KAUNAS UNIVERSITY OF TECHNOLOGY

VIKTORIJA EISINAITĖ

INNOVATIVE PIGMENT ENCAPSULATION AND STABILIZATION TOOLS FOR MEAT PRODUCTS

Summary of Doctoral Dissertation
Technological Sciences, Chemical Engineering (05T)

2017, Kaunas

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Scientific Supervisor:
Assoc. Prof. Dr. Ina JASUTIENĖ (Kaunas University of Technology, Technological Sciences, Chemical Engineering – 05T).

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Address: K. Donelaičio St. 73-402, 44249 Kaunas, Lithuania.
Tel. no. (+370) 37 300 042; fax. (+370) 37 324 144; e-mail doktorantura@ktu.lt.

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KAUNO TECHNOLOGIJOS UNIVERSITETAS

VIKTORIJA EISINAITĖ

INOVATYVIOS PIGMENTŲ ĮKAPSULIAVIMO IR STABILIZAVIMO PRIEMONĖS MĖSOS GAMINIAMS

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Mokslinė vadovė:

Doc. dr. Ina JASUTIENĖ (Kauno technologijos universitetas, technologijos mokslai, chemijos inžinerija, 05T).

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Adresas: K. Donelaičio g. 73-402, 44249 Kaunas, Lietuva.
Tel. + 370 37 300 042; faks. + 370 37 324 144; el. paštas doktorantura@ktu.lt.

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1. INTRODUCTION

Over the past decade, the world observed a growing trend in meat consumption. Nevertheless, consumers expressed great concerns regarding meat products consumption. It is believed that a healthy image of meat is tarnished by its negative association with cardiovascular diseases and cancer. In this way, a wide range of meat products related to negative associations could be avoided by reducing unhealthy ingredients, such as synthetic food additives, or by replacing them with natural alternatives which have the same technological and functional properties. That is why clean labelled meat products with natural vegetable-based ingredients are growing in popularity.

Vegetables are excellent sources of dietary fiber, nitrates, pigments, or bioactive compounds, such as antioxidants, vitamins, phenolics, and glucosinolates. Vegetables or their extracts may be used as antioxidants or antimicrobial agents and are safer compared to their synthetic analogues. However, compounds isolated from vegetables could have lower stability as well as off-odor, taste, or color that could negatively affect the final product.

Synthetic red colorants in meat products could be replaced with a natural pigment betanin, extracted from beet root (*Beta vulgaris*). However, industrial application of this pigment is limited because of its sensitivity to various environmental and technological factors. In order to increase stability of this pigment, an encapsulation technology was offered. Double emulsions that could be used as a fat reduction and modification strategy could be used as an encapsulation technique, too. However, they are also notoriously difficult to prepare and their stability as well as encapsulation efficiency is an intrinsic challenge.

As an alternative to synthetic nitrite that is used as a preservative and color stabilizer in meat, vegetables that naturally accumulate a high amount of nitrates (for example, celery, parsnip, parsley, and leek) could be used. It was reported that celery extracts and concentrates had been previously used in hot smoked hams and sausages. It is important to note that such vegetables must be used together with microorganisms that have nitrate-reducing properties in order to reduce nitrate to nitrite.

The aim of the thesis was to create pigment encapsulation and stabilization tools, to characterize them, and to investigate the possibility of their application in meat products.

The following tasks were set in order to achieve the aim of the thesis:

1. To create a stable double water-in-oil-in-water emulsion by modeling the water/oil interface composition;

2. To investigate the effect of osmotic pressure, the water/oil and oil/water interfaces composition, and the type of emulsification system on the stabilization mechanism of double emulsions with encapsulated beetroot juice;
3. To use the double water-in-oil-in-water emulsion as a multifunctional tool in order to improve the composition of fat and red color in meat systems;
4. To investigate the influence of nitrates from freeze-dried vegetables on changes in meat systems during the fermentation process and to evaluate their influence on meat safety;
5. To investigate the influence of nitrates from freeze-dried vegetables on the formation and changes of different myoglobin forms in meat systems during fermentation in order to keep the red color of meat;
6. To investigate the possibility of changing synthetic nitrate and nitrite to nitrate from freeze-dried vegetables in cold smoked sausages in order improve their quality and safety.

Scientific novelty of the research. Physically stable double emulsions with encapsulated beetroot juice having high encapsulation efficiency (>98%) were produced while using two stages homogenization and a hybrid premix membrane emulsification system. It was obtained that the stability of double emulsions was influenced by the increase of viscosity as a result of the osmotic pressure differences between inner and outer water phases. The pigment betanin was also safely encapsulated in the water-in-oil-in-water emulsion and protected during the thermal treatment, thus, double emulsions were used not only to improve the composition of fat but also to form and stabilize the red color of meat.

The effect of nitrates from freeze-dried celery, leek, and parsnip on the formation and changes of different myoglobin forms in meat systems during fermentation was also investigated.

Practical significance of the research. Two innovative pigment encapsulation and stabilization tools that can replace food colorants and color stabilizers in meat products were created. The first one: double emulsions with encapsulated beetroot juice in the inner water phase were used not only to replace animal fat but also as a way to enhance the color of meat systems. Double emulsions that were developed had high encapsulation efficiency and were highly resistant to environmental and technological factors.

The second tool: freeze-dried celery, leek, and parsnip as an indirect nitrate source in meat systems. In addition to the red meat color formation, the inclusion of freeze-dried vegetables improved technological properties. A prototypical technology of cold smoked sausages with the addition of freeze-dried celery was

prepared. It allows replacing the added nitrite while maintaining the same technological properties, quality, and safety.

Defended claims of the dissertation

1. It was obtained that the stability of double emulsions was influenced by the increase of viscosity as a result of the osmotic pressure differences between inner and outer water phases.
2. The double emulsion with encapsulated beetroot juice could be used in meat products in order to replace animal fat and keep the red color of meat.
3. Nitrates from freeze-dried celery could be used instead of the added nitrate or nitrite, maintaining the same functional and technological properties of cold smoked sausages.

Structure of the dissertation. The dissertation is written in Lithuanian. It consists of the following parts: introduction, review of literature and its summary, research objects and methods, results and discussion, conclusions, a list of references which consists of 209 items, and a list of publications on the dissertation topic. The entire dissertation consists of 119 pages including 22 tables and 56 figures.

Publication of the research results. The research results have been published in 4 scientific articles in the journals indexed by Thomson Reuters (WOS), in 3 reviewed periodical scientific journals included into the list of other databases and were presented in 5 international conferences.

2. OBJECTS AND METHODS

2.1 The research scheme

The overall structure of the research is represented in Figure 1.

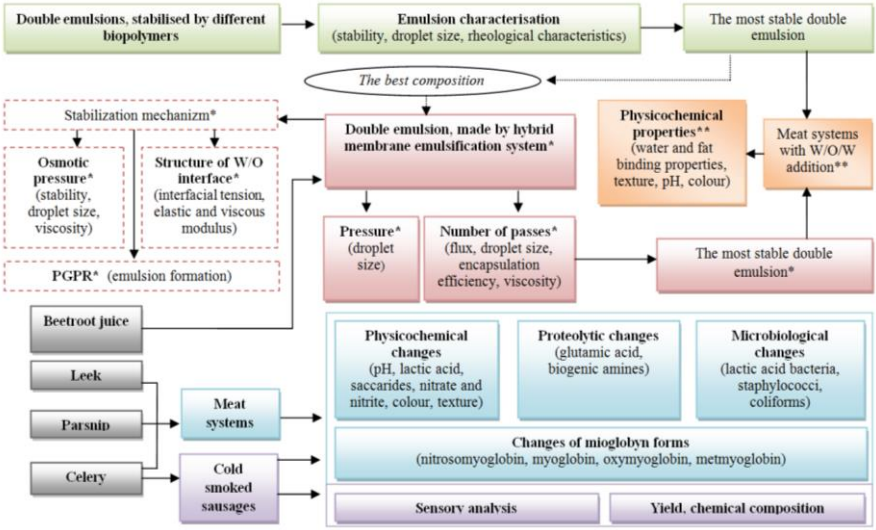

Figure 1. The research scheme. * - experiments performed at the Wageningen University, Food Process Engineering group (the Netherlands); other experiments performed at Kaunas University of Technology, Department of Food Science and Technology; ** - experiments performed in both Universities.

2.2. Research objects and methods

All methods applied in the research and the principle of each method are briefly introduced in Table 1.

