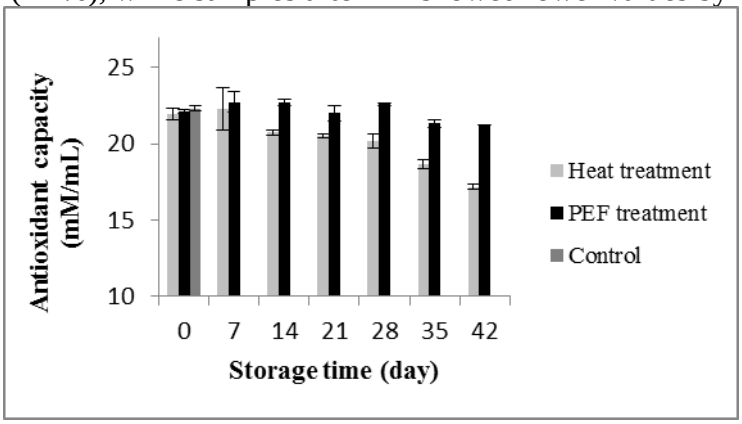

Figure 3. Antioxidant capacity of elderberry juices during storage

The storage time influenced the pigment concentration as well, as TA decreased continuously during the experiment. The lowest values were detected after 42 days in both types of samples, however, PEF-treated juice preserved its pigment content better (607.8 mg CGE/L) than HP-treated sample (588.6 mg CGE/L).

The change of antioxidant capacity in elderberry samples is shown in Figure 3. The values decreased minimally after the treatments compared to the control sample. However, values changed similarly to the total polyphenol and the total anthocyanin content as expected during the storage. PEF-treated juices had higher FRAP values during the whole experiment, moreover, after 42 days, these sample preserved almost the initial capacity. HP treatment combination with storage caused higher degradation in the samples.

Table 1. Change of some physical and chemical parameters during storage

	Control	HP/PEF						
Storage time (day)	0	0	7	14	21	28	35	42
pH values	4.25	4.3 / 4.4	4.4 / 4.3	4.3 / 4.2	4.2 / 4.2	4.2 / 4.2	4.3 / 4.2	4.3 / 4.3
Conductivity (mS/cm)	1.80	1.78 / 1.75	1.81 /1.82	1.80 / 1.81	1.76 / 1.79	1.76 / 1.82	1.72 / 1.77	1.82 / 1.82
Nonenzymativ browning index	2.083	2.179 / 2.139	2.236 / 2.007	2.195 / 2.078	2.111 / 2.105	2.049 / 2.085	2.133 / 2.208	2.171 / 2.195
Colour difference (ΔE*)	-	0.08 / 0.05	0.14 / 0.08	0.21 / 0.15	0.22 / 0.19	0.15 / 0.15	0.14 / 0.10	0.22 / 0.17

Table 1 shows some investigated parameters after HP and PEF treatments and during 42-day-long storage.The pH and conductivity values were almost constant during the experiment, therefore the two investigated preservation technologies did not have effects on these parameters. Non-enzymatic browning index increased minimally after treatments and during storage compared to the control sample. Regarding colour parameters, ΔE* values were under the level of 0.5, so no visible colour difference was observed after treatments and during storage in elderberry juices.

CONCLUSIONS

Pulsed electric field treatment applied with laboratory equipment is able to achieve microbial inactivation, producing a juice with a shelf-life of 42 days at 4°C. Furthermore, after PEF treatment, samples had higher total polyphenol and total anthocyanin content compared to the control sample, and the juices preserved its valuable compounds better than heat-treated samples during storage. According to these results, PEF technology is a promising alternative to food preservation by heat pasteurization, and produces elderberry juice with higher quality.

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PULSED ELECTRIC FIELD PRESERVATION OF NEUTRAL AND ACIDIFIED RED BEET JUICES – RESEARCH FOR THE OPTIMAL SETTINGS

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SUMMARY

The goal of our research was to gain microbiologically stable (<10 CFU/mL) red beet juice applying pulsed electric field (PEF) treatment. We conducted experiments changing the working parameters of the machine (electric field strength: 25-32 kV/cm, total treatment time: 120-300 μs, pulse duration: 2-3 μs, flow rate: 0.63-0.92 mL/s), and the properties of the raw material (pH, initial microbial load). By this work we determined the borders of the Pulsed Electric Field equipment with the transformed flow system. The physical properties (colour, conductivity) and the biochemical characteristics (pH, antioxidant capacity, total polyphenol content, non-enzymatic browning index) of the samples were only slightly different after PEF treatment, in contrary to heat pasteurisation, where colour change was visible.

INTRODUCTION

Pulsed Electric Field (PEF) is an emerging non-thermal technology in the field of juice preserving. The technique is based on the irreversible electro-permeabilisation of the bacterial cell membrane.This pasteurisation method causes minimal or no detrimental changes in nutrient value, physical and sensory properties, therefore foods preserve their fresh-like quality after treatment contrary to heat pasteurisation technique, which causes several degradation processes in valuable components. There are a lot of studies on immediate effects of PEF-treated fruit juices, however, vegetable juices like red beet *(Beta vulgaris rubra)* are less researched area. In this study, the CFU reduction by different working parameters of the machine was investigated. Furthermore, effects of PEF and conventional heat treatment (HT) on physical and chemical properties of freshly squeezed red beet juices were compared.

MATERIALS AND METHODS

Red beet juice preparation

Red beetroot (purchased from the local market) was peeled and squeezed. The juice was centrifuged and filtered to gain pure fluid without pulp to avoid the inhomogeneity of the raw material, which could cause arching under voltage. The juice was diluted to 12.5% with distilled water to reach the conductivity of 1.7-1.9 mS/cm, which is suitable for PEF treatment. In case of acidifying, the pH value was lowered by citric acid to pH=4.2.

Pulsed electric field treatment

The PEF treatment was carried out in a continuous flow laboratory scale system (OSU 4B, Ohio State University, Ohio, USA). The treatment system consists of six co-linear electrode pairs in series, each one with two stainless steel electrodes separated by an insulator gap of 0.29 cm. The original flow system of the machine was changed from syringes to bottles, where the flow of the fluid is held up by vacuum. The flow rate could be set by a valve on the tube of the vacuum pump. The nominal maximal electric field intensity of the machine is 34 kV/cm, which is built up by 10 kV output voltage on 0.29 cm gap distance. However, by our experiments, the practical maximum was 30 kV/cm. The applied waveform was bipolar square wave, with 2 or 3 μs long pulses and 10 or 15 μs delay time, respectively. The total treatment time could be changed between 100 and 300 μs.

Microbiological, physical, and biochemical characteristics

- Mesophilic aerobic total count was determined on PCA (Plate Count Agar – Merck) incubated at 30 °C for 3-5 days, according to the standard MSZ EN ISO 4833.
- Total polyphenol content was determined based on method of standard *MSZ 9474-80* with Folin-Ciocalteu reagent.
- Determination of antioxidant capacity (FRAP) was made according to the method of *Benzie and Strain* (1996).
- Colour was monitored by digital chroma meter CR 400 (Konica Minolta, Japan), L*, a*, b* were recorded, and colour difference (ΔE) was calculated using the following equation: $\Delta E^* = (\Delta a^{*2} + \Delta b^{*2} + \Delta L^{*2})^{1/2}$.
- pH values were measured by digital equipment (Testo 206, Lenkirch, Germany).
- Conductivity was determined by Voltcraft LWT-03 ATC digital instrument (Conrad Electronic, Munich, Germany).
- Non-enzymatic browning index (NEBI) was measured according to the method of *Cohen et al.* (1998).

RESULTS AND DISCUSSION

Keeping the original pH value of the red beet juice no satisfying reduction in the numbers of colony forming units could be achieved. Though the initial microbial load of the acidified juice was lower, further decrease was observed due to PEF treatment. The result obtained at the lower pH was satisfactory. The magnitude of the CFU reduction was also higher with increased initial microbial load. (Table 1.)

Table 1. Selected experimental results of CFU reduction

Electric Field Strength (kV/cm)	Treatment Time (μs)	Pulse Duration (μs)	Flow Rate (ml/s)	pH	n_0 (CFU/ml)	n (CFU/ml)	$\log(n/n_0)$
25	120	2	0.92	6.2	$1.73x10^4$	$1.64x10^4$	-0.02
25	120	2	0.92	6.2	$5.10x10^7$	$8.10x10^4$	-2.8
25	250	3	0.67	6.2	$1.10x10^6$	$6.0x10^3$	-2.26
25	250	3	0.67	4.16	$1.90x10^2$	2	-1.98
25	300	3	0.67	6.2	$1.10x10^6$	$3.0x10^3$	-2.56
25	300	3	0.67	4.16	$1.90x10^2$	1	-2.28

Total polyphenol content was not affected by HT or PEF (Fig. 1).

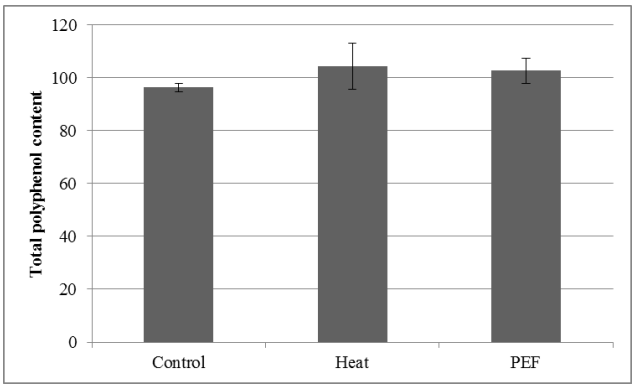

Figure 1. Total polyphenol content of untreated control, heat treated (90 °C, 30 s), and PEF treated samples (28 kV/cm, 150 µs treatment time, 2 µs pulse width)

Antioxidant capacity was not decreased significantly by HT or PEF (Fig. 2).

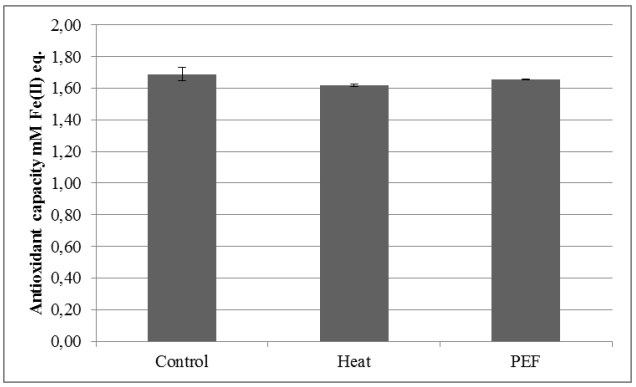

Figure 2. Antioxidant capacity of untreated control, heat treated (90 °C, 30 s), and PEF treated samples (28 kV/cm, 150 µs treatment time, 2 µs pulse width)

In case of PEF treatment, the colour differences were not visible ($\Delta E<0.2$) compared to the untreated samples, whereas the colour difference between the untreated and the heat pasteurised samples was notable ($\Delta E=1.2$).

The pH value was not changed by either technology.

Conductivity remained constant during all experiments.

In case of heat treatment, NEBI increased by 18%. In contrary to heat pasteurization, PEF treatment did not cause significant difference in the non-enzymatic browning index (Fig. 3).

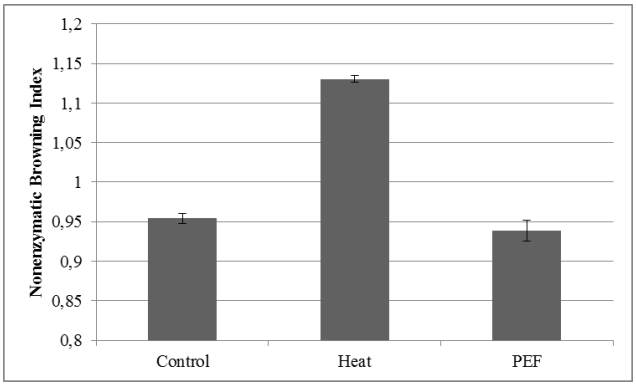

Figure 3. Non-enzymatic browning indices of untreated control, heat treated (90 °C, 30 s), and PEF treated samples (28 kV/cm, 150 µs treatment time, 2 µs pulse width)

CONCLUSIONS

The microbial counts of PEF treated red beet juices decreased at all applied working parameters, but not to the same extent. With the maximal energy input not causing electric arching in the treatment chamber the satisfying result (< 10 CFU/ml) could not be reached to begin a 28 days long cold storage. With the lowering of the pH of the red beet juice from 6.1 to 4.2, the PEF technology was able to reduce the microbial contamination further, though the initial total plate count of the acidified juice was already lower with orders of magnitudes.
Total polyphenol content was not decreased by either technology.

Antioxidant capacity was not reduced significantly.

Advantage of the PEF technology over traditional heat pasteurization is that it preserves the original colour of the red beet juice.

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THE FUNCTION OF THE LOCAL PRODUCTS IN THE FOOD CHAIN

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SUMMARY

International surveys of recent years show that both consumers and producers have increasing demand for local products. Report made by the EU Commission in 2013 affirms that local production is profitable in economical, environmental and social respect in the EU. The EU level legal framework regarding the production and commerce/trade of local products already exists. However there are large differences among the local productions in the Member States. These are likely due to differences among Member States in farm structures, distribution channels, national traditions and consumer behavior. Accordingly, elaboration of the detailed rules of the operation of local production systems should be delegated to Member States competence. In order to make provisions motivating the manufacture of local products, consumer expectations and preferences must be known. A massive research study was carried out in 2014 in the form of a consumer questionnaire/survey. The aim of the study was to map what kinds of aspects are relevant for consumers during food purchase. We make proposals on the basis of the results of the survey for the members of the local food production chain.

MATERIAL AND METHODS

The research was carried out in July 2014 with a personal questioning method based on a structured questionnaire in Budapest and 10 country side towns. The 1000 respondents were selected with quota sampling method based on the 2011 census' data- made by Hungarian Statistic Office. The selected categories were gender, age, qualification, region and abode of the respondents. There were four categories with different number of respondents (aged younger 29, aged 30-39, aged 40-59, aged older 60), seven categories by region (Central-Hungary, Central-Dunántúl, West-Dunántúl, South-Dunántúl, North-Hungary, North-Alföld, South-Alföld).

The consumer perception and knowledge of the quality labels and local products were assessed mainly by a 5-grade attitude Likert-scale. There were several open question as well for a more detailed analysis of the food purchasing habits. The error-filtered data was analyzed with the help of the SPSS statistical program package. Beyond the descriptive statistical results, the relations among the variables were studied using cross-tables.

RESULTS AND DISCUSSION

Aspects of purchasing food with quality label

The main purpose of the study were the following:
- to identify what the customers find the most important source of information about food,
- to identify the aspects of selecting food,
- to identify the premises of the food purchase.

The most trusty information resource is the labeling of the products. 88 % of the respondents state that the labeling is their prime information source. The second most important are the commercials, with only 33%. The role of printed and electrical informative articles are infinitesimal.

The respondent ranked from 1 to 5 how often they purchased at the different premises. The purchases are made mostly in the specialist's shops (3.4) followed by the hypermarkets (3.19) (e.g. Auchan, Tesco, Interspar) and the supermarkets (3.12) (e.g. CBA, Real, SPAR). The discounts (Aldi, Lidl, Penny), the small shops and buying directly from the producers scored only 2.5-2.8. Hawkers, mobile shops and the online shopping are infinitesimal (1.46-1.15).

The two main aspects of the food purchasing are the taste and the safety of the. The price is forced back to the third rank. The place of provenance, the producer and the trade name are less important aspects for purchasing food. Least of all but according to the rankings still important aspects are the brands, durability of food and the appearance of the product.