Table 1. Research objects, analyzed properties, and characterization of methods

Objects of the research	Property analyzed	Method and it characterisation/principle
Spontaneously formed W/O emulsion	Droplets formation	Optical microscopy. Detection of PGPR induced spontaneous W/O emulsification (water droplets in the continuous oil phase).
W/O interface	Interfacial tension	Pendant drop tensiometry. Surface tension of a water droplet surrounded by a continuous oil phase was determined by fitting the shape of the drop in a captured image to the Young-Laplace equation which relates interfacial tension to the drop shape.
	Dilatational elastic (E') and viscous (E'') modulus	Pendant drop tensiometry. Dilatational frequency sweeps (0.002–0.1 Hz) were performed at the water/oil interface with a constant amplitude (20%) in order to characterize the response of the surface tension against relative surface area change.
W/O/W emulsion stabilized with different emulsifiers *and* W/O/W emulsions with encapsulated beetroot juice	Stability	Creaming and thermal stability. The stability of double emulsions after storage (creaming stability) or after heating at 70^{o}C for 30 min (thermal stability) were recorded in terms of a phase separation and expressed as a percentage of initial sample height.
	Droplet size	Static light scattering. The droplet size distribution calculation based on Mie theory as a function of light intensity versus light angle.
	Microstructure	Optical microscopy. Visualization of the double emulsion morphology.
W/O/W emulsion with encapsulated beetroot juice	Flux	Calculated from the formula. The flux across the packed bed was calculated from the mass flow rate.
	Encapsulation efficiency	Spectrophotometric method. The double emulsion sample was centrifuged and the absorbance of the filtered outer water phase was measured. The calibration curve was used to calculate the actual juice concentration that was released during centrifugation.
	Water activity	Measurement of so called "not chemically bound" water in double emulsion. Measurements based on the relationship between the water vapor pressure of the sample and the saturation pressure of pure water at the same temperature.
Meat systems with double emulsion	Color	Colorimetry. The color was evaluated using the Commission Internationale de l'Eclairage (CIE) L* (lightness), a* (redness), and b* (yellowness) system.
	Water and fat binding properties	Thermal treatment. It was evaluated by water and fat released from meat systems after the thermal treatment.
	Hardness, Cohesiveness	Texture profile analysis.The sample was compressed in the Y-axis direction 40% of the sample's original height. The force time curve was derived and peak forces for the first and second compressions were used for calculations.
Meat systems and cold smoked sausages	pH	Potentiometric. pH electrode placed directly in homogenized samples by measuring potential difference between two electrodes.
	Hardness, cohesiveness	Texture profile analysis. Sample compression with the 20 mm diameter aluminum cylinder.

Continuation of Table 1

	Glucose, fructose, saccharose	Spectrophotometric. The determination is based on the formation of NADH measured by the increase in light absorbance at 340 nm.
	L-lactic acid	Enzymatic/spectrophotometric. The amount of NADH formed during enzymatic reactions is stoichiometric to the amount of L-lactic acid. The increase in NADH is determine by means of its light absorbance at 340 nm.
	Nitrate/nitrite	Potentiometric with ion selective electrode. Measurement of electromotive force as a response to the NO_3^- ion concentration.
	Color	Colorimetry. The color was evaluated using the Commission Internationale de l'Eclairage (CIE) L* (lightness), a* (redness), and b* (yellowness) system.
	Coliforms, Lactic acid bacteria, Staphylococci	Agar well diffusion method. Inoculation on agar medium and calculating the total viable count.
	L-glutamic acid	Enzymatic/spectrophotometric. Enzymatic reactions that cause the formation of INT-formazan, stoichiometric to the amount of L-glutamic acid. The amount of INT-formazan measured by the increase in absorbance at 492 nm.
	Biogenic amines	HPLC. Sample extraction, derivatization, detection, and quantification of different BA's.
	Myoglobin forms (OxyMb; DeoxyMb; MetMb)	Spectrofotometric. Relative forms of myoglobin were extracted with phosphate buffer. The absorbance of the filtered supernatant was read at different wavelengths, corresponding to oxymioglobin, deoxymioglobinm, and metmioglobin.
	Nitrosomioglobin (NOMb)	Spectrofotometric. NOMb was extracted with the acetone/water mixture and the absorption was measured immediately after filtration at 540 nm.
Cold smoked sausages	Yield	Weight loss. The yield of sausages was determined by calculating the difference in weight measured before and after the thermal treatment.
	Water activity	Measurement of so called "not chemically bound" water in cold smoked sausage samples. Measurements based on the relationship between the water vapor pressure of the sample and the saturation pressure of pure water at the same temperature.
	Hardness, cohesiveness, chewiness	Texture analysis. Samples were compressed in the Y-axis direction 75% of the sample's original height. The force time curve was derived and peak forces for the first and second compressions were used for calculations.
	Sensory evaluation	Sensory analysis. The sensory analysis was performed by selected and trained panelists while applying a profile test of organoleptic characteristics. Intensity and acceptability of sensory properties were evaluated.

3. RESULTS AND DISCUSSION

3.1 Double emulsions as an encapsulation system for natural pigments

3.1.1. Effect of the emulsifier type and concentration on the double emulsion (W/O/W) properties

Initially, W/O/W emulsions containing different whey protein isolate (WPI) (0.5%; 1.0%; 1.5%) and carboxymethilcelullose (CMC) (0.25%; 0.30%; 0.35%) concentrations in the external water phase were characterized. The creaming stability of emulsions depended on the concentration of emulsifiers that were used. In the case of WPI, the emulsion with only 0.5% WPI remained stable even after 305 h (Figure 2). However, when the emulsifier concentration was increased up to 1.5%, a more extensive phase of separation was observed and this could be related to a higher concentration of the non-adsorbed emulsifier that promoted Ostwald ripening or droplet flocculation (Klang and Valenta, 2011; McClements, 1994; Weiss, et al., 2000). A previous study of Mun et al. (2010) reported about stable double emulsions with a relatively high amount of WPI (2%; 4% or 6%) if compared it to our research.

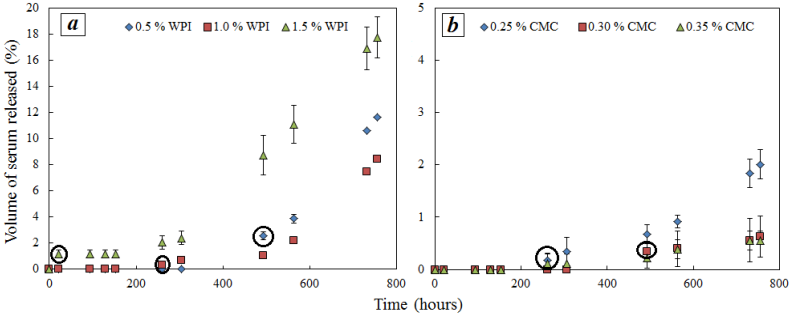

Figure 2. W/O/W emulsion stabilized with WPI (a) and CMC (b) stability as monitored by measuring released serum volume (%) during storage. The beginning of serum separation is marked by round black circles

As in the case of WPI, the effect of CMC concentration on the double emulsion stability was observed. At higher CMC concentrations (0.30–0.35%) double emulsions were physically more stable (Figure 2), but even in the most unstable double emulsion with CMC at the end of storage volume of the released serum was ~4 times less in comparison with the most stable WPI-stabilized emulsion (8.47 ± 0.00%). Such results were similar to the results provided by Pays et al. (2002) who also reported that double emulsions stabilized with CMC

indicated more resistance to the phase separation in comparison with WPI-stabilized emulsions. The reason for that was different origin of these emulsifiers as well as different stabilization mechanisms. The action of CMC was more of a stabilizing agent rather than emulsifying agent increasing viscosity of the aqueous phase and slowing down possible double emulsion destabilization processes (Coffey et al., 1995) that were confirmed by higher viscosity (2.095–4.381 $Pa \cdot s^n$) in double emulsions (Table 2).

Irrespective of the composition, double emulsions had a heterogeneous structure that was confirmed by microscopic images (not presented). Oil droplets of different size filled with water inside with two phases separating the interface layer were clearly visible. Double emulsions prepared with 0.5% WPI and 0.25% CMC had the highest droplet size (36.19 μm and 39.01 μm, respectively). Other emulsions had a mean droplet size (~30 μm) which was still very large and could be related with the uneven "two step" homogenization process used for the preparation of W/O/W emulsions. In the case of the CMC droplet, size had no negative effect on the double emulsion stability as the phase separation was slowed down by the increase in viscosity.

Table 2. The average droplet size and rheological properties of double emulsions stabilized by different emulsifiers

Type of emulsifier	Emulsifier concentration, %	Average droplet size (d_{32}), μm	Span factor	Rheological properties		
				n	Viscosity index κ ($Pa \cdot s^n$)	R^2
WPI	0.5	36.19±1.86c	1.54	0.546	0.509±0.087b	0.926
	1.0	31.32±1.32b	1.53	0.626	0.816±0.024c	0.871
	1.5	26.64±2.13a	1.78	0.600	0.321±0.041a	0.935
CMC	0.25	39.01±3.22d	6.69	0.549	2.095±0.043d	0.948
	0.30	28.92±1.70b	1.70	0.622	2.132±0.317d	0.992
	0.35	25.19±1.62a	1.33	0.622	4.381±0.478e	0.959

Results are presented as the mean value ± standard deviation; mean values followed by the same letters within the same column are not significantly different ($p>0.05$)

The results indicated that 1.0% of WPI and 0.35% of CMC were the most suitable concentrations for the formation of stable double emulsions. Despite the obtained positive results, the average droplet size of structures such as the double emulsion was too high and needed to be reduced without changing the amount of emulsifier, especially, in the systems with WPI. For this purpose, different emulsification systems could be used.

3.1.2. Effect of emulsification systems on the properties and encapsulation efficiency of double emulsion (W/O/W) with encapsulated beetroot juice

Freshly prepared double emulsions had the typical opaque appearance of emulsions and were pink in color. Their droplet size was in the range of 10–100 μm (~30 μm) as was expected for the rotor stator system (Ultra-Turrax) that was used for emulsification. Such systems yielded polydisperse emulsions with droplets typically larger than 10 μm because of a rather chaotic liquid movement (Urban et al., 2006). In the microscopic image presented in Figure 3a, filled double emulsion droplets of various sizes are clearly visible. Irrespective of their size, all droplets have a typical structure of double emulsions consisting of small water droplets located inside an oil globule.

Despite the size of droplets, the emulsions were quite stable during storage (even after 2 weeks of storage) and this was most probably due to their viscosity that increased during storage. In general, the size of emulsion droplets, the density difference, and viscosity of the continuous phase affected the creaming rate (Stokes' law) when the most important factor was the size of a droplet (Walstra, 2003).

Immediately after the preparation, emulsions flowed rather freely and their viscosity reached (0.03 Pa·s), but after 2–3 h of storage, emulsions became stiffer and viscosity increased considerably (2.9 Pa·s). During this storage time, the particle size d_{32} increased slightly from 32 to 34 μm and water activity decreased from 0.9993 to 0.9814. Such results could be associated with the swelling of internal water droplets in oil droplets during storage to re-equilibrate the chemical potential (water activity was different in the internal and external water phases). Although the effect on droplet diameter was not significant at first glance, the effect on droplet volume, which increased 3 times to the droplet size, was significant. For the values given here, the volume of oil droplets increased 20%. Since the initial volume ratio water to oil in the primary emulsion was 1:4, this also implied that the volume of water has doubled after equilibration, and this will have an effect on the viscosity of oil droplets that now are packed with 40% water in the internal phase (coming from 20%). As indicated, water transfer had a great influence on the volume ratio between the primary emulsion and the outer water phase which initially was 40:60. Since the volume fraction of internal water droplets increased 20%, the total volume fraction of the droplet phase (that started as the primary emulsion) was 48%. Consequently, the outer water phase was reduced to 52 vol. %.

3.1.2.1. Effect of pressure on oil droplet size in double emulsion prepared by hybrid membrane emulsification system

The size reduction, which was due to the droplet break-up governed by localized shear forces, interfacial tension effects, and steric hindrance between droplets, occurred with all applied pressures (Sahin et al., 2014). As illustrated in Figure 3, which shows a microscopic structure of the double emulsion before and after premix emulsification, droplet sizes that can be seen in the microscopic images correlated well with droplet size measurements.

As shown in Table 3, there were small differences ($p<0.05$) in the droplet size of double emulsions emulsified at different pressures, with smaller droplets corresponding to higher applied pressures. This was similar to the results that were reported by Sahin et al. (2014) who found out that higher droplet size reduction was achieved at higher applied pressure, which was associated with higher shear stresses inside the pore labyrinth because of the increased flow velocity.

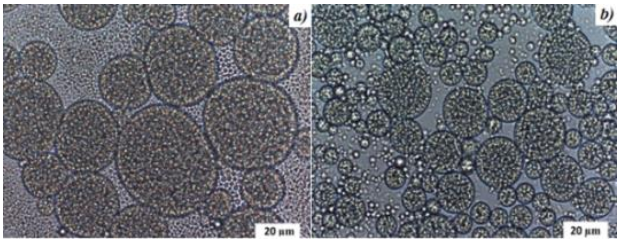

Figure 3. Microscopic image of the double emulsion with native beetroot juice and 0.5% WPI before and after premix emulsification (1 pass at 300 kPa pressure) (magnification x100)

All emulsions that were obtained after emulsification had a span of 2-3, which made them more polydisperse as was previously reported for double emulsions prepared with regular surfactants for which the span was typically around 1 (Sahin et al., 2014). Apparently, the composition of our emulsions did not allow narrower droplet size distributions. One day of storage caused a considerable increase in the droplet size of emulsions (Table 3), much more than of 2 h described above. As was already mentioned, this is associated with water migration from the outer water phase to the inner water phase that led to the swelling of oil droplets. For the emulsion prepared at 200 kPa, the increase in the droplet size after 1 day corresponded to the volume increase of 120% of oil droplets, leading to even more extreme volume ratios than mentioned above [we now calculate 88:12 for the droplet phase relative to the outer water phase which corresponds to mayonnaise]. Similar behavior has been reported for double

emulsions by Bahtz et al. (2015) who showed that the actual swelling was caused by the osmotic pressure difference between W_1 and W_2, and this process started after a certain lag time that was determined by the oil phase viscosity.

Table 3. Droplet size of double emulsions with native beetroot juice immediately after emulsification and after 1 day of storage at room temperature (20°C) before and after 1 pass through the packed bed premix emulsification system operated at different pressures.

Applied pressure, kPa	After emulsification		After 1 day storage	
	d_{32}, μm	Span	d_{32} μm	Span
Before	31.95±0.10dA	1.39	39.14±0.10dB	1.38
200	24.26±0.35cA	2.49	31.63±0.27cB	2.39
300	23.23±0.06bA	2.80	28.79±0.14bB	2.43
400	21.39±0.08aA	1.98	27.04±0.37aB	2.67
500	21.19±0.12aA	2.73	29.64±0.61bB	2.05

Values are reported as means ± standard deviation; lower case letters indicate significant ($p<0.05$) differences in particle size d_{32} between different passes, and upper case letters indicate significant ($p<0.05$) differences in particle size d_{32} immediately after emulsification and after 1 day of storage

3.1.2.2. Effect of number of passes on the properties of double emulsions prepared by hybrid membrane emulsification system

Since the observed differences in the droplet size of emulsions treated by different pressures at one pass were not considerable, it was decided to change the number of passes only at 300 kPa pressure. Premix emulsions were prepared with native and two times concentrated beetroot juice, and 0.5% and 1.0% WPI in the outer water phase, and the effect on the flux, droplet size, stability and encapsulation efficiency was evaluated.

It was found out that the flux decreased quite dramatically with increasing number of passes for all emulsions. For example, in the emulsion with native juice and 0.5% WPI, the highest flux value was obtained during the first pass (65.18 ± 3.22 m/h), while for the fourth pass the flux was as low as 1.18 ± 0.18 m/h, which is still a very reasonable flux when compared with values found in literature (Nazir et al., 2010). It is clear that changes in flux cannot be explained only by a change in viscosity. Sahin et al. (2014) found out that the flux after the first pass was the highest and for other passes flux values were slightly lower and they related this effect to the presence of emulsion droplets that (slightly) accumulated in or before the bed.

While considering just the first pass, the flux reported here was 8 times lower than reported by Sahin et al. (2014), but those emulsions had much lower viscosity (3.6 mPa·s), and with respect to that, the hydraulic resistance of the systems (flux x viscosity) was the same. Also, in experiments described here, the dispersed phase volume fraction was much higher, especially when taking the

swelling effect into account which is time dependent, and this most probably had led to the obstruction of the column when operated multiple times.

While the first pass led to the droplet refinement at constant span when using 0.5% WPI. At 1% WPI, this effect was lost and more polydisperse emulsions were formed (Table 4). That was also the case for a higher number of passes at 0.5% WPI when the formation of a small second peak with droplets <1 μm and a bigger third peak with droplets ≥100 μm was observed, and it clearly indicated that emulsions were not sufficiently stable to survive these process conditions. When using concentrated juice, these effects were even more noticeable; most probably, the water transfer towards the internal water phase was even faster than for the native beetroot juice, and that has led to very polydisperse emulsions and large droplets. This could also partly explain why the emulsion prepared with 1% WPI was more polydisperse; because droplets were smaller and the water transfer was faster due to the higher interfacial area that was available.