To differentiate among the different aspects we created pairs of questions. The respondents had to decide which aspect was more important for them. The opinions on the content of the labeling were not excessively different. The several respondents thought the content of the labeling gave enough information on place of provenance. Among the quality of the food and the place of provenance the Quality of the product was the more important aspect for the larger share of the respondents. The place of provenance was important as well since the most of respondents checks the place of provenance. There was no real difference among the place of provenance and the price. Both aspects were equally important for most respondents.

Most corespondents were willing to try new food by their own admission. They didn't insist on traditional food all the time. But most of them buys traditional food directly from the small producers and not from big food business operators. Most of the consumers trust in the small producers. They conceived that the products of the small producers were tasty and fresh contrary to the product of the big producers.

The person of the small producer was a more valuable guarantee then the existence of independent auditing systems.

No uniform opinion was formed on the price of the small producer's products. There were neutral opinion on the purchase from the producer or from bigger stores. Most of respondents indicated that they have trusted producers, where they regularly shop, but they still preferred to buy everything in the same place. According to the answers, the respondents cannot state unambiguously that the producers sell their self-made products on the marketplace.

Interest in local food products

The labeling "local product" would increase the trust of the most consumers (78.7%) towards local products.

This question was analyzed by cross table analysis. One demographic parameter showed significant correspondence with the trust on local products. The respondents from the country side small towns trusted strongly on the local products than respondents from the big country side towns end the capital.

One of the main purpose of the research was to identify the consumers' preferences when purchasing local products. There were five aspects in the survey and respondents could add additional aspects as well. Several aspects could have been chosen simultaneously. The

highest ranked was getting acquainted with new tastes (68.22%). Another Important aspect was the support the local business, generate new jobs (63.55%). More than half of the respondents (55.76) thought buying local food could maintain or strengthen the local economy (tourism, catering). Environmental protection (less environment charging) was an important aspect for more than one third of respondents (37.90%). An important message is that 37.07% of the respondents find a close relation to the producers essential.

It is important how local products are being purchased. The acceptance or rejection of the current practices of selling has an important message for the development of the marketing methods in the future. Most consumers (78.9%) buy local products in the market, directly from the producers. A bit less than half of the consumers (47,66%) purchase local products in the shops of the producers. One quarter of the consumers have taken part the “pick yourself” movements. Nowadays only 22.43% purchased from the farms directly. The other marketing methods - wayside marketing 7.68%, home delivery 6.54%, communal initiative 4.36%, webshop, online purchase 3.01% - has less importance in the marketing of local products.

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We think it is a positive feedback on the timing of the research that 65,89% of the respondents had purchased local food directly from the producer within a month before the survey was taken. The cross-table analysis pointed out that there was a significant connection among the age and the direct purchasing. The elderly shopped at local producers more often that the younger respondents (cross-table 14).

CONCLUSIONS

The aim of the study was to reveal and explore consumers’ perceptions relating to food. It sought to investigate consumers’ aspects when choosing food. The most trusted information source for the respondents is the labeling of the products. It is an important message to the marketing experts. The consumer takes the product from the shelf whose labeling is comprehensive.

Food purchasing is made by mostly women. They are willing to buy in the specialist’s shops and on the market. Important aspect is to purchase a lot of different kind of products at the same place, so supermarkets and hypermarkets are popular. The respondent purchase now and then directly from the producers, however the role of mobile shops and online shopping are infinitesimal in food marketing.

The results of the surveys made in the latest decade show that the price was the most relevant aspect of food purchasing. According the results of our research the taste of the food and the food hygiene are the most relevant aspects for the consumers.

There was not any previous orientation on the “local product” before the questionnaire. The respondent did not have any problem formulating their answers to the questions. The “local product” means for them in most cases a product produced by a local producer and is connected to a geographic area. The answers of the respondents from the country side reflect the unequivocally that “local product” label would enhance the consumers’ trust.

The most determining aspect of the local products is their taste. It must represent typical flavours from an area. Social awareness appears while shopping local products. Consumers believe that buying local food can strengthen local economy. The most popular place of purchase of food are the markets. Also popular are the sales in the production sites (on-farm

sales, pick-your-own movement), which are not recognized only food purchasing. The food itself is an important part of a complex program. The consumer buys a pleasant experience as well.

In our study we found that local food reaches the consumers through two main-channels. In the first case, when the consumer only wants to purchase food wide range of products need to be offered at the same place. The consumer can then be assisted by experts in choosing the right products. We believe that farmers' markets and farmer-shops could take this part in the future. There are several good examples of market places at national level where consumers can obtain local products directly from experts namely from the producers. Several good examples can be found for the other case when food purchase is combined with buying the pleasant experience. Both cases are important tools of country side development.

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IMPACT OF GROWING CONDITIONS OF RED BEETROOTS (*BETA VULGARIS L.*) FOR THE NUTRITIONAL CHARACTERISTICS

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SUMMARY

Nowadays the healthy diet is more and more widespread, so the entry of the nutrients, antioxidants and pigments in the body is important. The beetroot is rich in vitamins and minerals, and it can be grown both in summer and winter, so it has prominent part in healthy diet between the winter vegetables. It is worth to produce the beetroot in nutrient-rich soil because the tubers can effectively absorb the minerals, that is the growing conditions significantly affect the nutritional characteristics of the beetroots.
During the investigation the nutritional characteristics of three different beetroots (Cylindre, Detroit and Alto F1 varieties) can be compared, which have been grown the treated and untreated soil with fertilizer. During the experiments the total polyphenol content, the antioxidant capacity, the betanin and betaxanthin content were determined in the different beetroots varieties.

INTRODUCTION

The beetroot is native to the Mediterranean area and ancestor of wild shape of *Beta vulgaris L.* var maritime. The Greek and Romans knew and cultivated the beet root in the II-III. centuries and consumed it. It came to known in the XVII. century in Hungary.
The beetroot is one of the most ten powerful vegetables regards antioxidant capacity. The polyphenol content for the total dry matter is 50-60 mol/g (VINSON et al., 1998; KAHKONEN et al., 1999).

Two major groups of colour components of the beetroots are the red betacians and the yellow betaxantins. Besides the red pigments the beetroots contain anthocyanins which responsible for the purple shade. In the beetroots the largest part of the betacianin is betanin. The pigments of the red and yellow beet have been proved that the red components have larger free radical scavenging capacity than the yellow's and it increases fluently with the function of the pH.

Clinical research results of KAPADIA and co-workers (2011) show the growth of prostate and breast cancer cells slow down with 12% due to the betacyanin effect.

NAGY-GASZTONYI and co-workers (2005) investigated the water soluble nitrate content various red beet varieties. This content was measured as a function of irrigation and mineral supplement in three years. In the first year, the nitrate content increased in the roots following the irrigation. The activity of nitrate-reductase in the leaves was also significantly higher after irrigation in different red beet varieties. The mineral supplement causes in most of the carrot varieties increase of the nitrate content. The impact of water supply showed various tendencies in nitrate accumulation in different red beets. The results of three years showed that the seasonal and varietal differences were also remarkable.

The aim of our study was to investigate the different nutritional characteristic and compare these parameters in case of the treated and untreated beetroots.

MATERIAL AND METHODS

Plant materials

In the experiment the spring sowing of Cylindre, Alto F1 and Detroit varieties are realized in conventional soil by the Experimental and Research Farm of Department of

Vegetables and Mushroom Growing (Corvinus University of Budapest). The cultivated land was divided into two parts and only the one half was treated with fertilizer (0.50kg/10m^2). The applied fertilizer contained 27% N, 5% MgO and 7% CaO. Following the harvest the beetroots were washed, cleaned and peeled. The beetroots were cut into three parts, so separately top, middle end and peel parts. Further, the four parts were treated separately from each other. The mash was made from comminuted raw material with mixer and the pressing was realized by mechanical compression.

Analytical methods

The determination of soluble solids content has happened with using of the refractometric measurement method (DBX ATAGO ATAGO and PR-301 models). Three independent measurements were realized in case of each samples.

The colour measurement was realized with Konica Minolta CR 400 type handheld digital colour measuring device which is on the base of the CIELab system. The lightness factor (L*), the a*coordinate indicates (the transition from red to green) and the b* (from blue to yellow) were determined. The determination of the polyphenol content has been carried out with the method of Singleton and Rossi (1965). Applied measurement device was the Hitachi U-2900 spectrophotometer to the spectrophotometric measurement.

The determination betacyanin and betaxanthin content have been carried out according to the spectrophotometric method by Castellar and his colleagues (2003) and Stintzing and his colleagues (2005). The absorbency of the sample solutions to 2ref% diluted has been measured on 535 nm and 484 nm. Distilled water was used instead of blank samples.

The determination of the antioxidant capacity happened with the FRAP method. The determination of FRAP value was realized with Benzie and Strain method (1996).

RESULTS

The morphological characteristics of three beetroot varieties can be found in the Table 1. The data contain the average values of the 10 pieces beetroots.

Comparison of the treated and untreated beetroots it stated for the Cylindre and Detroit varieties can be observed substantial difference for the masses.

Table 1: Morphological parameters of the investigated beetroot varieties

Morphological parameters	Cylindre		Alto F1		Detroit	
	Untreated	Treated	Untreated	Treated	Untreated	Treated
average weights of the 10 pieces (kg)	0.196	0.357	0.140	0.141	0.189	0.357
average length of the 10 pieces (m)	0.636	0.749	0.622	0.583	0.659	0.706

Table 2: Dry matter content of the investigated beetroot varieties

Parts of the beetroots		Cylindre		Alto F1		Detroit	
		Untreated	Treated	Untreated	Treated	Untreated	Treated
Ref (%)	Top	14.20	10.00	10.37	11.23	13.11	12.80
	Middle	13.63	9.13	9.57	11.07	11.97	12.70
	End	14.80	9.60	11.17	11.27	13.17	13.60
	Peel	14.43	9.90	11.09	11.90	12.90	13.80

For the dry matter content of the investigated beet varieties the Cylindra beets showed higher values by the untreated version, and the distribution in the each parts are relatively even. For the Alto F1 and Detroit beets the data showed no substantial difference between the

treated and untreated form. The distribution in the each beet parts showed no remarkable difference (Table 2).

For the polyphenol content investigation the distribution was varied between the different parts of beetroots, but only minor differences can be observed between the treated and untreated versions, except the end and peel part of Alto F1 beets (Figure 1).

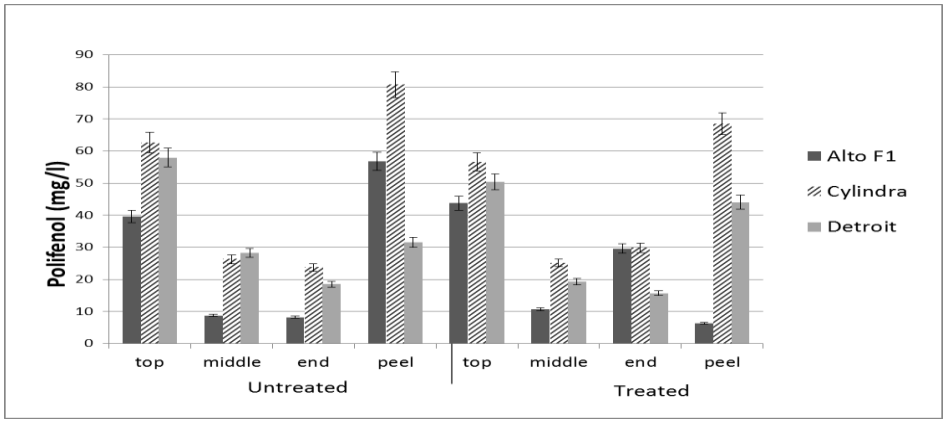

Figure 1: The polifenol content of the investigated beetroot varieties

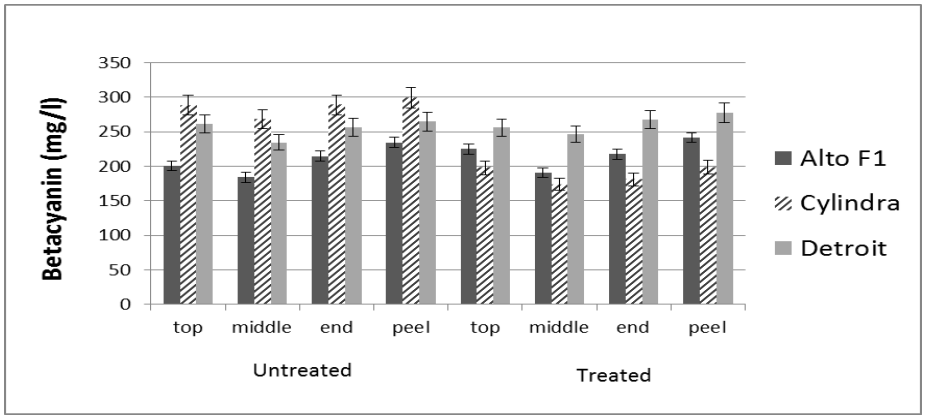

Figure 2: The betacyanin content of the investigated beetroot varieties

For the investigation of betacyanin content similar results were obtained in each parts of Alto F1 and Detroit beetroots depend on the treated and untreated versions. In case of the Cylindre significant difference can be observed between the treated and untreated versions, since the betacianin content give much lower values in the treated version (Figure 2).

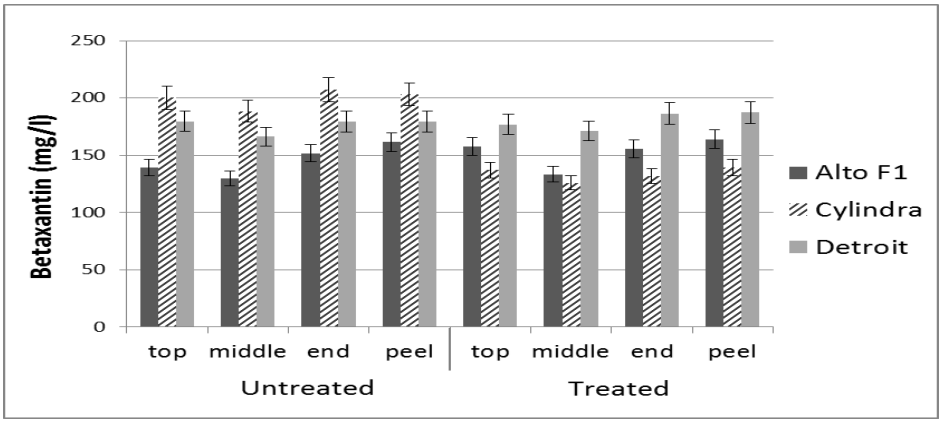

Figure 3: The betaxantin content of the investigated beetroot varieties

Also similar results can be observed for the investigation of the betaxantin content, because in case of the Alto F1 and Detroit beet varieties were no significant difference between the treated and untreated versions in contrast the Cylindre roots showed much lower values in case of the treated versions (Figure 3).

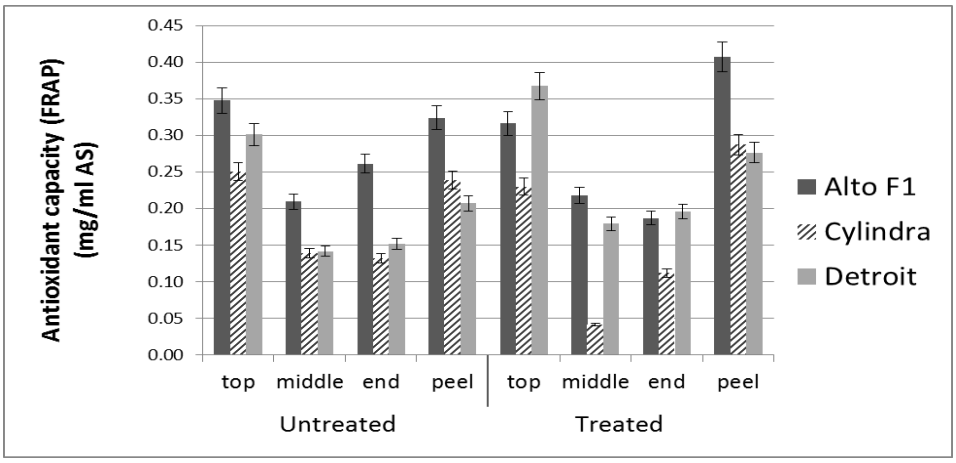

Figure 4: Antioxidant capacity of the investigated beetroot varieties

For the measurements of the antioxidant capacity much more varied results were provided in the different part of each beet varieties. For the Cylindre the antioxidant capacity values were less favourable in case of the treated versions, except in the peel part. For the Alto F1 and Detroit the distribution of antioxidant capacity showed differences since outstanding amounts were demonstrated in the peel of the Alto F1 and in the top part of Detroit.