According to Nazir et al. (2013), at all applied pressures and, especially, at the elevated pressures, the droplet size reduction for single emulsions was the highest after the first pass, but significant further reduction occurred till the third pass after which only minor reduction took place. Our results were very different and this was because of the increase in viscosity mentioned above which led to low fluxes. As a result, high residence times in the system and a higher probability of coalescence of droplets occurred.

Table 4. The average droplet size and droplet size distribution of double emulsions with different formulations before and after emulsification through the packed-bed premix emulsification system at 300 kPa pressure and for different number of passes.

Number of passes	Native juice; 0.5% WPI		Concentrated juice; 0.5% WPI		Native juice; 1.0% WPI	
	d_{32}	Span	d_{32}	Span	d_{32}	Span
Before	31.95±0.10e	1.39	38.61±0.03d	1.11	27.17±0.05e	1.41
1	20.35±0.02d	1.03	24.87±0.03b	1.28	21.36±0.26d	2.65
2	14.53±0.43a	1.75	21.67±0.31a	4.07	10.44±0.11a	2.64
3	15.04±2.04ab	2.77	31.99±2.09c	3.42	11.82±0.50b	4.37
4	17.04±2.01bc	4.37	34.28±1.17c	2.64	10.61±0.50a	5.19
5	17.97±1.06c	8.56	-	-	13.36±0.07c	5.48

Values are reported as means ± standard deviation; lower case letters indicate significant ($p<0.05$) differences in particle size d_{32} between different passes

The initial encapsulation efficiency slightly depended on the double emulsion formulation but was always high and reached 98–100% (Table 5), which was even higher as reported by Kaimainen et al. (2015) who found 89% for water extracts of betalain in the double emulsion. It was found out that the best encapsulation efficiency was reached after the first pass in all three emulsions (95.53–100%). Further passes led to lower encapsulation efficiency

(75.08–92.15%) and some phase separation, but it should be mentioned that all values in Table 5 are high and very acceptable. Despite of the increase in the droplet size recorded at a higher number of passes, the internal phase was not expelled. Most probably, the droplets interacted with each other either directly or through the glass bead bed, of which the latter effect may have led to the blockage of the system resulting in low fluxes.

Table 5. Encapsulation efficiency in double emulsions with different formulation before and after emulsification for different number of passes

Number of passes	Native juice; 0.5% WPI	Concentrated juice; 0.5% WPI	Native juice; 1.0 %WPI
Before	98.62±0.08d	100.00±0.00d	95.76±0.10d
1	100.00±0.00e	100.00±0.00d	95.53±0.12d
2	100.00±0.00e	89.68±0.12c	93.85±0.10c
3	92.15±0.05c	81.21±0.08b	91.21±0.03b
4	86.89±0.10b	75.08±0.15a	89.17±0.05a
5	84.50±0.12a	-	89.01±0.11a

It was found out that all emulsions showed shear thinning behavior and have affected the emulsification process positively. While analyzing the data of experiments carried out with emulsions containing 0.5% WPI, viscosity at first increased with the number of passes, most probably because of the water transfer to the internal water phase. Starting from the third pass, viscosity decreased again and that was related to the instability of the system and phase separation was visible. These effects are not desirable in a real product.

It can be noticed that for the viscosity of the emulsion with 1.0% WPI a similar trend as for 0.5% was visible but the reduction in viscosity at a high number of passes was very low. The increase in viscosity occurred due to the previously mentioned osmotic swelling, but in this case droplets were better stabilized by a higher concentration of WPI, leading to very high encapsulation efficiency (Table 5). As was already clear from the encapsulation results, double emulsions prepared with concentrated juice were not stable enough to keep high encapsulation efficiency at a higher number of passes, and the phase separation was observed. Because the osmotic pressure difference was even higher in this system, this might have led to an extensive swelling, and eventually phase separation would occur leading to lower viscosity.

3.1.3. Effect of osmotic pressure, lipophilic emulsifier, and water/oil interphase composition on double emulsion stability

Influence of osmotic pressure on the properties of double emulsions

The osmotic pressure in the internal water phase (juice) was a result of carbohydrates (and salts) that were naturally present in beetroot juice. Using the Van't Hoff equation, we have calculated that the osmotic pressure of beetroot juice with 10% total solids (mostly glucose) was to be 1.486 MPa (20^{o}C, density 1.4500 kg/l). As juice was used in the primary emulsion formulation (the inner water phase), glucose was used in the outer water phase in order to influence the osmotic pressure differences. Coarse double emulsions were prepared with different glucose concentrations (0.5%, 1.0%, and 3.0%) in the outer water phase, corresponding to 0.682, 1.364, and 4.038 MPa osmotic pressure, respectively. We have recorded that viscosity of emulsions with added glucose was much lower (0.15–0.65 Pa·s) compared to the emulsion without glucose (3.60 Pa·s). These results confirm that a significant increase in viscosity of W/O/W was related to the swelling effect which was caused by the osmotic pressure differences in the water phases. It should be mentioned that the addition of 3.0% glucose led to unstable emulsions which allowed determining only viscosity.

Effect of lipophilic emulsifier (PGPR) on emulsion stability

Oil phases containing various PGPR concentrations were brought into contact with a water phase in the absence of any agitation. Initially, both phases were clearly separated but 24 hours later, at the contact line between them, a layer of droplets was formed (seen as a white layer indicating small droplets; Figure 4a). This effect became more important for the increase of PGPR concentration in the oil, and the formation of large structures in the bottom (water) phase could be seen. In the control system (without PGPR (0%), pure oil and water; Figure 4a), this was not observed, confirming that PGPR was responsible for the observed changes. This is further illustrated in the microscopic images of the oil phase after 24 hours showing small dispersed water droplets when PGPR was present (Figure 4b). This corresponds to the findings reported by Bahtz et al. (2015) who also found spontaneously formed water droplets in PGPR-containing oil with medium-chain triglyceride. Authors suggest two possible mechanisms: water diffusion into the oil phase until saturation is reached leading to the formation of clusters that nucleate to small droplets or water entrapment through the formation of PGPR reverse micelles.

According to the authors, the second argument seems more likely since the water solubility in oil is poor.

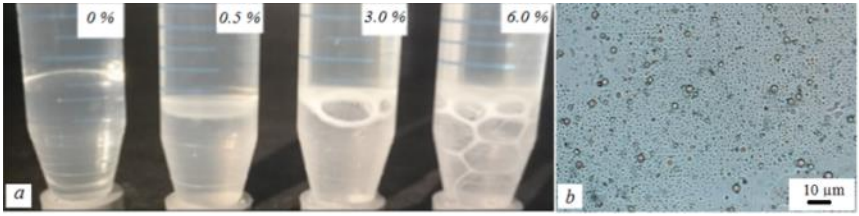

Figure 4. a. Images of contact lines between water and oil after 24 hours of storage (without mechanical force applied) with different PGPR concentrations (indicated as w/w%; in the image) in the oil phase. **b.** Microscopic image of water droplets formed in the oil phase containing 0.5 (w/w%) PGPR without mechanical force applied. Image taken after 24 hours of incubation. Magnification x40.

Influence of water-oil interphase composition on surface tension and interphase structure

As can be seen in Figure 5, surface tension considerably decreased when a higher juice concentration (>0.5 %) was used. For example, 2.0% of juice was enough to reduce interfacial tension from 22.11 ± 0.11 mN/m to 12.39 ± 0.51 mN/m (almost 2 times). This effect was even more noticeable when PGPR was added to the oil phase and it was strongly affected by the lipophilic emulsifier concentration (Fig. 5). These results were similar to the previously reported results by Gülseren and Corredig (2012;2014) who found out that the interfacial tension decrease was affected by the PGPR concentration increase. The reason for this could be more available surface active compounds presented in the mixed interfaces that resulted in higher absorption density at the juice/oil interface. As their concentration increased, the surfactant molecules began to orient themselves at the interface forming a monolayer. This increased the surface pressure and decreased both the interfacial energy and the interfacial tension, and when the solution concentration became close to the critical micelle concentration, no further adsorption at the interface occurred. Therefore, interfacial tension reached the final plateau value (Gao and Sharma, 2013).

In the presence of 0.01% PGPR, a clearly visible correlation between the surface tension decrease and the beetroot juice concentration increase was established (Fig. 5). However, different system behavior was obtained when the concentration of PGPR was increased to 0.05%; there was no clearly visible synergistic effect anymore. At a higher concentration, PGPR probably dominated at the interface and masked the effect of juice compounds.