CONCULSION

Based on the results of the morphological investigations higher yield can be achieved for the Cylindre and Detroit varieties with treatment, however in some cases the values of the inner parameters were unfavourably.

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STABILITY OF IMIDAZOLE DIPEPTIDES DURING FOOD PROCESSING

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SUMMARY

Carnosine and anserine are antioxidative dipeptides and the most abundant antioxidants in meats. Our earlier studies demonstrated the absorption of carnosine by determining its concentration in blood plasma after ingestion of meat. For optimized bioavailability the increasing knowledge about the stability of these components during processing is also important. It is known that carnosine is quite heat stable. Our previous experiments demonstrated that the freezing and freeze-drying had only a little effect on the carnosine and anserine level. The HHP (High Hydrostatic Pressure) is a promising technology for the preservation of meat products, and it shows a big potential for the innovative development of new products. In this work the contents of imidazole dipeptides of HHP treated (0-600 MPa, 5 min) beef and pork meat samples was determined. The detectable contents of the dipeptides decreased slightly in the pressurized samples, but significant differences ($p<0.05$) were not observed in each treatment.

INTRODUCTION

There are many functional compounds found in skeletal muscle of vertebrate animals. The imidazole dipeptides (carnosine and anserine) are prominent among them, because they have multifarious physiological functions and therapeutic effects, such as neurotransmitters in the brain (Tomonaga et al., 2004), buffering capacities (Harris et al., 1990), antiglycation (Alhamdani et al. 2007) and anti-ischemic effects (Stvolinsky and Dobrota 2000), modification of enzymic activities (Jonson and Hammer, 1989), antineoplastic effects (Holliday and McFarland, 1996) antioxidant and membrane protective effect (Stuerenburg and Kunze 1999). The meat is the main contributor of supply of imidazole dipeptides in humans. The absorption of carnosine was investigated and verified in rat (Tomonaga et al., 2007), in minipig (Bauchart et al. 2007) and in pig models (Ma et al., 2010) and also in human studies (Park et al. 2005). Carnosine and anserine were suggested as biomarker of meat intake (Dragsted 2010). These dipeptides - as natural antioxidant in meat - are effective in preventing oxidative rancidity and undesirable colour changes during the storage of meat (Kohen et al. 1988) so they could be used as potential markers of meat quality. Das et al. (2005) found that carnosine preblending extended the shelf life of ground buffalo meat under refrigerated storage. Ma et al. (2010) reported that dietary supplementation with carnosine improves antioxidant capacity and quality of pork meat. The imidazole dipeptide content of meat varies from a few hundreds to a several thousands of ppm depending on the species of the animal (Zapp and Wilson 1938) metabolic type of the muscle (Cornet and Busset 1999, Mora et.al. 2007), gender, age, breeding (Intarapichet and Maikhuntod 2005) and others.

The high anserine/carnosine ratio is typical of the poultry meat, in contrast with pork and beef meat (Peiretti et al., 2011). The imidazole dipeptides are fairly heat stable (Maikhunthod and Intarapichet, 2005), and unlike other endogenous polypeptides, they are relatively resistant to the hydrolytic breakdown of many common proteases (Bellia et al., 2011). In contrast with raw meat samples, few studies have described the effect of food technologies on imidazole dipeptides and the carnosine and anserine contents of processed food products (Hermanussen et al., 2010).

The aim of this study is to assess the contents of imidazole dipeptides in selected meat samples and the stability of them during high HHP treatment. Capillary electrophoresis

method was used for determination carnosine and anserine level in raw meat samples and meat based food products.

MATERIAL AND METHODS

Meat samples: Raw meat samples of different species and cuts together with the processed meat samples were purchased from local supermarkets.

HHP treatment: The extraction of imidazole dipeptides: Muscle and food samples were finely ground by a meat cutter. 5g of this sample was homogenized with 10 ml of distilled water. The homogenates were centrifugated at 20000 g for 30 min. at 4 °C. The supernatant was deproteinized by treatment in boiling water for 10 minutes then centrifugated at 5000 g for 10 min. at 4 °C and the filtered through 0.45 μm membrane.

CZE quantification conditions of carnosine and anserine: A BioFocus 2000 System with UV detector was used for the experiments. The sample was separated in uncoated fused-silica capillary with dimension of 50 μm I.D. and effective length of 45.5 cm, under voltage of 15 kV. As carrier electrolyte 0.01-0.1 M phosphate buffer (pH 2.5) was used. The dipeptides were detected without derivatization at 200 nm. The concentrations were determined from peak area with calibration and the relative standard deviations were determined from three independent samples.

RESULTS AND DISCUSSION

A number of Hungarian meat products were analyzed using the capillary zone electrophoretic method. Figure 1. summarizes the imidazole dipeptides contents of 20 Hungarian frequently purchased meat products. The characteristic carnosine/anserine ratio was observable also in the case of poultry meat based products (e.g. turkey breast ham, chicken breast ham, poultry frankfurter). The imidazole dipeptides were still detectable in intensely heat treated canned products with low meat content (e.g. lunch meat). The highest level of imidazole dipeptides was found in the smoked ham, and the lowest in a liverwurst. Because the carnosine and anserine are fairly heat stable most of the meat product contained significant amounts of imidazole dipeptides, depending on the quality of the raw material.

The detectable contents of the dipeptides decreased slightly in the pressurized beef and pork meat samples, but significant differences ($p<0.05$) were not observed in each treatment (Fig.2).

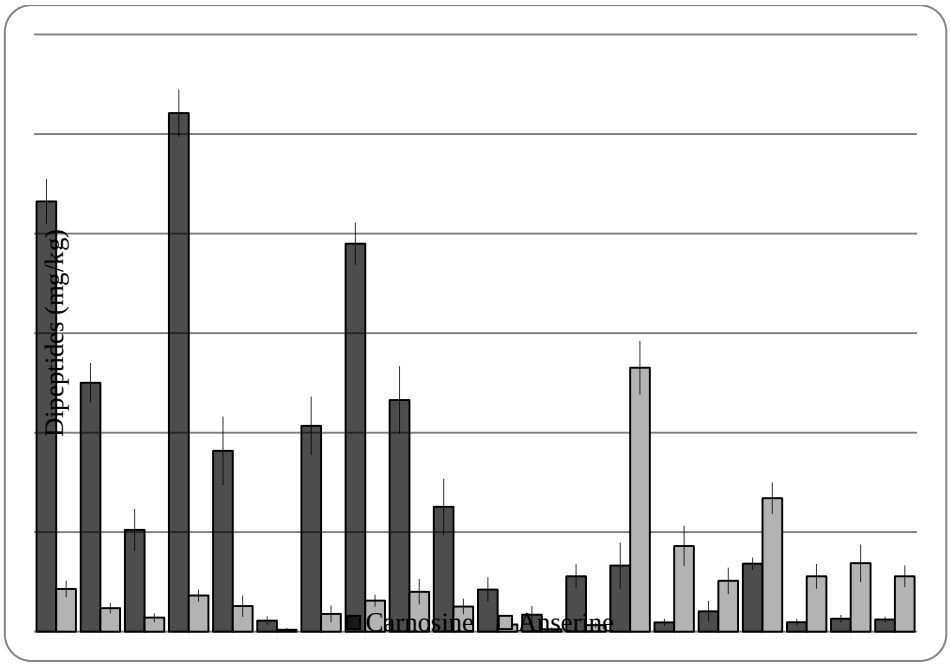

Figure 1.: Level of carnosine and anserine in meat products determined by capillary zone electrophoresis. Each value represents the mean of three different extracts from the sample, and labelled error bars indicate the standard deviation for each measurement. 1: stuffed pork chop, 2: salami (pork), 3: Debrecen sausage, 4: smoked ham, 5: Prague ham, 6: liverwurst (1), 7: lunch ham, 8: backed ham, 9: chop ham, 10: Wienerwurst, 11: liverwurst (2), 12: lunch meat, 13: Bologna sausage, 14: turkey breast ham (1), 15: turkey breast ham (2), 16: chicken breast ham (1), 17: chicken breast ham (2), 18: turkey ham, 19: smoked poultry sausage, 20: poultry frankfurter.

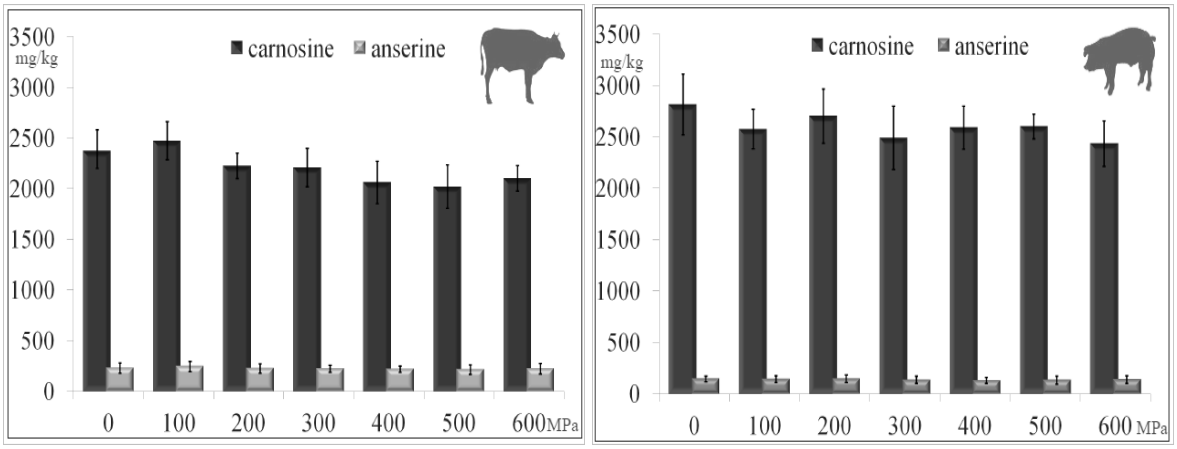

Figure 2.: Level of imidazole dipeptides (mg/kg) in HHP treated (0-600 MPa) beef striploin (a) and pork leg (b) samples determined by capillary zone electrophoresis. Each value represents the mean of three different extracts from the sample, and labelled error bars indicate the standard deviation for each measurement.

CONCLUSIONS

The developed CZE method is useful for determination of imidazole dipeptides in meat and also in intensely heat treated canned products. The carnosine and anserine content of meat products is determined mainly by the quality of the raw material because the imidazole dipeptides are fairly stable during food processing. The results may provide a scientific basis for the development of high-quality meat products which are fit into a healthy diet.

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THE OCCURENCE OF *FUSARIUM* MYCOTOXINS IN CASE OF CEREALS HARVESTED FROM TWO REGIONS IN HUNGARY

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SUMMARY

The Fusarium mycotoxins such as: deoxynivalenol, zearalenone and T-2 are frequent contaminations of grain crops in Middle and Eastern Europe. This survey has been prepared to state and prove this fact. We have examined 116 cereal samples for deoxynivalenol (DON), zearalenone (ZEA) and T-2 mycotoxins in samples collected from the different areas of Hungary in two Hungarian regions (north and south Transdanubia). The main point of view to choose the grains were which are used mainly in human and animal feeding in Hungary. These cereals are: maize, wheat, barley and oat. The inspection method was indirect competitive ELISA. Concentrations of deoxynivalenol, zearalenone and T-2 were determined. The examination stated that maize was the most contaminated grain regarding DON (86%), ZEA (41%) and T-2 (55%) toxins.

INTRODUCTION

The Fusarium mycotoxins cause significant losses in case of grains and this contamination occurs before harvest (Tanaka et al., 1988; Creppy, 2002; Geraldo et al., 2006). The extent of the affect of these mycotoxins often depends on the climate conditions, and is widely changeable depending on the influence of the different climate zones (Sforza et al., 2006). Cereals and cereal-based products are the main source of mycotoxin intake in EU population (Schothorst, van Egmond, 2004). The consumption of grains and products contaminated with Fusarium mycotoxins may lead to intoxication, called mycotoxicosis. This can be the cause of different illnesses and it may has teratogen, carcinogen, immune-suppressive impact on humans (IARC, 1993; Canady et al., 2001; Kabak et al., 2006).

The applicable and reliable methods to detect and quantify mycotoxins are the cromatographic (and its analytical equipment the high performance liquid chromatography HPLC-equipment), and the ELISA method (Schaafsma et al., 1998; Krska et al., 2007). The advantage of ELISA method is that it is fast, accurate, sensitive and reproducable. The specificity of the method depends on the preparation of the sample, the efficiency of the mycotoxin extraction, and depends on the antibody applied (Barna-Vetró, Solti, 2001).

The aim of the present study is to collect data regarding deoxynivalenol, zearalenone and T-2 contamination of grains (wheat, maize, barley and oat) harvested from different fields located in two Hungarian regions, and to describe which cereal is mainly concerned regarding contamination.

MATERIALS AND METHODS

Samples

We have used 116 cereal samples for our measurements which derived from the different territories of Hungary (two regions: north and south Transdanubia). Cereal samples were collected from the following north Transdanubian counties: Győr-Moson-Sopron, Komárom-Esztergom, Vas county, and south Transdanubian counties: Somogy, Tolna, Baranya county. The examined grains were: wheat, maize, barley, oat (29-29 samples for each grain).

Determination of mycotoxins

This experiment was carried out with ELISA (Enzyme Linked Immunosorbent Assay) method, which is based on antigen-antibody reaction. Ridascreen kits were used for preparation of the measurements (DON, ZEA, T-2 kit). Mycotoxin concentrations were determined using competitive ELISA test kits as instructed by the kit manufacturer. Ridascreen test kits were provided by R-Biopharm /Darmstadt, Germany.

RESULTS AND DISCUSSION

Altogether 116 cereal samples were examined: 29 maize, 29 wheat, 29 barley and 29 oat samples. The determined number (No) and percentage of positive samples are shown in Table 1. In maize DON was quantified in 25 (86%), ZEA in 12 (41%), T-2 in 16 (55%) samples. Wheat was also found to be contaminated with DON, ZEA, T-2 toxins: 21 (72%), 5 (17%), 9 (31%) samples respectively. In barley DON was quantified in 14 (48%) and T-2 in 4 (14%) samples. In oat DON and T-2 were quantified in 8 (27%), 3 (10%) samples.

Table 1.: Number (No.) and percentage of positive samples of DON, ZEA, T-2 mycotoxins in cereals.

Mycotoxin	Cereal	No. of positive [a]/ No. of total samples	Percentage of positive samples (%)
DON	Maize	25/29	86
	Wheat	21/29	72
	Barley	14/29	48
	Oats	8/29	27
ZEA	Maize	12/29	41
	Wheat	5/29	17
	Barley	0/29	0
	Oats	0/29	0
T-2	Maize	16/29	55
	Wheat	9/29	31
	Barley	4/29	14
	Oats	3/29	10

[a] Mycotoxin concentrations are above Limit of Quantification (> LOQ).

The average (mean), minimum (min) and maximum (max) concentrations with the accompanying standard deviations (SD), displayed per an investigated mycotoxin and cereal, are shown in Table 2.

Table 2.: Concentrations of mycotoxins in cereals harvested on different fields in two Hungarian regions.

Mycotoxin	Cereal	Mean of positive samples (µg/kg)	SD (µg/kg)	Min (µg/kg)	Max (µg/kg)
DON	Maize	1894	1421	225	2963
	Wheat	478	205	230	1880
	Barley	339	89	240	429
	Oats	272	81	222	359
ZEA	Maize	267	164	54	565
	Wheat	83	15	50	98
	Barley[a]	<50			
	Oats[a]	<50			
T-2	Maize	69	27	55	146
	Wheat	64	12	54	87
	Barley	58	11	52	79
	Oats	56	12	50	81

[a] Mycotoxin concentrations are under Limit of Quantification (<LOQ).

CONCLUSIONS

Toxin contamination levels sporadically higher than allowed might be associated with some factors and climate conditions, known to be important for molds and mycotoxin formations. During the examination of the permissible levels it can be established that there has been a significant difference between the mycotoxin limit values meant for human and animal feeding, which may mean an indirect risk to human health and may rise food safety problems.

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COMBINATION OF HIGH HYDROSTATIC PRESSURE AND HEAT TREATMENT ON LIQUID EGG WHITE

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SUMMARY

Minimal processings (combinations of thermal treatments and high hydrostatic pressure - HHP) of liquid egg white (LEW) were studied. The effects of different treatment's parameters to color, viscosity, pH and microbial state were investigated by the authors. Fresh and homogenized LEW was prepared for samples for our study.

For prior experiment 300 and 350 MPa, 300 s HHP and 57°C 600 s thermal treatments were applied, in this case we used fresh and homogenised LEW. For the experiment homogenized LEW's were treated between 279,3 and 400 MPa HHP combined between 45,9 and 74,1°C. The experiment was composed a Central Composite Design – CCDA

Our results show that the different combinations of minimal processings have several effects to the quality of LEW. Based on our applied model there are significant different effects of treatment's parameters that statistically is proven.

INTRODUCTION

High hydrostatic pressure (HHP, or High Pressure Processing HPP) belongs to the physical, non-thermal so called „minimal processing" food preservation methods. During HHP-treatment food products packed in flexible airtight packaging materials and immersed in liquids are under equal hydrostatic pressure in whole volume (isostatically) according to Pascal principle, therefore treatment parameters are independent from size and figure of products (Dalmadi, Farkas, 2006; Farr, 1993; Lechowich, 1993; San Martin et al. 2002). Adverse effects of high temperature can be ignored and the quantity of used chemicals (preservatives) can be reduced or completely omitted. HHP-treatment can evolve new techno functional properties, e.g. in case of liquid egg white (LEW) better foaming ability develops (Oey, Lilly 2008; Rico, Martin-Diana, 2007).

After the II World War egg production launched a sharp increase that shows nowadays a growing trend too, estimated in 2015 volume of production may be greater than 72 million tons that means 1260 trillion pieces of shell eggs (Deutsche Stiftung Weltbevölkerung 2009; FAO, 2005). The growing demand justify the development of new products which are easy to store and use, have extended shelf-life and are fully satisfying the aspects of food safety.

Often we crash the problem by heat treatment of egg products, that there may be surviving microorganisms which can multiply in refrigerated storage. We can solve this problem with combination of HHP and heat treatment based on research results (Pilavtepe et al., 2008; Wang et al., 2013).

The aim of our study is to investigate how the different heat and HHP treatment parameters and combinations of the treatments influence some properties of liquid egg white (LEW), so colour, pH-value, viscosity and microbiological state. Results were analysed statistical assisted with Central Composite Design – CCDA.

MATERIALS AND METHODS

We used two different kind of liquid egg white: one was bought at a supermarket and broken at the laboratory by hand, the other is homogenized LEW from production line of **Capriovus Ltd.** Physical investigations were performed from LEW with a maximum storage

time 24 hours between 0-4°C, microbiological samples were prepared from 21 days refrigerated storage LEW consciously increased microbiological load. For every treatment combination 3-3 samples were prepared. Each sample was 150 ml, packaged in polyethylene bags and hermetically sealed. The combinations of treatment's parameters are included in **table 1** for **previous-experiment**

Table Hiba! A hivatkozási forrás nem található.**.** Treatment parameters of prior experiment

Sample coding	Temperature of heat treatment (°C)	Time of heat treatment (min)	Pressure range of HHP (MPa)	Time of HHP (min)
Control	-	-	-	-
57°C	57	10	-	-
350 MPa.	-	-	350	5
53°C 350 MPa	53	10	350	5
57°C 300 MPa	57	10	300	5
57°C 350 MPa	57	10	350	5

The **main experiment** was evolved from the experiences of previous-experiment. In this case only homogenized LEW was used taken from production line of Capriovus Ltd. Combinations of HHP and heat treatments are shown in **table 2**. In every combination heat treatment was the first step followed by HHP. To pasteurization laboratory water bath was applied and for cooling ice water was used. HHP treatment was carried out in a RESATO FPU 100-2000 equipment at Corvinus University of Budapest, Department of Refrigeration and Livestock Products Technology. Increasing of pressure was 100 MPa/min.

Table Hiba! A hivatkozási forrás nem található.. Treatment parameters of the main experiment

Sample coding	Temperature of heat treatment (°C)	Time of heat treatment (min)	Pressure range of HHP (MPa)	Time of HHP (min)
45,9°C, 350 MPa	45,9	10	350	5
50°C, 300 MPa	50	10	300	5
50°C, 400 MPa	50	10	400	5
60°C, 279,3 MPa	60	10	279,3	5
60°C, 350 MPa	60	10	350	5
60°C, 420 MPa	60	10	420	5
70°C, 300 MPa	70	10	300	5
70°C, 400 MPa	70	10	400	5
74,1°C, 350 MPa	74,1	10	350	5

Microbiological tests examined the mesophilic aerobic microbial count. Nutrient agar and an incubation of 48 h and 30°C were used. Colour measurement was performed with a Konica Minolta CR-400, from results ΔE_{ab}^{*} color difference was counted. Consort C831 pH-meter was used to evaluate pH-value, viscosity was measured by Rheomat 115.

Statistical analyse of the experiment was carried out by Central Composite Design – CCDA. Setting up model only 11 treatments were necessary.

RESULTS AND DISCUSION

The **pH-value** of samples during pre-experiment (**figure 1**.) showed no basically changes after treatments, but fresh and homogenised LEW gave significant differences. Every case homogenised samples had lower pH-values, on the contrary pH-values of samples during main experiment (**figure 2**.) were similar to fresh LEW from pre-experiment. The effects of combined treatments had no significant changes in pH-value called our statistical modelling.

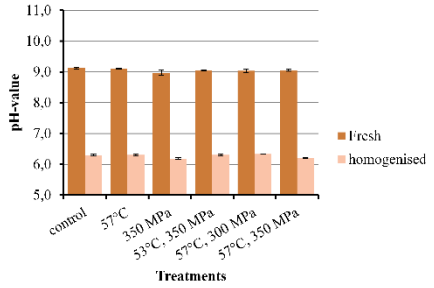

Figure 1.: The pH-value of samples during pre-experiment

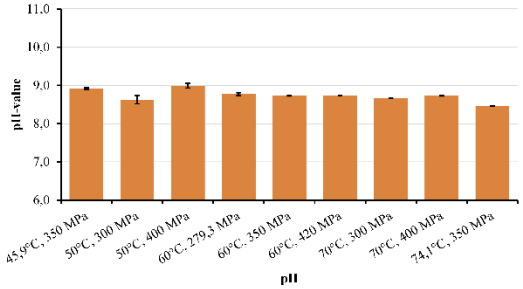

Figure 2.: The pH-value of samples in main experiment

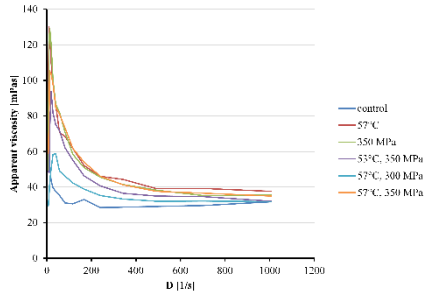

Figure 3.:. Apparent viscosity during the pre-experiment

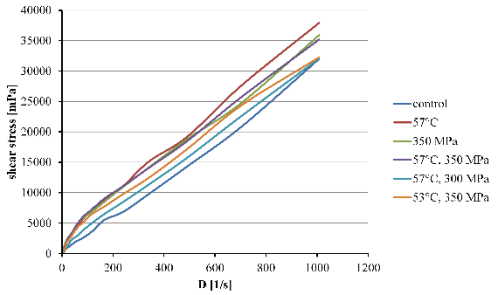

Figure 4.: Shear stress during the pre-experiment

Viscosity of LEW during pre-experiment is show on **figure 3** and **4.** As it is expected, viscosity of homogenised samples is lower than from fresh egg white. Changes caused by treatments were less by homogenised samples. Apparent viscosity of samples treated in main-experiment is lower, if higher temperatures were used in heat treatments (**figure 5**.). Accordingly to apparent viscosity stear stress (**figure 6.**) is higher with higher temperature and pressure of treatments.

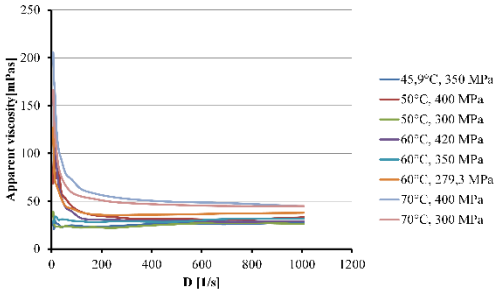

Figure 5.: Apparent viscosity of treated samples

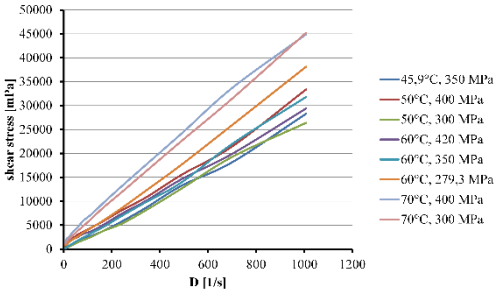

Figure 6.: Shear stress of treated samples

Microbiological load of samples decreased by every treatment of pre-experiment (**figure 7**.). Least effective was HHP treatment (350 MPa) without heat treatment, in homogenised samples plate count had a greater reduction. The effects of combined treatments of main experiment were greater with increasing of treatment parameters (**figure 8**.). Heat treatments of 70°C combined with HHP were able to reduce CFU at least 8 orders of magnitude. There were statistical significant differences between effectiveness of combined treatments.

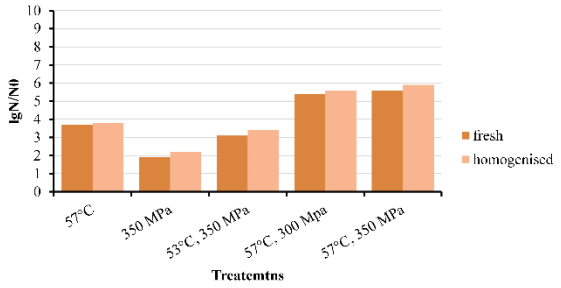

Figure 7.: Microbiological load of samples of pre-treatment

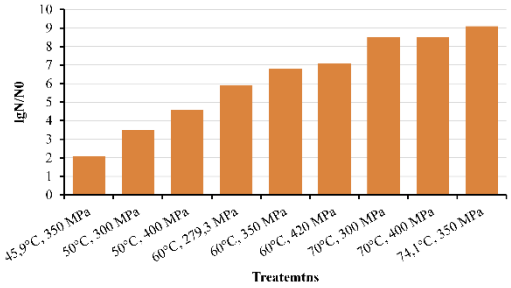

Figure 8.: Microbiological load of samples of experiment

CONCLUSION

The combinations of heat and HHP treatments were examined. Based on our results it can be seen that different combinations of heat treatments and HHP treatments influence product properties diverse. The pH-values of samples were not changed by treatments. Viscosity characteristic has deteriorated, the influence of that changes to techno functional properties have to be examined. We experienced a convincing decrease of microbiological load that may extend shelf-life of LEW. Appropriate food safety is not enough to produce marketable products, we have to develop fresh similar properties which lead to good usability of liquid egg white.

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EXTENDED SHELF-LIFE OF FRESHLY SQUEEZED VEGETABLE AND FRUIT JUICES BY THE APPLICATION OF HIGH HYDRISTATIC PRESSURE

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SUMMARY

Our study is about extended shelf-life of freshly squeezed vegetable and fruit juices treated in industrial HHP environment. 9 different recipes were produced from carrot, orange, grapefruit, beetroot, lemon, parsley, celery, celery sticks, apple, mint, ginger, spinach and water. Juices were treated with 500 MPa, for 120 s, or with 600 MPa, for 180 s and stored at 2-5°C for 35 days. The colour, pH-value, microbiological stability and sensory evaluation of the samples were measured. pH-values of red group were increased, in yellow and green groups slightly declined. The colours (CIELab colour system) of every sample changed at least highly visible during storage time. In every case microbiological state was appropriate, 6 samples were used to a challenge test with Aspergilllus niger F882 an Saccharomyces cerevisiae Y801 that establishes HHP is able to reduce microbiological load at least eight orders of magnitude. Sensory tests pointed out that after 35 days the samples were like freshly squeezed, but for Hungarian customers vegetables juices are unusual that's why this product group has to be acquaint.

INTRODUCTION

High hydrostatic pressure, HHP (also known as High Pressure Processing HPP) is a cold pasteurization technique which offers an opportunity to preserve food products quality with high content of minerals and vitamins and freshness. HHP is a „minimal processing" technology which promising for products with high water activity, like juices.. To overcome such shortcomings of heat processing, different processing technologies including the applications of high pressure have been developed as the non-thermal emerging technologies for juice processing (Soliva-Fortuny et al. 2009). Alternatives to the traditional thermal processing, which do not involve direct heat impact, are investigated in order to obtain safe products, but in the same time with fresh-like quality attributes (Knorr et al., 2011). To fight wodn such shortcomings of heat processing, different processing technologies including the applications of high pressure have been developed as the non-thermal emerging technologies for juice processing (Soliva-Fortuny et al. 2009).

The food industry continues to be interested in the use of minimal processing technologies as a way of meeting consumer demand to extend shelf-life, without the need for chemical preservatives and without compromising the wholesomeness and safety of foods. The retention of fresh-like characteristics and nutritional quality are particularly important in certain foods, such as juices, and can be used in the marketing strategy for premium added-value products (McKay et al., 2011). Epidemiological studies have reported that there is a significant positive association between consumption of polyphenols and reduced risk of chronic diseases such as viral infections, cardiovascular disease, diabetes, cancer, inflammatory activities and Alzheimer's disease (Galati, et al., 1994; Hertog et al., 1993; Liu, 2003; Scambia et al., 1994; Temple, 2000; Willett, 2002).

As it is shown, many studies reported about the positive effect of HHP in laboratory conditions, however our work focused on HHP treatment on executing industrial equipment. The aim of this experiment was to support the viable industrial production.

MATERIAL AND METHODS

Samples were prepared between industrial conditions by Presh Food System Ltd., after squeezing and mixing the samples were bottled in 0,2 l PET bottles and HHP treated. Processed juices were stored at 2-5°C. For the assessment the samples were grouped in 3 groups after its colour (and ingredients): red (containing beetroot), yellow (containing orange) and green (containing apple). The ingredients, coding, groping and treatment parameters of juices are shown in **table 1**. The used treatment parameters were chosen accordance with possible contamination of ingredients.