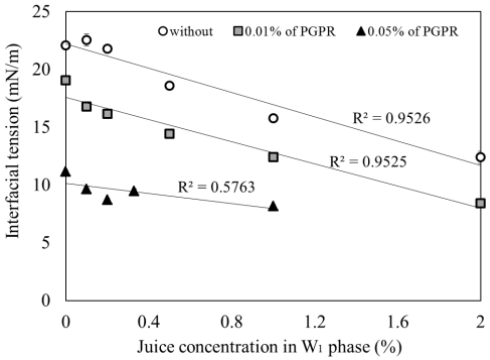

Figure 5. Effect of different beetroot juice and PGPR concentrations on interfacial tension, as measured by drop tensiometry. Values were taken after 3 h of equilibration and are the average of at least two experiments ± standard deviation

Viscoelastic interface properties are important because, according to Gaonkar (1991), interfacial tension is an indicator of adsorption rather than the structure of the interfacial film and lowered interfacial tension does not always lead to enhanced stabilization. The linear viscoelastic response to the applied deformation was also confirmed by *Lissayous plots*, which showed the surface pressure versus deformation (data not shown). Still, as can be seen from Figure 6, the elastic behavior dominated at the interfaces as the elastic modulus (E′) was higher than the loss modulus (E″) in all samples almost at all applied frequencies (≥0.01 Hz).

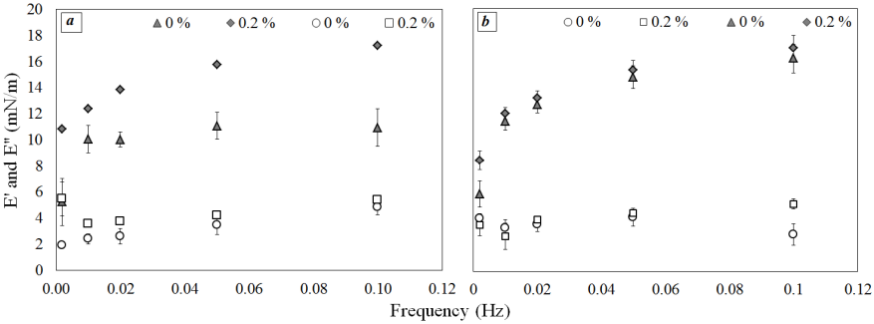

Figure 6. Elastic (E'_d) (filled markers) and loss (E''_d) modulus (empty markers) as a function of applied frequency (0.002; 0.01; 0.02; 0.05; 0.1) at a constant amplitude 20%; a) PGPR concentration – 0.01%; b) PGPR concentration – 0.05%

According to Amine et al. (2014), higher interface elasticity could slow down the coalescence rate. What is more, such elastic behavior could be a reason of higher

swelling capacity that droplets could reach during water diffusion and, due to this fact, our findings were very positive. It is important to note that the effect of juice compounds was visible only at the interface as well as in the case of interfacial tension, where 0.01% of PGPR was presented.

3.2. Characterization of meat systems made with double emulsions addition

Values of the total fluid, water, and fat released showed little variation neither as a function of fat amount (7 or 11%) nor as a function of the added double emulsion ($p>0.05$) (data not shown). The fact that the insertion of the double emulsion had no negative effect on water and fat binding properties was in line with the good stability of W/O/W emulsions found in earlier stages of our work which apparently can also be achieved while including a double emulsion in a meat product, in the same way as was reported by Cofrades et al. (2013). The use of solid animal fat versus double emulsions prepared with liquid sunflower oil influenced the hardness and cohesiveness of meat systems (data not shown). The hardness of meat systems made with W/O/W emulsions was reduced compared to the control samples, especially in meat systems with coarse and fine double emulsions. Such results were similar to the findings of Serdaroğlu et al. (2016) and Freire et al. (2016). To be more precise, no differences in cohesiveness of meat systems (~0.7 or ~0.9, depending on the emulsion type) were found. These results are contrary to the findings of Serdaroğlu et al. (2016) who reported that the decrease in cohesiveness from 0.8 to 0.3 occurred when beef fat was replaced by 10% of a double emulsion stabilized with sodium caseinate.

Table 6. Color of meat systems (internal) with different double emulsions after thermal treatment at 70^{o}C for 30 min

Meat system	L*	a*	b*
DE, stabilized with different emulsifiers			
C-7	71.52±0.03a	7.50±0.21e	13.70±0.05d
DE-WPI-7	76.65±0.57c	5.26±0.11bc	12.77±0.07c
DE-CMC-7	79.21±0.44d	5.45±0.08c	12.54±0.08c
C-11	73.17±0.35b	6.67±0.07d	13.50±0.08d
DE-WPI-11	79.23±0.10d	4.24±0.03a	12.17±0.02b
DE-CMC-11	80.68±0.10e	5.14±0.01b	11.19±0.36a
DE with encapsulated beetroot juice			
C-7	57.55±0.28c	2.40±0.10b	13.12±0.25d
DE-C-7	55.47±0.33a	8.84±0.03d	11.78±0.18c
DE-F-7	56.70±0.24b	8.39±0.08c	11.55±0.25bc
C-11	58.18±0.22d	2.19±0.07a	13.20±0.24d
DE-C-11	57.14±0.07bc	9.17±0.12e	11.31±0.03ab
DE-F-11	55.85±0.29a	10.12±0.03f	11.10±0.13a

Values are reported as means ± standard deviation.

After the thermal treatment, the redness of all meat systems decreased because of chemical changes in myoglobin. In samples made with DE (stabilized with different emulsifiers), the color of meat systems was negatively affected (Table 6) as redness values were lower and brightness values were higher in comparison with control samples that were similar to the research by Cofrades et al., 2013 and Jiménez-Colmenero et al. (2010). However, in meat systems made with double emulsions (with encapsulated beetroot juice), redness values were significantly higher, irrespective of the type and amount of the added emulsion (Fig. 7).

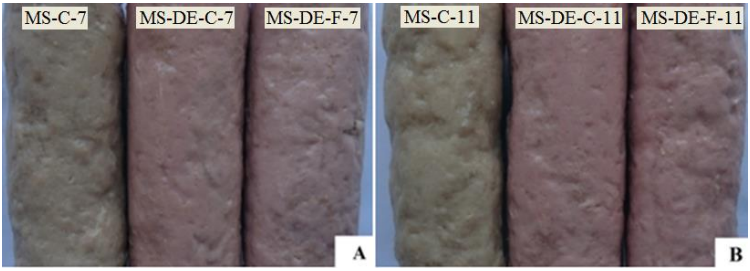

Figure 7. Meat systems color after thermal treatment: (A – with reduced fat content (7%); B – with normal fat content (11%)

3.3. Effect of nitrates from freeze-dried vegetables on meat fermentation

3.3.1. Effect of nitrates from freeze-dried vegetables on safety of meat systems during its fermentation

It was found out that due to sugar fermentation, which was carried out by lactic acid bacteria naturally present in meat, the amount of lactic acid increased (0.038–0.085 g/100g) while lowering pH values. After fermentation, pH values were noticeably lower in meat batters with freeze-dried vegetables (4.77–5.02) in comparison with controls (5.55–5.70). In order to prevent the growth of undesirable spoilage and pathogenic microorganisms, the pH value during fermentation must decrease below 5.5 (Edward, 2008). This value has been reached only in samples with freeze-dried vegetables as a consequence of carbohydrates fermentation that is naturally present in vegetables. It should be mentioned that the rate of pH and carbohydrates decreased, while the rate of lactic acid increased was the same regardless of *Staphylococcus* species that were used for fermentation.

Our results showed that the rapid increase in staphylococci and lactic acid bacteria content was observed in the initial stage of fermentation (after the first

day) (data not shown). Later, the growth of staphylococci slowed down as they sometimes have difficulties competing with lactic acid bacteria which became dominant microflora during meat fermentation. Such increase was obtained irrespective of the fact that during the meat batters formulation only *Staphylococcus* was added. It was also determined that in meat batters, which were fermented with *Staphylococcus carnosus*, a higher amount of these microorganisms was during the whole fermentation process. In the control (without *Staphylococcus* addition), it was the lowest amount of *Staphylococcus*, but during the fermentation process, the same growth kinetic was obtained and in the end of the process it reached 6.7 log CFU/g as well as in other meat systems (5.1–7.3 log CFU/g) (Fig. 9).