Table 1.: Coding, ingredients and treatment parameters of samples

Code	Sensory code	Ingredients	Pressure, MPa	Time, s
1.	031	apple, beetroot, carrot, water, lemon, ginger	500	120
2.	518	apple, beetroot, spinach, celery, parsley, water, lemon	600	180
3.	713	green apple, spinach, cucumber, parsley, water, lemon, ginger	600	180
4.	882	green apple, celery, cucumber, water, lemon, ginger	600	180
5.	976	apple, ginger, lemon	500	120
6.	455	orange, carrot, water, ginger	500	120
7.	642	orange, ginger	500	120
8.	351	grapefruit	500	120
9.	294	apple, orange, grapefruit	500	120

To measurement of pH-value Testo 206 pH-meter was used. Color measurement was carried out with Minolta CR-400 and colour differences were calculated to the color of untreated control samples. From colour factors (L*, a* and b*) were tested by two way ANOVA. Sensory evaluation was made by 12 laic panellists, they had to work in a 5(figure 5) point system and create a preference order.

In microbiological challenge testes *Aspergilllus niger* F882 and *Saccharomyces cerevisiae* Y801 were used. Native (untreated, natural microbiological loaded), inoculated untreated and treated samples were prepared and stored between the same conditions like the other samples.

RESULTS AND DISCUSSION

The **pH-value** of red and green samples (**figure 1** and **3**) increased during 35 days of storage but the pH of yellow samples (**figure 2**) decreased or stayed the same values. It can be seen that the combinations of ingredients influence the change of pH, not the time of storage. Colour differences ΔE_{ab}^{*} of every samples compared to control were at least visible, ΔE_{ab}^{*} grew during storage time. Juices containing beetroot became significantly darker.

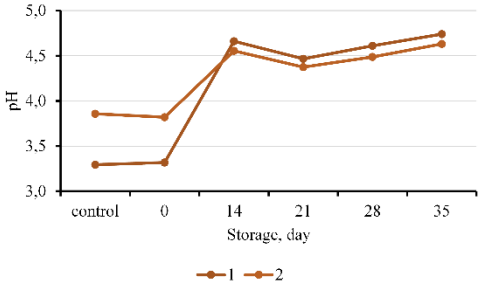

Figure 1.: The pH-value of red samples

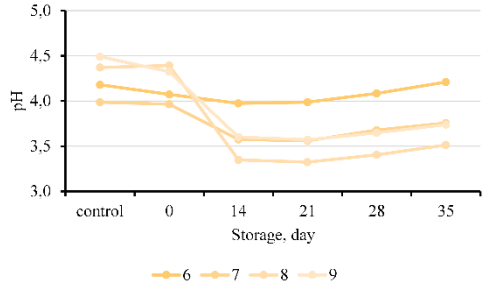

Figure 2.: The pH-value of yellow samples

Sensory evaluation of juices showed freshly-like characteristic of juices. Sedimentation of samples had a mixed reputation but as **figure 4** shows during storage this

prosperity became less and less liked by panellists. The intensity of odour (**figure 5**) had no tendencies, but quality of odour (**figure 4**) stand stabile mostly by every sample. Taste intensity (**figure 7**) was liked by panellists, but the quality (harmony) of taste (**figure 8**) was inadequate, it can be explained by the fact that in Hungary mixed juices from vegetables and fruits are unknown for consumers.

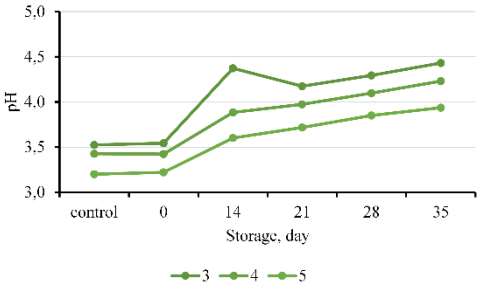

Figure 3.: The pH-value of green samples

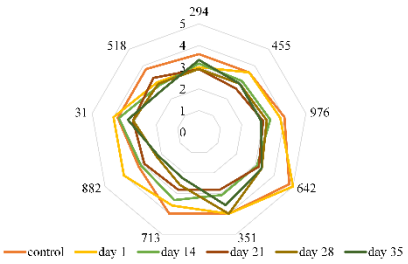

Figure 4.: The sedimentation of samples by opinion of panellists

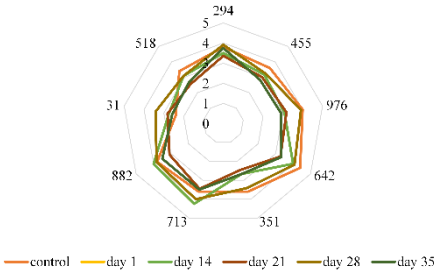

Figure 5.: The intensity of odour

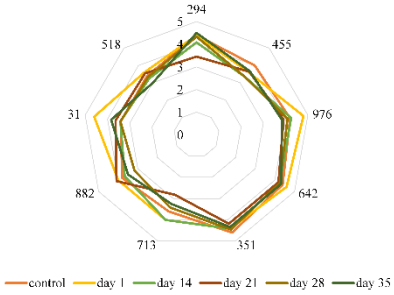

Figure 6.: The quality of odour

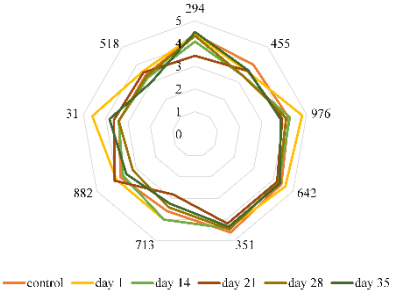

Figure 7.: The intensity of taste

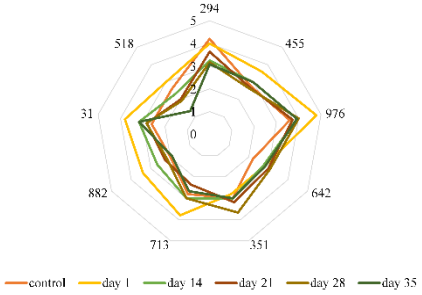

Figure 8.: The quality of taste

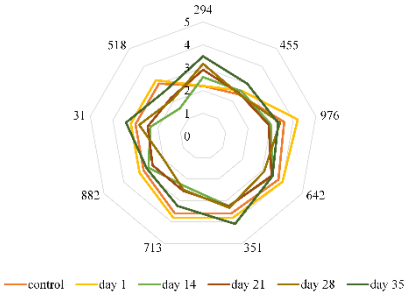

Figure 9.: The furiousness during tasting

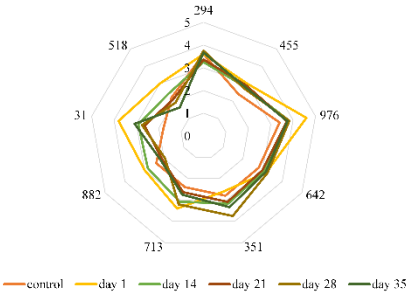

Figure 10.: The overall impression

In **figure 9** furiousness of juices is shown: during storage time the preferences of panellists were very variable, but it has a downward trend. The overall quality of juices (**figure 10**) of six samples had the best judgment on the first day and several control samples

had the worst assessment. Juices with beetroot were unliked because of their getting brown colour.

Microbiological load of juices is shown in **figure 11**. As it can be seen, native microbial state (mesophyll aerobic plait count) of juices was between 3-4 logCFU/ml, in challenge test it was increased to 8,5-9 logCFU/ml. During storage the load of inoculated control samples gron to 12 logCFU/ml, in contrast in HHP treated inoculated samples the microbiological load remained constant, there was no microbial growth. The results of microbiological experiment shows that HHP is able to extend shelf-life of freshly squeezed juices.

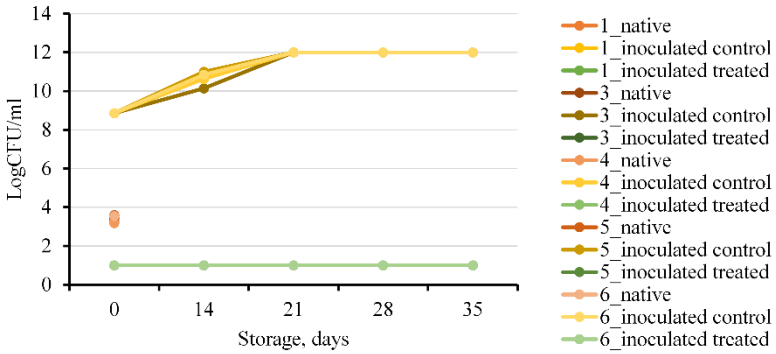

Figure 11.: Microbiological load of samples during storage time

CONCLUSIONS

In our study is shown that the shelf-life of freshly squeezed fruit and vegetable juices can be extended by the applications of high hydrostatic pressure. HHP combined with 2-5°C storage temperature may grant microbiological stability and essentially unchanged, freshly-like sensorial quality. The colour and pH-value show statistically significant changes, but in long run these changes are stabilized

According to our results 500 MPa, 120 s and 600 MPa 180 s HHP treatments in industrial equipment combined with 2-5°C storage temperature may allow at least 21 DAYS shelf-life of freshly squeezed juices. Different compositions and different storage temperatures may be examined for validation of industrial applications.

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THE SOCIAL COST OF FOOD BORNE DISEASES: A SCOPING REVIEW OF METHODOLOGIES

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SUMMARY

Food borne diseases are important economic problems worldwide. Though in most countries the true impact of these diseases is still unknown, a vast amount of social resources are being spent annually to prevent health damages due to food consumption. In recent years, researchers worldwide have been working to develop a methodology that will allow for the quantification of the differences between national database incidents and true incidents, thus, providing the necessary information to estimate the cost of diseases at a national level. However, these studies use different methods and vary by perspective which can often limit the comparability of the results. The purpose of the present study is to summarize the results of some significant international research on methodologies.

INTRODUCTION

The examination of the high cost resulting from food-borne diseases has become very important globally due to the large number of cases and the heavy burden they place on society. In order to estimate the economic burden of a disease, the true incidence, the outcomes and component costs incurred due to health damages should be identified.

However, data on food borne illnesses – especially the cases due to food consumption in households - are often not consistently collected and reported in databases in many countries. In a WHO report, the registered number of food-borne diseases even in developed countries is estimated to be merely 10 to 30%, which is referred to as 'the tip of the 'iceberg' (Stréterné et al. 2008). Between 1987 and 2000, two British researchers Skirrow and Brazier used similar sources to determine the occurrence of some important food-borne diseases. Despite the differences between their individual methods, their results provided valuable information on the differences between recorded and true incidents. During this period, officially nearly 10,000 people became infected with Salmonellosis. However, the total number of infections was 106,000, as registered in a 1995 report (Brazier et al. 1995). Different potential outcomes of a disease lead also to high medical costs and bears heavy social and economic burden, thus, is worth to map all of the potential transition that can occur between different health states.

As a vast amount of social resources are being spent annually to prevent health damages and outcomes worldwide, governments require appropriate methods and information to prioritize resource allocation. However, it is difficult to summarize indirect and direct costs across multiple studies. Considering some significant international studies, researchers use different methods and methods vary by perspective which can often limit the comparability of the results. It is evident that there is a lack of standardization in cost calculation in the literature. The categories of costs are incurred by individuals or households, industry, and the regulatory and public health sector.

METHODS

This short review was conducted to identify the reliable data sources used for estimating the true incidence of food-borne illnesses and their outcomes as well as the component costs incurred due to health damages.

Our study was accomplished by: (1) selecting the relevant literature, (2) and summarizing the results. All relevant data collected in the study were subsequently grouped into themes.

RESULTS AND DISCUSSION

True incidence of food-borne illnesses and a special multiplier factor

As proved by numerous international studies, reported illnesses are only a fraction of the true incidents (FSA, 2011). Their real number is significantly larger as compared to statistical data. Calculation of a multiplier factor to correct reported cases is an extremely complex task, the realisation of which requires laboratory tests, research data and survey results from representative samples. A comparison of the multipliers for food-borne diseases registered in the national database shows marked differences within individual countries. Results by researchers of Danmarks Tekniske Universitet in order to correct the number of statistical incidents of different diseases, specific multipliers should be used (7.1 in the case of Salmonellosis and 12 in infections with Campylobacter). In Hungary, according to a recent study, in the case of gastrointestinal diseases, factor of 125-200 can be acceptable (Krisztalovics & Kasza 2007).

Description of health outcomes

The outcomes of foodborne infections and the probabilities of transferring to these outcomes following infection can be described in an outcome tree or a stochastic Markov model, in which transition probability should be applied to each stage of the disease from its beginning (Gulácsi et al. 2005).Outcomes trees are developed on the basis of a literature review of other burden-of-diseases studies which associate specific outcomes with food borne infections (Havelaar et al., 2012). In this respect, these models are often significantly different.

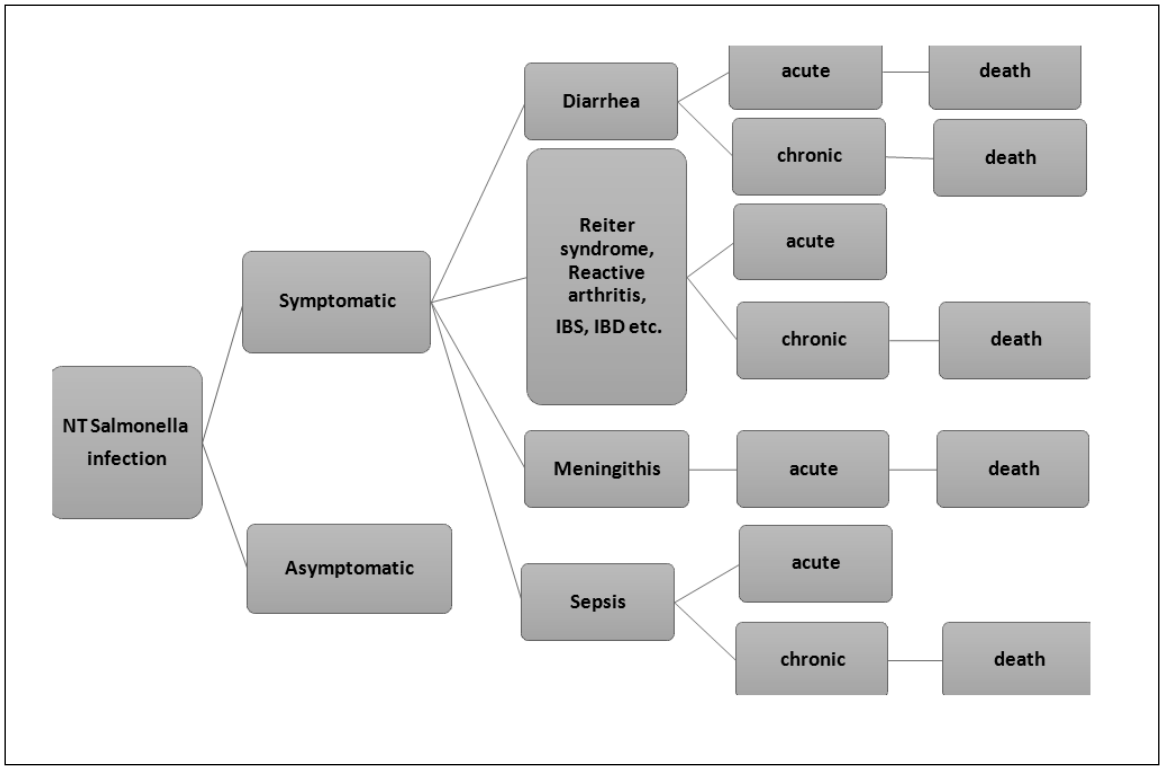

Figure 1. Possible outcomes of non-tifoid salmonella infections on the basis of a literature review (Sources: Mearin et al. 1995, Thomson et al. 1995, Dworkin et al. 2011, Spiller et al. 2007, Pires et al. 2014)

Calculation of social costs

In order to estimate the cost of diseases at a national level, these studies use different methods and vary by perspective which can often limit the comparability of the results. Some of the most often used methods in cost of illness studies are:

- Cost-of-Illness (COI): this method aims to identify direct, indirect as well as „soft costs" of households and companies. This approach includes various aspects of the disease impact on the health outcomes in a country, communities and even individuals. It helps to understand the effect of diseases on morbidity, mortality, quality of life (QOL), and financial aspects as well (Buzby, 1996).
- DALY (Disability Adjusted Life Years): DALY measures „in quality of life years" the gap between current health status due to disease and an ideal health situation. It is calculated as the sum of the years of life due to premature mortality and the years lost due to disability) (Lopez et al, 2006).
- QALY: Similarly to the DALY method, QALY can also measure the impact of health interventions – but contrary to the DALY- it focuses on the "gains" and expected improvement in health state. It investigates the impact of different health interventions on the quality and length of life of the patient. QALY can compare different health interventions under the same or different conditions (Kaló & Nagyjánosi, 2009)
- WTP (Willingness-to-Pay): it is a measure of human preferences. WTP represents the sums of the maximum amounts that people would be willing to pay to avoid illnesses and outcomes.
- RIA (Regulatory impact assessment): RIA is a systemic approach to help decision-makers to identify the positive and negative effects of proposed and existing regulations and non-regulatory alternatives. RIA includes a wide range of methods (OECD, 2007).

CONCLUSIONS

In recent years, researchers worldwide have been working to develop a methodology that will allow for the quantification of the differences between national database incidents and true incidents, thus, providing the necessary information to estimate the burden of food-borne diseases. The majority of international studies determine the true occurrence of food-borne diseases on the basis of data supplied by national health authorities and supervisory bodies (WHO, 2012). These databases represent valuable sources of information for public health economics, however, in these information sources significant differences are emerged when compared to the occurrence of true incidents. Public surveys on households also indicate that in case of a food-borne disease with mild symptoms, patients are often reluctant to visit their GP's, which leads to the increase of unreported cases. In this respect it is worth to mention that consumers' health consciousness has a considerable influence on changes in the number of reported cases (Motta et al. 2014).

In order to estimate the total burden of a disease, it is also vital to know its outcomes that can occur. Each new outcome leads to high medical costs and bears heavy social and economic burden. The methods most commonly used to calculate social costs of diseases are the so called COI, DALY and WTP. These methods vary by perspective and use different unit of measurement which can limit the comparability of the results.

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TRACKING AND COMPUTER-AIDED SIMULATION OF MALTING OF EINKORN (*TRITICUM MONOCOCCUM L.*)

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SUMMARY

Einkorn hasn't been malted at large-scale in Hungary before, thus the aim of our research was to make and optimize a preliminary computer-aided model about industrial malting of einkorn. The micromalting was performed with the same parameters that were used at the model. Samples were taken during micromalting. Physical and chemical properties were measured. Data of measurements were statistically analyzed. The bulk density values of einkorn samples are lower than other cereals; weight loss is normal compared with barley; compared with other cereals the ash contents are higher; starch, protein and fat contents didn't change. In the computer-aided model the germination is the time bottleneck. A second-order polynomial model was fitted to correlate the relationship between independent variables and response of the computer-aided model. The optimization gives realistic parameters at the minimum point of the polynomial model.

INTRODUCTION

Einkorn (*Triticum monococcum L.*) is ancient wheat and it is cultivated potentially in organic farming. (Kovács, 2008; Løje et al., 2003) Einkorn is regarded high-nutritional value cereal, especially considered for its high protein, mineral and antioxidants content. (Brandolini et al. 2008; Fogarasi et al., 2013) The aim of a Hungarian project consortium (Alkobeer_TECH-08-A32-2008-0423) was to develop an einkorn based organic and functional beer. The members of the consortium were the Centre for Agricultural Research, the Hungarian Academy of Sciences; the Körös-Maros Biofarm Ltd.; the Department of Brewing and Distilling, Corvinus University of Budapest and the Biokontroll Hungária Nonprofit Ltd. At the Corvinus University of Budapest, Department of Brewing and Distilling, usability of einkorn in the brewing industry was researched, because the brewing industry is optimized to use of barley. The detailed tasks of the Department of Brewing and Distilling were the following: elaboration of micromalting process, preparing of technological procedures at laboratory level, pilot brewing experiments, laboratory analytical tests (mineral composition) and quality stability testing in the course of production cycles. Because einkorn hasn't been malted at large-scale before in Hungary, the aim of our research was to make and optimize a preliminary and realistic computer-aided model about industrial malting of einkorn. The final computer-aided model is based on the latest research results of the consortium project, the literature sources and the own developed tracking of micromalting process. With the using of the model, energy and material balances and economic calculations can be performed.

MATERIALS AND METHODS

Micromalting

Experiential micromalting has been performed with a Schmidt-Seeger micromalting system (Schmidt-Seeger GmbH, Germany). Malting of einkorn has been optimized by Response surface methodology (RSM) at Department of Brewing and Distilling, Corvinus University of Budapest. The *Table 1.* (data from Fogarasi et al., 2012**)** shows the applied parameters for the malting of dehulled einkorn.

Table 1.: Malting parameters of dehulled einkorn

Malting step	Duration (h)	Air temperature (°C)
I. Steeping	4	22
Air rest	12	22
II. Steeping	4	22
Germination	120	20-22
I. Kilning (Wilting)	24	55
II. Kilning	4	65
III. Kilning	2	75
IV. Kilning	4	85

Sample handling

During the micromalting process, samples were taken before and after all process steps. After sample taking, the samples for bulk density measurements were executed immediately, but the samples for analytical measurements were placed in a convection oven for a specified time and temperature (*e.g.* 3 hours at 105°C). The samples were dried to constant weight. The aims of the drying are to preserve the actual chemical content of samples and prevent spoilage, because rapid drying at 105°C stops biochemical processes (*e.g.* germination, respiration) and reduces the moisture content. After drying, the samples were vacuum packed to reduce moisture absorption and oxidation during storage. Then the samples were stored in room temperature under dry conditions. Before chemical measurements, the samples were grinded with an EBC mill (mesh sieve was set at 1 mm).

Measurement of physical properties

The following chemical physical of samples were measured: weight loss and bulk density.

Measurement of chemical properties

The following chemical properties of samples were measured:

- Moisture content (mass based drying method)
- Ash content (dry ashing method)
- Starch content (Ewers polarimetric method)
- Carbohydrate (monosaccharide and disaccharide) composition with own developed extraction and HPLC method
- Crude protein content (Kjeldahl method)
- Crude fat content (Soxhlet method)
- Fibre content (indirect method)

Statistical analyses

Data of physical and chemical properties were statistically analyzed to compare the samples that derived from the different steps of micromalting process. After One-way ANOVA (α=0.05), the Tukey's HSD post hoc test (α=0.05) was performed in IBM SPSS Statistics 20.0.0.

Computer-aided process simulation

Computer-aided process simulation of the industrial malting was performed in SuperPro Designer 8.0 with educational license.

Box-Behnken Design

Box-Behnken Design was performed in STATISTICA 8.0. The four factors were the following: selling price, einkorn purchase cost, capacity and labour cost. The response was the payback time. Then the observed results were calculated in SuperPro Designer with different set of experimental factors.

Payback time function by least squares method

The 4-D second order polynomial payback time function has 15 unknown coefficients. In this method we used not normalized independent variables (selling price, einkorn purchase cost, capacity and labour cost) instead of normalized ones. SuperPro large-scale malting process simulation provided more than 15 experimental results. As a result of that we received an overdetermined linear equation system of the coefficients of quadratic polynomial. This linear equation system could be solved by Matlab R2009a in the sense of least square method. It means that the error vector is the shortest. Thus the provided quadratic polynomial approximates the SuperPro computed payback time values as the least squares method.

Optimization of polynomial method

The aim of the optimization was to find the response (payback time) and the independent variables (selling price, einkorn purchase cost, capacity, labour cost) of the polynomial model (that was fitted with Box-Behnken Design and the least squares method) at the minimum point. The payback times were calculated in many mesh points of 4D space of four factors. After that the minimum of payback time was determined. The optimization was performed in Matlab R2009a.

RESULTS AND DISCUSSION

The results of the tracking of malting of einkorn are the following:

- The rate of the weight loss is normal compared with barley.
- The bulk density values of einkorn samples are lower than the values of other cereals. At steeping, the bulk density was increased and at germination the bulk density decreased. At wilting and kilning, the bulk densities were further decreased.
- There are statistically significant differences between the means of ash contents. Compared with other cereals, the ash contents of einkorn are higher, this result also confirms that einkorn has high mineral content.

- There are no statistically significant differences between the means of starch contents, but the trend is decreasing.
- In case of carbohydrate (monosaccharide and disaccharide) composition, there are only statistically significant differences in disaccharide contents, the trend is increasing. The reasons of the increasing trend are the degradation of starch.
- There are no statistically significant differences between the means of crude protein contents.
- There are no statistically significant differences between the means of crude fat contents.
- The changing of the fibre content shows a decreasing trend based on dry basis.

In the computer-aided model it was found that the unit procedure with the longest duration is the kernel storage and the germination is the time bottleneck. The optimization method gives realistic parameters at the minimum point.

CONCLUSIONS

The computer-aided model doesn't include the following procedures: pre-treatment of grain, wastewater treatment, waste heat and CO2 recovery. These procedures should be added to the model before industrial application. The model could be optimized for other types of grains as well.

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EFFECT OF ACID WHEY CONCENTRATE ON THERMOPHYSICAL AND RHEOLOGICAL PROPERTIES OF MILK-BASED ICE-CREAM

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SUMMARY

The aim of the present study was to examine the influence of hydrolyzed, nanofiltered concentrate of the ultrafiltered permeate (HNF concentrate) of acid whey on the quality characteristics of milk-based ice cream. Thermophysical properties were determined by differential scanning calorimeter, consistency was measured by oscillatory rheometer, and sensorial quality was evaluated by scoring method. It was concluded that the acid whey did not increase the melting of the product, and reduced the freezing point. Cryoscopic temperature, onset point, and glass transition temperature gradually decreased as the quantity and proportion of acid whey increased in the ice-cream. Rheological results indicated that using HNF, acid whey produced more creamy and smooth ice-cream. However, because of its characteristic taste, maximum 20% of milk could be replaced by HNF acid whey in milk-based ice-creams.

INTRODUCTION

Quality of ice-cream can be determined by the thermophysical, rheological, and sensorial properties. Thermophysical properties (i.e. heat flow curves, endothermic and exothermic peaks, onset point, transition temperature, etc.) can be measured by DSC (Differential Scanning Calorimetry) method (Figura & Teixera, 2007). Using this technique, ice crystallisation, glass transition temperature, oxidation, and other chemical reactions are often examined (Dissanayake & Vasiljevic, 2009) and quantity of bound, freezable, and unfreezable water can also be determined (Alvarez et al., 2005). Unfreezable water content influences the instability of the system and alludes to the required storage temperature or temperature range of the ice-cream (Heys et al., 2008). Whey protein concentrate has significant effect on rheological properties of ice-cream as it changes the disperse microstructure of the product (Aktas et al., 1997). The rheological properties (elasticity, viscosity, hardness, etc.) of the ice-cream can be suitably analysed by oscillation rheometry like amplitude sweep test (Adapa et al., 2000). Temperature sweep (or dynamic thermoanalysis test) is a widespread method to examine changes in rheological properties of ice-creams at storage or consumption temperature and during melting (Mezger, 2006). The aim of the present study was to investigate the effect of HNF acid whey on thermophysical properties, rheological behaviour, and sensorial quality of milk-based ice-creams.

MATERIAL AND METHODS

Materials

Hydrolysed and nanofiltered concentrate of acid whey permeate (HNF AWh) was developed and provided by the Hungarian Dairy Research Institute. HNF AWh had 25.2 g/100 g dry material content, 0.9 g/100 g protein, 21.50 g/100 g carbohydrate, 11.5 g/100 g lactose, 2.65 g/100 g minerals, 1.53 g/100 g lactic acid, and pH of 4.45. In addition, commercially available milk (2.8 g/100 g fat content), cream (30 g/100 g fat content), sugar, ice-cream emulsifier (MEC3, Italy), and flavouring agent (vanilla paste, MEC3, Italy) were used for sample preparation.

Sample preparation

Control sample (H0) was produced without HNF AWh as follows: 66 g milk, 14 g cream, 15 g sugar, 1 g vanilla paste, and 4 g emulsifier were mixed and whipped by an electric whisk for 5 min and then frozen by an ice cream machine (CRM GEL5, Andretti Italy Group Kft, Budapest, Hungary) for 15 min and hardened in a shock freezer (Nortech QCF 103, Normann S.R.L., Pordenone, Italy) at –35 °C. Six different milk-based ice-creams were produced using 0, 20, 40, 60, 80, and 100% of hydrolysed and nanofiltered concentrate of acid whey permeate (HNF AWh) to replace milk. Samples are indicated as H0, H20, H40, H60, H80, H100, according to their HNF AWh content.

Sensory evaluation of ice-cream samples was performed immediately after production. Melted ice cream samples were used for DSC measurements. For rheological measurements ice-cream samples were frozen using a circle shaped mould 50 mm in diameter and 2 mm in height in order to minimize deformation prior to measurement.

DSC measurements

Thermophysical properties of samples were determined by a DSC 131 evo (Setaram Instrumentation, Caluire, France) differential scanning calorimeter and an immersion cooler (Flexi-Cool FC100, FTS, USA). Synthetic air (Messer Hungarogáz Kft., Budapest, Hungary) was used as the purge gas at a flow rate of 50 cm3 min–1. Samples of 50–70 mg were weighed and an empty aluminium DSC pan was used as reference. Temperature program was as follows: cooling from +30 °C to –50 °C by a cooling rate of 5 °C min–1, then keeping at –50 °C for 15 min, and finally heating from –50 °C to +30 °C by a heating rate of 2 °C min–1. Data collection and analyses was controlled by Callisto Processing 1.076 software (AKTS AG, Siders, Switzerland). Glass transition (Tg, °C), onset melting temperature (To, °C), latent heat of melting (peak area, ΔH, J g–1) and freezable water content were determined as Vajda el al (2013)

Rheological measurements

Oscillation rheological tests were performed using a Physica MCR 51 rheometer (Anton-Paar Hungary Ltd., Veszprém, Hungary) with a plate and plate measurement system consisting of P-PTD200 plate and PP50/S (plate 50 mm in diameter) measuring bob using 2 mm gap size. Amplitudo sweep test was performed increasing strain (0.005%-40%), at constant temperature (10°C), and at constant angular frequency (10 rad s–1).Temperature sweep method was performed in increasing temperature range (–15 °C - 0 °C) by a heating rate of 1 °C min–1 at constant strain (0.005%) and at constant angular frequency (10 rad s–1). All samples were measured five times in the same way. Results were recorded and analysed using Rheoplus software ver 3.2. (Anton-Paar Hungary Ltd., Veszprém, Hungary).

Sensory analysis

The organoleptic properties were evaluated by a sensory panel consisting of 15 trained panelists using a scoring system. A maximum of twenty points could be given for the texture, while ten points for the colour, taste, texture, total impression, creaminess, homogeneity, scoopability, and susceptibility to melting.

Statistics

Effect of HNF AWh was evaluated by comparing certain properties of each sample to the control sample by Student t-test at 95% confidence level using Excel (MS Office 2010, Microsoft Magyarország Kft., Budapest, Hungary) software.