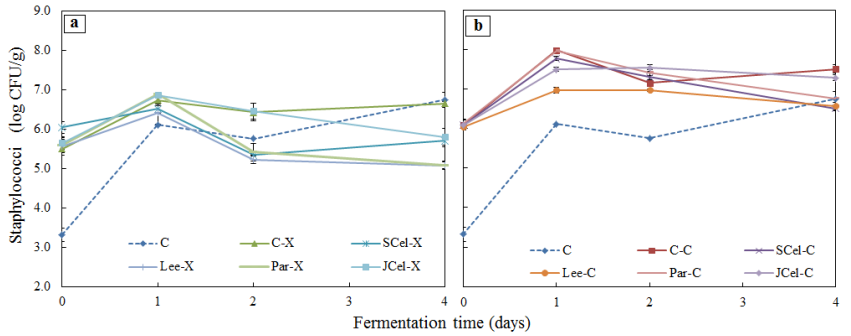

Figure 9. The amount of staphylococci in meat batters of different composition during the fermentation process fermented by a) *S. xylosus*; b) *S. carnosus*

In the control samples (C, C-X, C-C), the amount of lactic acid bacteria was significantly ($p<0.05$) lower during fermentation if compared with meat systems with added freeze-dried vegetables. In the end of the process, meat batters with vegetables contained 8.4–8.6 log CFU/g, while in control it was only 7.6–7.8 log CFU/g (Fig.10). It is believed that favorable conditions for lactic acid bacteria to grow in these samples were created because of the nutrients (carbohydrates) that were added in meat batters with vegetables.

During the first two days of fermentation, the number of coliforms increased from ~3 log CFU/g to ~6 log CFU/g. However, later in meat systems with vegetables addition, the amount did not change, while in the control samples it increased further and reached 7.0–7.8 log CFU/g. Differences in the growth kinetic could result from a higher amount of lactic acid bacteria and the following higher amount of lactic acid and lower pH values in samples with vegetables as coliforms are sensitive for an acidic environment. In addition, nitrite that had formed during the nitrate reduction could inhibit the growth of several aerobic and anaerobic microorganisms by limiting oxygen uptake,

breaking the proton gradient, and inhibiting metabolic enzymes (Tompkin, 2005).

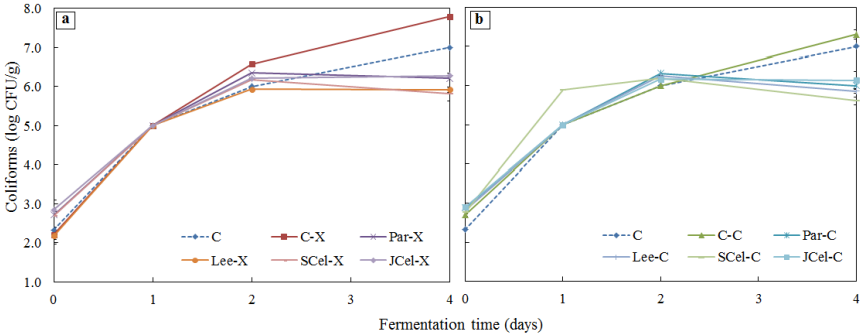

Figure 10. The amount of staphylococci in meat batters of different composition during the fermentation process fermented by a) *S. xylosus*; b) *S. carnosus*

Proteolysis was characterized by free glutamic acid (as a precursor of amino acids) and biogenic amines (as a product of amino acids decarboxylation). Free glutamic acid gradually increased throughout the process regardless of the meat batter composition. However, in the end of fermentation a significantly ($p<0.05$) higher amount of glutamic acid formed in treatments with added vegetables (0.052–0.085 g/100g) in comparison with controls (0.038–0.048 g/100g). This difference can be explained by initially higher amount of glutamic acid in these systems as freeze-dried vegetables naturally contained certain amount of free glutamic acid. Moreover, the higher amount of lactic acid bacteria could cause glutamine deamination that resulted in the increase of glutamic acid which indirectly contributed to the process by reducing pH which increased the activity of cathepsin D.

As the proteolytic process was confirmed by a progressive increase in the glutamic acid content, the next step was to determine biogenic amines (putrescine, cadaverine, histamine, tyramine, and spermine) that have formed during fermentation as a possibility of its formation cannot be excluded, especially, if we take into account the fact that free amino acids could be affected by certain microorganisms and promote decarboxylation reactions which resulted in the increase of biogenic amines. From a toxicological point of view, the most important biogenic amine-histamine levels in all samples were below the detection limits, for example, <5 mg/kg (data not shown). There was also a very small amount of spermine ranging from 12.58 to 32.74 mg/kg. The remaining amount of biogenic amines (putrescine, cadaverine, and tyramine) reached slightly higher levels (>100 mg/kg). Increasing levels of biogenic amines are often associated with the activity of lactic acid bacteria which leads to

increased acidity. Microbial enzymes that induced deamination and decarboxylation reactions are associated with the defensive microorganisms' reaction against acidic medium (Karovičová and Kohajdová, 2005). It is also clear that the proteolysis process was induced not only by the added microorganisms but also by the microbial and endogenous enzymes activity. What is more, in control without starter cultures, the increase in glutamic acid and biogenic amines was established.

Significantly ($p<0.05$) higher nitrate content during the fermentation process was in the control without *Staphylococcus* addition (C) and this confirmed the importance of microorganisms with nitrate reductase activity in meat fermentation process. After the first day of fermentation, a decrease in nitrate content (Figure 8) as well as an increase in nitrite content (data not shown) were found. Later, the amount of nitrate changed in two ways; i.e. it remained almost the same or slightly increased.

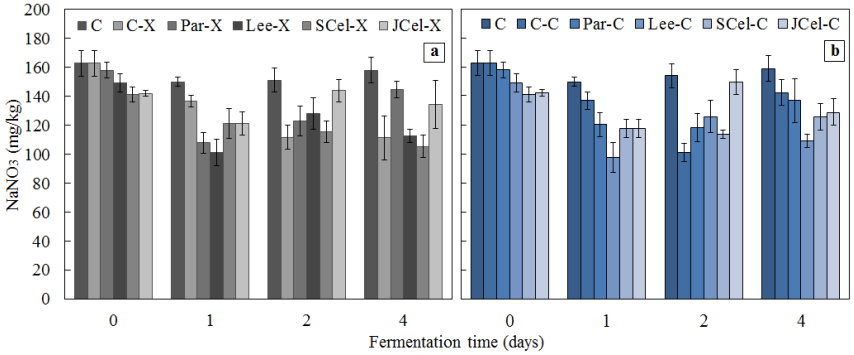

Figure 8. The amount of nitrate (expressed as $NaNO_3$ mg/kg) in meat batters during the fermentation process fermented by a) *S. xylosus* and b) *S. carnosus*

Such increase is most likely due to (highly reactive) nitrite that had formed during the nitrate metabolism, and, as it is known, it could oxidize back to nitrate (Honikel, 2008). Slow reduction of nitrates was also found in the studies of Tsoukalas et al. (2011) where freeze-dried leek powder was used as a source for nitrate in dry fermented sausages. However, during the fermentation process, the total amount of nitrate decreased 5–28% from its origin quantity depending on the type of meat batter. As was mentioned above, nitrate reduction slowed down after the first day, and as a result, residual nitrite levels in meat systems did not exceed 5 mg/kg.

3.3.2. Effect of nitrates from freeze-dried vegetables on myoglobin forms and the associated changes of meat color in meat systems during its fermentation

Changes in relative myoglobin forms during the meat fermentation process occurred only in the systems with parsnip, as the rest of the systems with vegetables established a similar trend.

The increase in staphylococci amount induced nitrate reduction to nitrite that transformed to nitrite oxide. Nitric oxide almost instantly reacts with myoglobin forming cured meat pigment nitrosylmyoglobin which rapidly increased to 42–86 mg/kg after the first day of fermentation (as can be seen in Figure 11a). Later, a decline in nitrosomyoglobin content can be clearly seen. It can be explained by its rapid oxidation or partial denaturation caused by lactic acid which amount increased during fermentation (Bozkurt and Bayram, 2006). However, in the end of fermentation, the amount of NOMb varied from 30 to 40 mg/kg, regardless of used *Staphylococcus* species or the source of nitrate (synthetic or from freeze-dried vegetables).

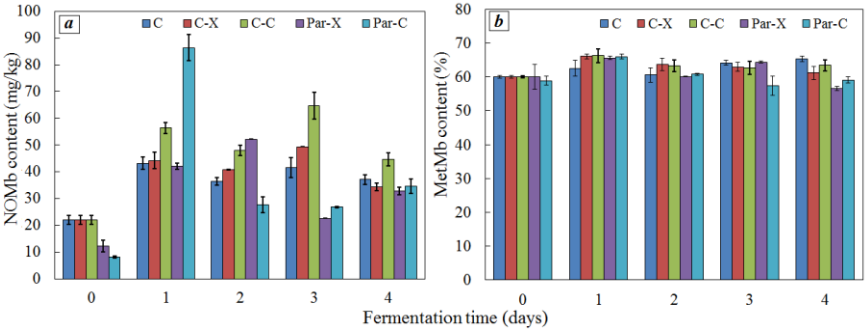

Figure 11. The amount of NOMb (a) and metmyoglobin (b) in meat systems of different composition during fermentation

While evaluating the relative content of chemical myoglobin forms, it was observed that oxidized myoglobin form, metmyoglobin, was predominant as its amount exceeded 55% (Figure 11b), while oxygenated (oxymyoglobin) and reduced forms (deoxymyoglobin) reached only ~20% and 25% (Figure 12a, b). As was mentioned above, nitric oxide that had formed in the meat matrix during the nitrate reduction influenced oxidative degradation of oxymyoglobin to metmyoglobin (Gøtterup et al., 2008), which was confirmed by the increase in metmyoglobin and the decrease in oxymyoglobin after the first day. The decrease in Oxymyoglobin was also related with the gradual oxygen decline

which microorganisms consumed during their exponential growth (Bozkurt and Bayram, 2006).