RESULTS AND DISCUSSION

DSC curves of HNF AWh and ice-cream samples (Fig. 1) showed similar tendencies. The first section of the curves from –40 °C to –35 °C provides information on Tg (glass transition) properties of the samples (Schenz, 1995). Beyond that an endothermic peak could be observed between –5 and 0 °C, which was reported to be the melting region of ice content (Alvarez et al., 2005). Glass transition (Tg, °C), onset melting temperature (To, °C), were decreasing tendency as the whey concentrate increased. The latent heat of melting (ΔH, J g–1) and freezable water content showed increasing tendency.

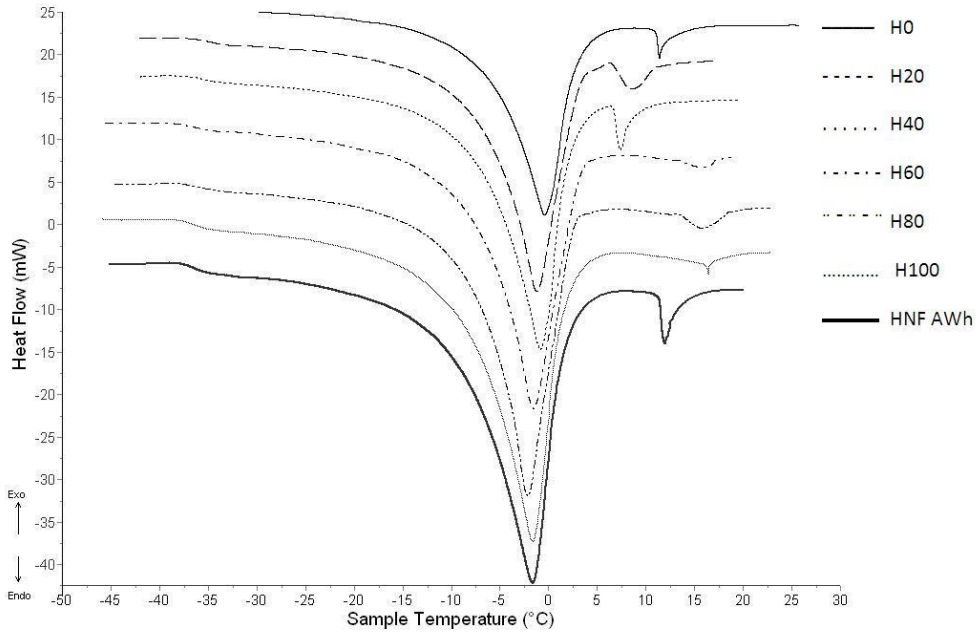

Figure 1. Differential scanning calorimetry melting curves for ice-cream samples prepared using HNF AWh.

Results of amplitude sweep and temperature sweep showed in Table 1.

Table 1. Rheological properties of ice-creams prepared with HNF AWh

	G0' (Pa)	G0" (Pa)	τ_{LVE} (Pa)	τ_{co} (Pa)	CV at -10°C (kPa.s)
H0	$1{,}31\cdot10^{6}\pm4{,}85\cdot10^{5}$[a]	$4{,}61\cdot10^{5}\pm5{,}14\cdot10^{4}$[a]	31,5±3,7[a]	1445±212[a]	188.6±21.1[a]
H20	$1{,}17\cdot10^{6}\pm2{,}78\cdot10^{5}$[a]	$4{,}57\cdot10^{5}\pm9{,}08\cdot10^{4}$[a]	24,9±8,2[a]	1097±231[a]	189.6±13.3[a]
H40	$8{,}77\cdot10^{5}\pm2{,}11\cdot10^{5}$[a]	$3{,}34\cdot10^{5}\pm8{,}52\cdot10^{4}$[a]	22,7±4,4[a]	673±159[b]	165.2±27.3[b]
H60	$7{,}47\cdot10^{5}\pm4{,}55\cdot10^{4}$[a]	$4{,}06\cdot10^{5}\pm3{,}52\cdot10^{4}$[a]	22,5±2,6[a]	560±71[b]	120.6±25.5[b]
H80	$8{,}02\cdot10^{5}\pm8{,}64\cdot10^{4}$[a]	$4{,}06\cdot10^{5}\pm8{,}23\cdot10^{4}$[a]	19,4±9,3[a]	466±66[b]	99.7±18.1[b]
H100	$7{,}48\cdot10^{5}\pm1{,}44\cdot10^{5}$[a]	$3{,}67\cdot10^{5}\pm8{,}06\cdot10^{4}$[a]	15,5±2,1[a]	309±65[b]	102.3±6.6[b]

x: Mean; SD: standard deviation; [a] there is no significant difference compared to the sample (0); [b] there is a significant difference compared to the sample (0);G0':initial storage modulus, G0" initial loss modulus, t_{LVE}: the end of the linear viscoelasticity range, t_{co}: crossover point, CV: complex viscosity at -10°C

Rheograms of samples made with varying quantities of acid whey showed similar shape, but G'0 and G"0 values were different. The ice-cream samples were in solid phase, it means

initial storage modulus was higher than initial loss modulus. As the concentrate of the whey increased the initial values of the storage and loss modulus, the end of the linear viscosity range, the crossover pint values and the complex viscosity values decreased. As AWh content exceeded 40% crossover points and complex viscosities of ice-creams measured at –10 °C (consumption temperature) were significantly lower than those of control, indicating that ice-cream samples at –10 °C became softer and easier to consume. Based on this results, the use of AWh produced more smoothly melting and creamier milk-based ice-creams.

Results of sensory analysis scoring test and organoleptic texture profile analysis were no significant differences between the colour and consistency of the samples made with various AWh contents. However, taste and overall impression scores gradually decreased when proportion of AWh exceeded 20% in the samples. Using more acid whey in the ice-cream, characteristic aftertaste was perceptible, thus sensory properties degraded. The overall impression was mainly influenced by the taste. The quality of the ice-cream depends on characteristics, such as creaminess, melting, homogeneity, and scoopability (Prindiville et al., 2000; Dooley et al., 2010). However, scores given during sensory test showed that there were no significant differences between the ice-cream samples; scores of creaminess, homogeneity, and scoopability decreased gradually with the increasing quantity of AWh.

CONCLUSIONS

Based on our results of thermophysical and rheological measurements, hydrolysed, nanofiltered concentrate of the ultrafiltered permeate (HNF concentrate) of acid whey could be added to milk based ice-cream to replace 40% of milk. However, sensorial analysis showed that because of its characteristic taste, maximum 20% of milk should be replaced by HNF acid whey in milk-based ice-creams.

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INFLUENCE OF DIFFERENT STORAGE TEMPERATURES ON THE PHYSIOLOGICAL RESPONSES OF JONAGORED APPLE CULTIVAR

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SUMMARY

Freshly harvested (Malus x domestica cv. Jonagored) apples were used for the determination of the effect of storage temperatures (2.5; 5; 10 and 20°C) and subsequent shelf-life on postharvest respiration, ethylene production, mass loss, textural properties and quality. Mass loss and textural changes (acoustic stiffness, impact firmness) showed clearly the positive effect of low temperature on quality. The higher the temperature, the higher the C_2H_4 and CO_2 production measured at 2.5; 5 and 10°C together with a moderate change through the cold storage period. At 20°C, continuous and significant decrease was found during the whole period. Significant and fast decrease of C_2H_4 production followed the steep rise after removal from cold storage during shelf-life of the cold stored apples independently on storage temperature. At 2.5 and 5°C stored apples showed a moderate respiration increase after removal from cold temperature in contrast to the slight decrease of the samples stored at 10 and 20°C.

INTRODUCTION

Apple is produced and consumed in high quantities and it is available throughout the year. As a seasonal and perishable product, it requires appropriate postharvest storage conditions to preserve its quality. Several factors (e.g. temperature, relative humidity, gas composition, ripening stage) influencing the effectiveness and possible duration of the postharvest storage of apples too, but one of the most important is temperature, heavily influencing metabolic activity of the fruit. These reactions ordinarily increase with increasing temperature up to 40ºC. Enzymatic reactions occur more slowly at low temperatures, extending fruit shelf life (Bron et al., 2005). In case of horticultural products, temperature dependent physiological processes affecting quality (respiration, textural changes, etc.) are investigated continuously (e.g. Abbott, 1999; Kidd & West, 1927 and 1945; Johnston et al., 2001 and Paull, 1999) and nowadays by novel, mainly non-destructive methods (e.g. De Ketelaere et al, 2006; East at al., 2008; Felföldi & Fekete, 2003; Hertog et al., 2004; Shmulevich et al., 2003; Weber et al., 2015). This experiment was aimed to demonstrate the impact of different storage temperatures to physiological responses as carbon dioxide and ethylene production and as well as the change of firmness and weight loss of apples.

MATERIALS AND METHODS

Freshly harvested apples (*Malus x domestica cv. Jonagored*) were sorted for uniformity (size, weight), appearance and absence of physical defects into four groups (marked from A to D according to the applied storage temperature). 18 apples per group were stored at 2.5 (A); 5 (B); 10 (C) and 20 °C (D) in temperature controlled refrigerators and each group was wrapped in LDPE foil.

Traditional Magness-Taylor penetration test (d=11mm, cylindrical probe) was carried out (data not shown) in order to evaluate fruit flesh firmness (N/cm^2). Total soluble solid content (Brix%) was also measured by an ATAGO PAL-1 digital refractometer (Atago Co. Ltd., Tokyo, Japan) at the beginning and at the end of the storage period (data not shown).

Physiological responses (CO_2 and C_2H_4 production, textural and mass loss changes) to different storage temperatures were evaluated non-destructively on a weekly basis.

Acoustic stiffness (S, $Hz^2 \cdot g^{2/3}$) and impact firmness (D, ms^{-2}, data not shown) of the apple samples were determined non-destructively at two opposite sides on each fruit, using an AWETA table top acoustic firmness sensor model DTF V0.0.0.105 (AWETA, Nootdorp, The Netherlands).

Respiration at the different storage temperatures (from 2.5 to 20°C) as carbon dioxide production was measured for about an hour in every 30 seconds in a closed respiratory system consisting several hermetically closed plexi glass containers (3 apples/container) equipped with FY A600-CO2H IR carbon dioxide sensors connected to an Almemo 3290-8 data logger (Ahlborn Mess-und Regelungstechnik GmbH, Germany). Results were expressed as milliliter of CO_2 produced per kilogram of fruit in 1 h ($mL \cdot kg^{-1} \cdot h^{-1}$).

The ethylene production of the apple samples was determined by an ICA-56 hand-held ethylene analyzer (International Controlled Atmosphere Ltd., UK) upon the measured ethylene production of the samples being held for about an hour in the above mentioned containers of the respiratory system. Results were expressed as microliter of ethylene produced per kilogram of fruit in 1 h ($\mu L \cdot kg^{-1} \cdot h^{-1}$).

Mass loss (%) of the samples stored at different temperatures was also determined as a percentage of initial weight.

Data were converted by means of routines in MS-Excel. SPSS for Windows 14.0 was used to perform statistical analysis at 95 % significance level and for the comparison of the dependent variables.

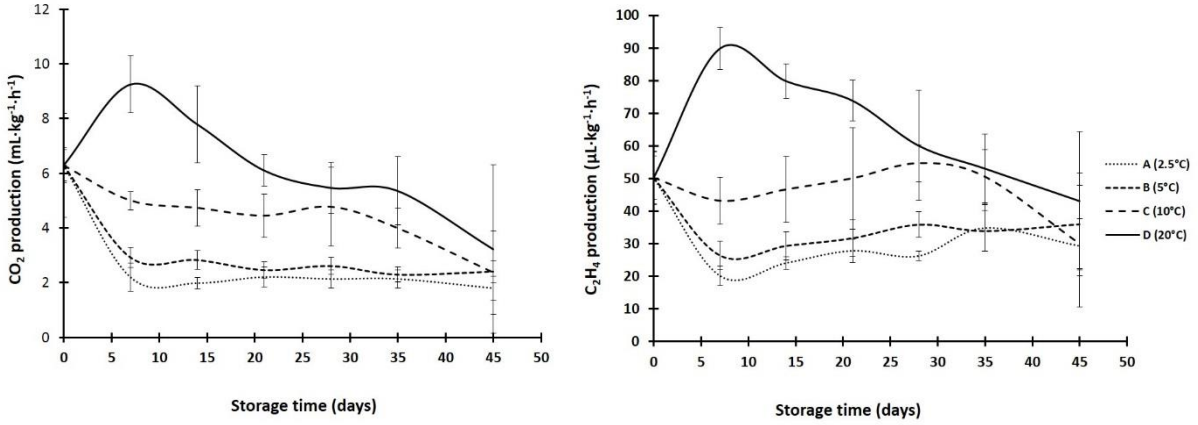
RESULTS AND DISCUSSION

The apples stored at 2.5°C (group A) remained in the best condition with low carbon dioxide and ethylene production (Fig.1A and B), maintaining firmness and weight (Fig.2A and B), showing significantly slower ripening with no fungus infections. Group B (5°C) showed similar results as group A, and both groups behaved well during the one week long shelf-life storage after the low temperature storage time due to positive effect of reduced temperature on overall quality. Cold storage of the samples at 10°C (C) resulted slightly higher quality losses compared to the samples stored at 2.5°C and 5°C. Samples of group D, stored at 20°C (simulating normal shelf-life conditions) ripened quickly compared to the other cold stored groups and started to deteriorate rapidly by the end of the experiment due to infections of fungus and rot. As shown in Fig.1 and 2, significant difference can be seen between groups D, C and A-B, indicating that higher temperatures have a measurable and significant negative impact on the quality and physiological responses of the stored apples.

a. Respiration and ethylene production

Fig.1A and B shows the change of respiration (produced CO_2) and ethylene production over storage time. Initial respiratory values and C_2H_4 production were determined for normal ambient temperature (20°C) for the freshly harvested apples. As expected, the higher the temperature the faster and more intensive the respiration (Fig.1A) and the ethylene production. Samples of group D (20°C) showed a sharp and intensive decline compared to group C (10°C) due to the higher temperature intensified internal metabolic processes, but both group A and B (2.5 and 5°C) remained on a relatively even and significantly lower level. The amount of produced carbon dioxide and ethylene reflects the speed of the metabolism, meaning higher values lead to faster ripening and decay. Taking into account the C_2H_4 production, Fig.1B shows a similar pattern as respiration versus storage time, but characterized by the less even nature of group A (2.5°C) and B (5°C). In case of ethylene (natural plant hormone conducing the ripening processes) similarly to carbon dioxide, the

higher produced values significantly shorten possible storage time. The production of both natural substances can be slowed or reduced by reduced storage temperatures as shown in Fig.1. Significant and fast decrease of C_2H_4 production (data not shown) followed the steep rise after removal from cold storage during shelf-life of the cold stored apples independently on storage temperature. At 2.5 and 5°C stored apples showed a moderate respiration increase after removal from cold temperature in contrast to the slight decrease of the samples stored at 10 and 20°C.

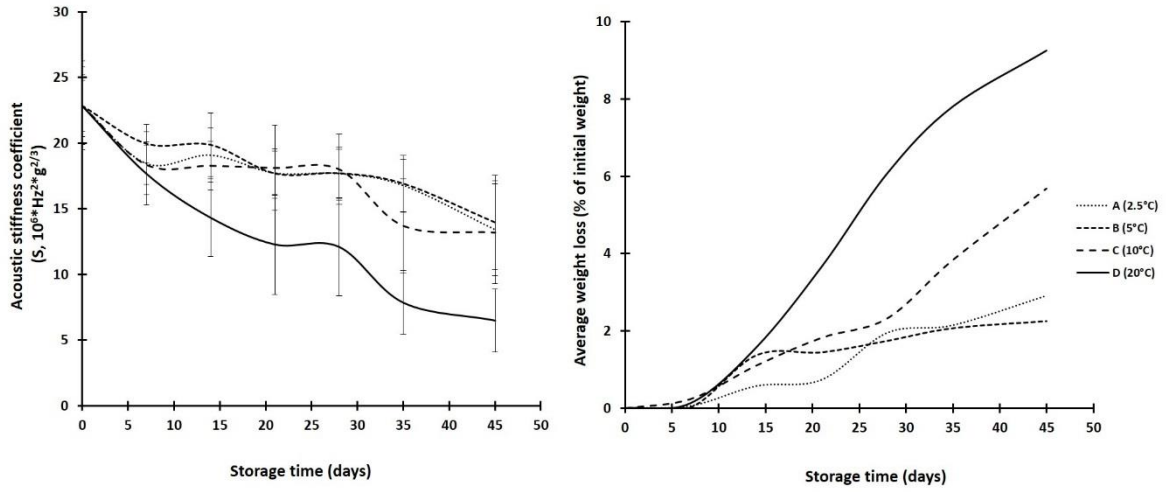


A B

Figure 1.: Respiratory activity (CO_2 production, left [A]) and ethylene production (right [B]) of Jonagored apple samples stored at different temperatures vs. time

b. Textural and mass loss changes

The data shown in Fig.2A represent clearly the highly positive and significant influence of reduced storage temperature on fruit quality and texture. The acoustic stiffness coefficient's (S) change is significantly different in case of cold stored samples (A, B and C) from at 20°C (D) stored samples representing a temperature and a water pressure deficit related relative humidity dependence. An overall textural degradation is shown over time independently from the different cold storage temperatures (from 2.5 to 10°C).



A B

Figure 2.: Acoustic stiffness coefficient change (left [A]) and average weight loss (% of initial weight) change (right [B]) of Jonagored apple samples stored at different temperatures vs. time

Acoustic stiffness coefficient is reflecting overall firmness of the fruit, showing the condition of the tissue and the effects of metabolism. The relatively short length of the experiment and the protective LDPE packaging of the groups led to only minor weight losses in all groups except group D (20°C) reaching nearly 9% by the end of the storage period (Fig.2B). Fig.2B also shows that group A (2.5°C) and B (5°C) remained very close to each other during most of the time, meaning no significant difference found between the two lowest temperatures.

CONCLUSIONS

Based on the gained experimental results, we conclude that significant difference was found between samples stored at 2.5°C and 20°C taking into consideration all the measured quality parameters as the physiological responses of CO_2, C_2H_4 production, textural changes and average mass loss of the stored apples. Additionally, little to no differences were found between the samples stored at 2.5°C and 5°C due to the low temperature difference between these two applied storage temperatures and to the thus reduced intensity of physiological responses. Storing apple samples at ambient temperature of around 20°C results in reduced quality (fruit firmness, ripening and increased mass loss, etc.). According to our results, it is clear that the applied reduced storage temperatures significantly and positively influenced quality and reduced the intensity of physiological responses as carbon dioxide and ethylene production and as well as overall textural and mass loss changes.

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OBJECTIVE CHILLING STRESS MONITORING OF SWEET PEPPER USING NONDESTRUCTIVE METHODS

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SUMMARY

Chilling injury represents a serious postharvest problem of horticultural products with tropical/subtropical origin (e.g. sweet pepper, banana). Fresh, LDPE packed green sweet peppers were stored at chilling (5°C), cold (10°C) and near room temperature (18°C) with subsequent shelf-life. Chilling stress' effects were monitored among others by chlorophyll fluorescence image analysis, surface colour measurement and nondestructive texture analysis. Increasing mass loss, decreasing acoustic stiffness indicated clearly the susceptibility to mass, texture and quality changes independent on low storage temperature. CIELab a and H° values showed a highly significant change during cold and shelf-life storage (steep rise and fall, respectively), reflecting the effect of optimal and room storage temperature. Chlorophyll fluorescence parameters F_0, F_m, F_v and F_v/F_m showed clearly from the 5th day of storage the negative effect of low temperature on photosynthetic activity and integrity of PSII, suggesting the effect of chilling injury without any visible symptoms even stored at 5°C.*

INTRODUCTION

Horticultural products, especially with tropical/subtropical origin, are sensitive to cold, but non-freezing temperature induced stress, called chilling injury. The quality, shelf-life and marketability negatively affecting symptoms mainly become visible and really severe after removal from chilled to typical retail conditions (Wang, 1994). Novel non-destructive optical methods such as chlorophyll fluorescence analysis, digital image analysis, UV/VIS, NIR or fluorescence spectroscopy and the texture measurement techniques offer the possibility to non-destructively characterise fruit responses (e.g. physiological status, stage of maturation) to different external stressors (e.g. chilling injury) to quantify or predict produce quality and their postharvest changes (Bron et al., 2004; Felföldi & Fekete, 2003; Herppich et al., 2012; De Ketelaere et al., 2006; Kosson, 2003; Nedbal et al., 2000; Zude, 2003; Zsom et al., 2014). This study was aimed to investigate the possible application of advanced non-invasive measuring methods for rapid chilling stress monitoring of sweet pepper.

MATERIALS AND METHODS

36 pieces of freshly harvested mature green sweet peppers (*Capsicum x annuum L.*) produced by Gartenbau K. Gentz (Werder, Germany) were divided into three groups according to uniform size, mass and maturity for the temperature treatments (A: 5 ± 0.5 °C, i.e. below chilling threshold temperature of 7 °C; B: 10 ± 0.5 °C; C: 18 ± 0.5 °C, i.e. conventional storage). Samples of the different treatments were packed in LDPE bags and stored in climate chambers at 95 ± 1 % RH. Cold stored samples were removed from cold conditions after one week to and stored at 18 ± 0.5 °C to simulated shelf-life for further 5 days.

FluorCam 700MF (PSI, Brno, Czech Republic) chlorophyll-fluorescence imaging system was used for the characterization of the changes in photosynthetic activity, integrity and efficiency of PSII under chilling injury conditions by the determination of fluorescence parameters (e.g. F_0, F_m F_v F_v/F_m) on two directly opposite sides of the pepper pod.

The portable fluorometer FluorPen FP 100-MAX (PSI, Brno, Czech Republic) enabled the rapid measurement of parameters of the chlorophyll fluorescence induction kinetics (Strasser et al., 2000). Measurements were carried out at two points per side (close to the stem at the shoulder and at the middle of the pod).

A portable handheld spectrometer, the Pigment Analyzer (CP, Germany) for UV/VIS-spectroscopy was used to characterize changes in contents and compositions of fruit pigments in response to low temperature stress (1st and 2nd derivatives of the remittance data, NDVI and NAI indexes).

Surface colour changes as changes in CIELab colour characteristics (L*, a*, b*, C* and H°) were determined by the use of Minolta CM-2600d spectrophotometer (Minolta Europe GmbH, Germany) with Ø 8 mm aperture. For data collection, the SpectraMagic^{TM} NX software was used. Sampling was carried out at the same points as of the FluorPen and Pigment Analyzer devices.

Non-destructive texture determination was carried out using the acoustic impulse response technique. According to Felföldi & Fekete (2003), pepper samples were tapped lightly on the top with a wooden stick. The acoustic response was collected by a microphone, recorded and finally analysed by Custom Fast Fourier Transform software. The acoustic firmness coefficient ($S = f^2*m*10^{-6}$ [Hz^2*g*10^{-6}]) was calculated. Precise mass loss (% of initial fresh weight) was determined with a computer connected Sartorius CPA2245-OCE digital balance (Sartorius, Germany) with 0.01 g accuracy.

Data were converted by means of routines in MS-Excel. SPSS for Windows 14.0 was used to perform statistical analysis at 95 % significance level.

RESULTS AND DISCUSSION

Despite of the relatively large standard deviations of raw data, mass loss changes (Fig.1A) clearly indicated the sweet peppers' susceptibility to fast mass and quality changes. For samples stored at 18 °C, significant difference occurred between cold stored and shelf-life samples, but only after the first week. In coincidence with the results of different sweet pepper varieties published by Zsom et al. (2014), the acoustic stiffness coefficient results (Fig.1B) clearly reflected the mass loss related textural changes. The acoustic stiffness measurement is suitable to objectively measure changes in quality by recording the decrease in fruit stiffness. Stiffness was not significantly different between samples stored at the chilling temperature of 5 C and those kept at the recommended cold storage temperature of 10°C. The decrease in stiffness of samples stored at and above 10°C was a bit less severe compared to the results of 5 C indicating a negative effect of low temperature storage on texture and quality.

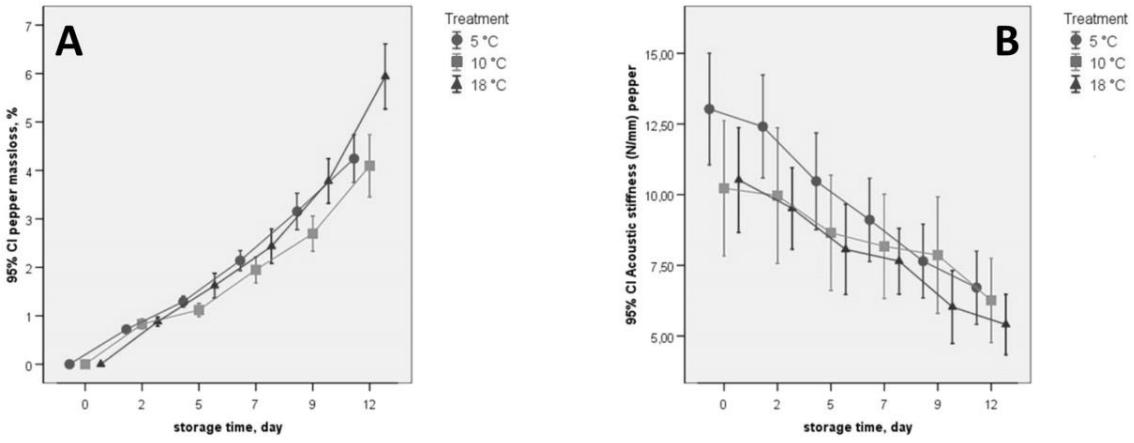

Figure 1.: The calculated mass loss (%, left [A]) and the acoustic stiffness coefficient (right [B]) change of sweet pepper samples vs. time

Only minor and insignificant changes in L*-values were measured at the different temperatures for one week (data not shown). Differences in L*-values were significant between the samples stored at 18°C (representing the inhomogeneous post-maturation) and those kept at 5°C, but not between cold stored samples (i.e. 5 and 10°C). Concomitant with the significant and steep increase in a* (data not shown), the light green base surface colour turned to yellowish-orange, especially after removal from cold storage. Hue values showed a highly significant change during the cold storage and the subsequent shelf-life, reflecting the effect of optimal and room storage temperature (Fig.2A). The NAI index significantly increased (Fig.2B) independently of storage temperature until the 7th day. In shelf-life, the temperature dependent rise (10 and 18 C) of NAI referred to the inhomogeneous postharvest ripening together with the significant difference between the effects of chilling temperature (5°C) and higher storage temperatures (10 and 18 °C).

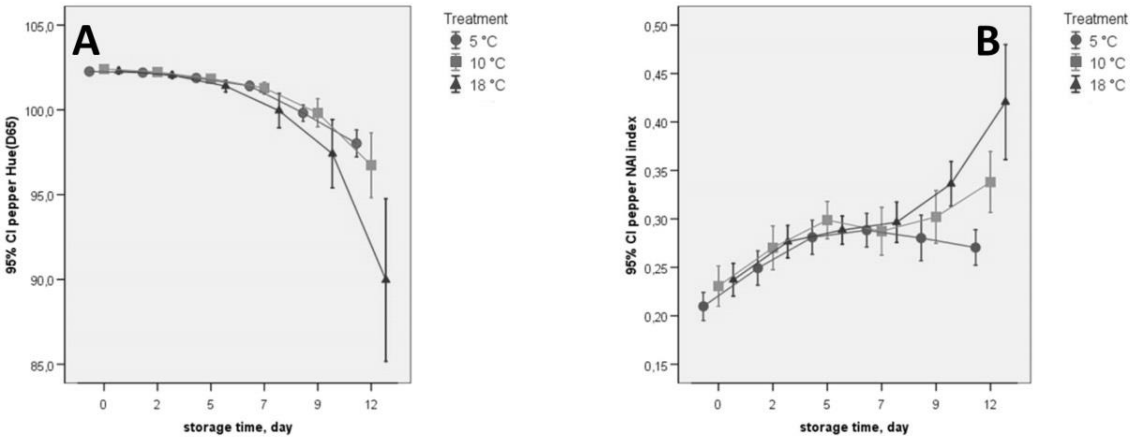

Figure 2.: CIE Hue° (left [A]) characteristic and NAI index (right [B]) of the sweet pepper samples vs. time

Independently of storage temperature, F_0 and F_m chlorophyll fluorescence values of pepper pods significantly increased, either measured by the imaging FluorCam system or the handheld FluorPen device (data not shown). This increase was followed by a likewise significant decrease in shelf-life within only two days (from day 7 to 9) (Fig.3). This clearly reflected the decrease of photosynthetic activity, suggesting effects of chilling injury together with clear significant change of F_v/F_m versus storage time, but without any visible symptoms of chilling injury (Fig.3).

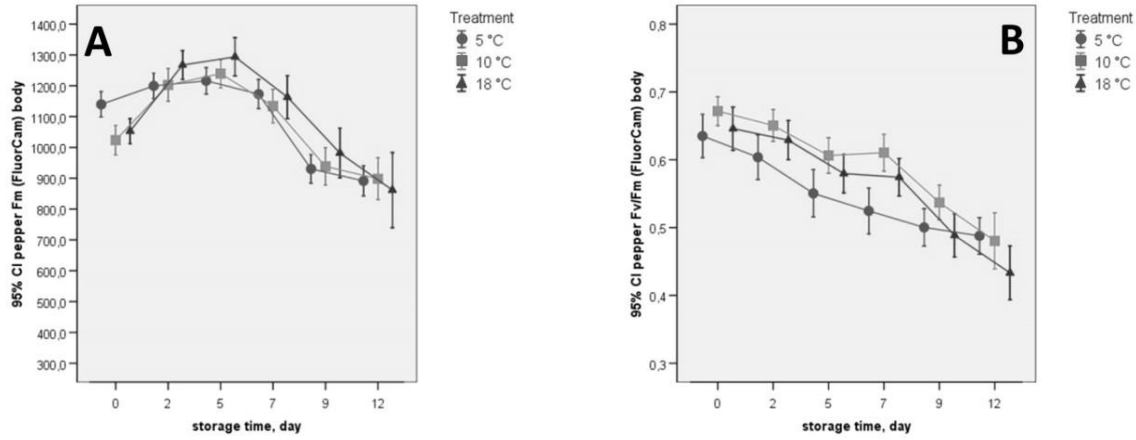

Figure 3.: The change of F_m chlorophyll fluorescence characteristic (left [A])) and F_v/F_m ratio (right [B]) of sweet pepper samples vs. time measured by the FluorCam imaging system

Significant difference was found in F_v/F_m referring to the integrity and photosynthetic activity of PSII on the 7th day between the samples stored at 5 °C and above. During the following days, this difference vanished in response to transfer to shelf-life conditions. F_0, F_v and F_m values obtained with the FluorPen device (data not shown), similarly increased as it was shown in case of FluorCam values until the 5th day. It was followed by a significant decrease, especially during shelf-life, but the measured FluorPen values of F_0, F_v and F_m are characterized with a different intensity (or magnitude) due to basic the differences of the measuring set-ups.

CONCLUSIONS AND SUGGESTIONS

All the applied non-destructive measuring methods were found to be suitable for the monitoring of the pepper postharvest quality changes during cold and subsequent shelf-life storage. Additionally, none of the well-known and characteristic symptoms of chilling injury (Lim et al., 2007) were observed during the 12 days of storage independently of temperature either during the 7 days long cold storage or during subsequent shelf-life.

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