In the end of fermentation, the amount of metmyoglobin was the lowest in systems with the parsnip addition (56.55–59.09%), while in controls with synthetic nitrates it was significantly higher (61.66–65.32%). The reason for that could be natural antioxidants that are naturally present in freeze-dried parsnip powder and slow down the formation of metmyoglobin. Later, oxymyoglobin and deoxymyoglobin systematically varied from one form to another until the end of the process.

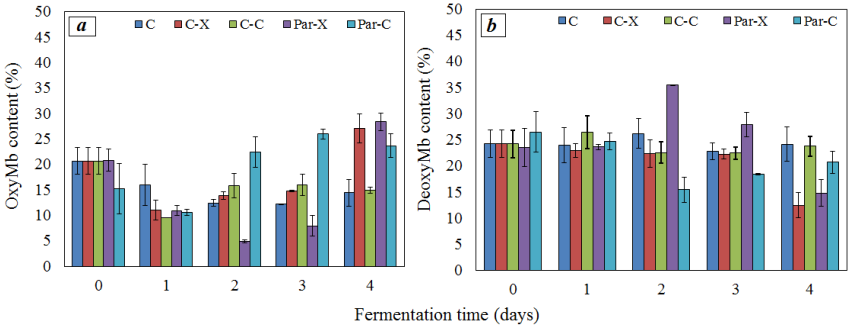

Figure 12. The content of a) oxymyoglobin and b) deoxymyoglobin in different meat systems during the fermentation process

It has been determined that 2 color parameters, L* and a*, are the most informative parameters for the color changes in meat and meat products (Mielnik and Slinde, 1983). A progressive increase in brightness (L*) was observed in all meat systems. Treatments with the leek and parsnip addition were brighter ($p<0.05$) than the remaining samples because of a bright (high L* values ~90) color of freeze-dried vegetable powders. Yellowness values (b*) slightly decreased in all systems as well as in the research of Perez-Alvarez et al. (1999), except in controls and meat batters with leek.

An increase in the redness value after the first day of fermentation was determined. Most likely it was because of the increase in nitrosopigments (this will be discussed in the next section). At the end of the fermentation process, all samples showed significant differences ($p<0.05$) in redness. Redness was lower in samples with the vegetables addition (5.68–11.63), in comparison with controls (17.32–18.30). The reason for that could be plant pigments providing specific color to vegetable powders.

3.3. Effect of nitrates from freeze-dried vegetables on the quality and safety of cold-smoked sausages

Cold smoked sausages were manufactured with the freeze-dried celery addition and compared with controls, where 150 mg/kg of nitrate or nitrite were added. In order to control the process, two types of starter cultures were used: *S. xylosus* only and a mixture of *S. xylosus* and *P. pentosaceus*.

It was obtained that in sausages with freeze-dried celery pH values were lower (~5.1) than in controls (5.3–5.4), but the final a_w values were similar in all investigated sausages, irrespectively of formulation (0.837 – 0.866). The addition of freeze-dried celery and synthetic nitrite caused a slight decrease in yield, as a result, its hardness increased while cohesiveness decreased. The decrease in pH, a_w, and the increase in the lactic acid and nitrate/nitrite reduction processes positively affected growth of gram-positive catalase-positive cocci, lactic acid bacteria, and effectively inhibited growth of Enterobacteriaceae, which are important from a technological point of view and from a food safety perspective.

The residual concentration of nitrate decreased during fermentation and ripening, but differences in nitrate and nitrite concentrations among samples in the end of the technological process were related to the microbial effect rather than to the origin of the added nitrate (synthetic or from celery). In sausages with *S. xylosus*, a lower amount of nitrates was found (~10 mg/kg), while in sausages with *S. xylosus* and *P. pentosaceus*, it reached 20–30 mg/kg. Moreover, since a* values were higher in the sausages with *S. xylosus* and *P. pentosaceus*, it was obtained that the starter culture mixture had a positive effect on the color red, while in sausages with the freeze-dried celery addition, the redness was less intense. As the changes in relative myoglobin forms as well as in nitrosomyoglobin were very similar in all samples, this was related with natural pigments that are present in celery (the color green). Despite this fact, these results only partly correlate with the sensory analysis as more red evaluated sausages with the added nitrite and those that contained a mixture of *S. xylosus* and *P. pentosaceus*. Also, after the sensory evaluation, it was concluded that neither starter cultures nor the freeze-dried celery addition had a negative effect on the overall sensory acceptability.

The progressive proteolysis was confirmed by the increase in free glutamic acid (from 0.01–0.02 g/100g to ~0.20 g/100g) which acts as a flavor enhancing agent and produces a unique taste (umami). In sausages with *S. xylosus*, the total concentration of biogenic amines was higher (>270 mg/kg) than in sausages with *S. xylosus* + *P. pentosaceus* (>130 mg/kg); such results were considered as positive since potential hazard for human health is related with sausages in which the concentration of biogenic amines exceeds 1000 mg/kg.

In general, the results showed that the addition of freeze-dried celery had no negative effect on the fermentation and ripening processes of cold smoked sausages. Therefore, freeze-dried celery can be used as an indirect source of nitrate and nitrite in the production of cold smoked sausages.

CONCLUSIONS

1. The stability of double emulsions (W/O/W) depended on the type of emulsifiers used, concentration, and storage time. Both emulsions, stabilized by whey protein isolate (WPI) and by carboxymethylcellulose (CMC), were stable for ~10 days, but after storage time, the destabilization processes began. Despite the fact that the average size of a droplet was similar in both emulsions (d_{32} ~30 μm), a higher stability has been established in emulsions with CMC. Higher viscosity indexes (2,095–4,381 $Pa \cdot s^n$) showed that CMC increased viscosity of the continuous phase by slowing down movements and possible interactions between fat droplets.
2. It was found out that the double W/O/W emulsion with encapsulated beetroot juice, produced with 6% polyglycerol polyricinoleate (PGPR) and 0.5% WPI, demonstrated high physical stability and encapsulation efficiency (100%). As a result of the osmotic pressure difference, viscosity of emulsions increased from 0.03 Pa·s to 2.90 Pa·s during the storage. After hybrid membrane emulsification, the average droplet size decreased from ~32 μm to 21.19–24.26 μm, depending on the applied pressure. The droplet size decreased until the 3rd pass, further passes influenced the increase in the droplet size, the decrease in stability and encapsulation efficiency. When the concentration of beetroot juice increased during the water/oil interphase from 0% to 2%, the interfacial tension decreased from 22.2 mN/m to 12.7 mN/m.
3. Double emulsions (W/O/W) used in meat systems instead of animal fat had no negative impact on water and fat binding properties and reduced hardness of meat systems. Meat systems, where double emulsions with encapsulated beetroot juice were added, had a significantly higher ($p<0.05$) red value (8.39–10.12), in comparison with control (2.19–2.40).
4. The nitrate reduction process in fermented meat systems was slow and did not depend on the type of nitrate used (synthetic or from freeze-dried vegetables). Meat systems with vegetables had lower pH values (4.77–5.02), a higher amount of lactic (0.9–1.0 g/100 g) and free glutamic acid (0.05–0.09 g/100g), but toxicologically dangerous biogenic amine-histamine levels were below the detection limit (<5mg/kg) in all meat systems. The amount of coliforms at the end of fermentation declined only in meat systems with vegetables.

5. It was found out that nitrates from freeze-dried vegetables influenced a lower amount of the brown pigment metmyoglobin (from 56.55 to 59.09%), compared with systems containing synthetic nitrate (61.66 to 65.32%). The amount of nitrosomyoglobin significantly increased after the first day of fermentation when the fastest growth of staphylococci, which is characterized by high nitrate reductase activity, was obtained. Later, the amount of nitrosomyolgobin decreased to 30–40 mg/kg and it was related with partial denaturation or oxidation.
6. Synthetic nitrite or the nitrate replacement with nitrate from freeze-dried vegetables in cold smoked sausages influenced lower pH values (5.04–5.10). The residual nitrate content in sausages depended on the type of nitrate (synthetic or from freeze-dried vegetables) and starter cultures (*S. xylosus* or *S. xylosus* together with *P. pentosaceus*) used in the sausages production. The total levels of biogenic amines (<150 mg/kg) was lower in sausages fermented with starter cultures mixture. The amount of red myoglobin forms was very similar in all systems, however, lower redness values (8.51–9.32) were in sausages with freeze-dried celery and that is related with green pigments that are naturally present in celery. The sensory evaluation showed that the freeze-dried celery addition had no negative effect on cold smoked sausages properties.

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INFORMATION ABOUT THE AUTHOR

Viktorija Eisinaitė was born on the 11th of December, 1988, in Šilutė (Lithuania).

In 2007: graduated from Pagėgiai Secondary School, Šilutės region, Lithuania.

2007–2011: Bachelor studies at Kaunas University of Technology (Faculty of Chemical Technology), Bachelor's in Chemical Engineering.

2011–2013: Master studies at Kaunas University of Technology (Faculty of Chemical Technology), Master's in Chemical Engineering.

2013–2017: Doctoral studies at Kaunas University of Technology (Faculty of Chemical Technology).

2015 06–2015 09 and 2016 06–2016 09: internship at the Wageningen University in Food Process Engineering group (Wageningen, Netherlands).

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INOVATYVIOS PIGMENTŲ ĮKAPSULIAVIMO IR STABILIZAVIMO PRIEMONĖS MĖSOS GAMINIAMS

REZIUMĖ

Pastarąjį dešimtmetį pasaulyje didėja mėsos suvartojimas. Nepaisant to, 2012 metais Europos Sąjungoje atlikta apklausa parodė, kad vartotojams mėsos sektorius atrodo mažiausiai patikimas iš visų maisto produktų sektorių. Manoma, kad tai lėmė mėsos gaminių siejimas su širdies, kraujagyslių ir vėžinėmis ligomis. Daugelio su mėsos gaminiais susijusių neigiamų asociacijų būtų galima išvengti sumažinant nesveikų sudedamųjų dalių, pvz., sintetinių maisto priedų, kiekį ar pakeičiant juos natūraliais junginiais. Vieni pagrindinių junginių, kuriuos norima pakeisti, yra konservantas ir mėsos spalvos stabilizatorius nitritas ir sintetiniai maistiniai dažikliai. Nitritai yra siejami su kancerogeninių N-nitrozo junginių susidarymu, o sintetiniai dažikliai gali sukelti alergijas, pasižymi neigiamu poveikiu vaikų elgesiui. Šių junginių naudojimas maisto produktuose yra griežtai reglamentuojamas. Būtent todėl pastaruoju metu padaugėjo tyrimų, kuriuose mėsos produktai ruošiami su natūraliais augaliniais komponentais, tokie gaminiai gali būti charakterizuojami kaip turintys „švarią etiketę“.

Viena pagrindinių sąlygų modifikuojant produktų sudėtį yra ta, kad naujas komponentas turi pasižymėti analogiškomis technologinėmis ir funkcinėmis savybėmis ir nepabloginti esamo produkto saugos ir kokybės. Daržovės jau nuo seno žinomos kaip nitratų, pigmentų, skaidulų ir bioaktyviųjų junginių šaltinis. Daržovės, jų ekstraktai gali būti naudojami kaip antioksidacinės ar antimikrobinės medžiagos. Tokios medžiagos yra saugesnės nei sintetiniai jų analogai. Tačiau iš daržovių išskirti ir sukoncentruoti bioaktyvieji junginiai gali pasižymėti mažesniu stabilumu, turėti pašalinį kvapą, skonį ar spalvą ir dėl to daryti neigiamą įtaką gaminio kokybei.

Žinoma, kad sintetiniai raudonos spalvos dažikliai gali būti pakeičiami natūraliu pigmentu betaninu, kuris išskiriamas iš burokėlių (*Beta vulgaris*). Tačiau šis pigmentas yra labai jautrus aplinkos ir technologinio proceso veiksniams. Todėl jo panaudojimas mėsos produktuose tampa labai ribotas. Siekiant to išvengti ieškoma pigmentų apsaugos ar stabilizavimo būdų. Vienas jų yra įkapsuliavimas. Perspektyvia įkapsuliavimo matrica laikomos dvigubosios emulsijos, kurios gali būti naudojamos ne tik bioaktyviesiems junginiams įkapsuliuoti, bet ir riebalų kiekiui mažinti bei riebalų rūgščių sudėčiai mėsos produktuose keisti. Dėl dviejų tarpfazių, kurie turi būti stabilizuojami, šių emulsijų stabilizavimas ir įkapsuliavimo efektyvumo didinimas vis dar išlieka nemenku iššūkiu mokslininkams.

Kaip alternatyva mėsos produktuose naudojamam sintetiniam konservantui ir spalvos stabilizatoriui nitritui gali būti naudojamos didelį nitratų kiekį sukaupiančios daržovės, pvz., salierai, porai, pastarnokai ir petražolės.

Literatūroje nurodoma, kad salierų koncentratai ir ekstraktai buvo sėkmingai panaudoti karštai rūkytų kumpių ir dešrų gamyboje. Svarbu, jog tokių produktų gamyboje būtų naudojami nitratredukcinėmis savybėmis pasižymintys mikroorganizmai ir būtų užtikrintas ilgesnis gamybos procesas (pvz., fermentinės, šaltai rūkytos dešros), kad natūraliai daržovėse esantys nitratai būtų redukuojami iki chemiškai aktyvaus nitrito, kuris atliktų konservanto ir mėsos spalvos stabilizatoriaus funkciją. Šis darbas skirtas sukurti priemonėms, kurios galėtų būti naudojamos raudoniesiems mėsos pigmentams stabilizuoti mėsos produktuose.

Darbo tikslas – sukurti pigmentų įkapsuliavimo ir stabilizavimo priemones, jas charakterizuoti ir ištirti jų pritaikymo mėsos gaminiuose galimybę.

Darbo tikslui pasiekti suformuluoti šie **uždaviniai**:

1. Sukurti stabilią dvigubąją V-A-V (vanduo-aliejus-vanduo) emulsiją, modeliuojant A-V (aliejus-vanduo) tarpfazio sudėtį.
2. Ištirti dvigubųjų V-A-V emulsijų su įkapsuliuotomis burokėlių sultimis stabilizavimo mechanizmą, įvertinant osmosinio slėgio, V-A (vanduo-aliejus) bei A-V (aliejus-vanduo) tarpfazių sudėties ir gamybos būdo įtaką.
3. Panaudoti dvigubąją V-A-V emulsiją kaip daugiafunkcę priemonę riebalų sudėčiai gerinti ir mėsos spalvai stabilizuoti mėsos sistemose.
4. Ištirti nitratų iš liofilizuotų daržovių įtaką mėsos sistemų pokyčiams fermentacijos metu ir įvertinti jų įtaką mėsos saugai.
5. Ištirti nitratų iš liofilizuotų daržovių įtaką skirtingų mioglobino formų susidarymui ir kitimui mėsos sistemose fermentacijos metu, siekiant išlaikyti raudoną mėsos spalvą.
6. Įvertinti galimybę pakeisti sintetinį nitratą ir nitritą nitratais iš liofilizuotų daržovių šaltai rūkytose dešrose siekiant pagerinti jų kokybę ir saugą.

Darbo mokslinis naujumas. Taikant dviejų pakopų homogenizavimą ir modifikuotą membraninį emulsavimą pagamintos dideliu įkapsuliavimo efektyvumu (>98 %), terminiu bei gravitaciniu stabilumu pasižyminčios dvigubosios V-A-V emulsijos su įkapsuliuotomis burokėlių sultimis. Nustatyta, kad dvigubųjų V-A-V emulsijų su įkapsuliuotomis burokėlių sultimis vidinėje vandens fazėje stabilumą nulėmė laikymo metu padidėjusi klampa dėl osmosinio slėgio skirtumų vidinėje ir išorinėje vandens fazėse. Dvigubosiose emulsijose įkapsuliuotas raudonos spalvos pigmentas betaninas buvo apsaugotas terminio apdorojimo metu ir todėl V-A-V emulsijos mėsos sistemose buvo panaudotos ne tik riebalų sudėčiai gerinti, bet ir raudonai mėsos spalvai stabilizuoti.

Taip pat nustatyta nitratų iš liofilizuotų salierų, porų ir pastarnokų įtaka skirtingų mioglobino formų susidarymui ir kitimui mėsos sistemose jų fermentacijos metu.

Darbo praktinė vertė. Sukurtos dvi inovatyvios pigmentų įkapsuliavimo ir stabilizavimo priemonės, kuriomis mėsos gaminiuose gali būti pakeičiami sintetiniai maisto dažikliai ir spalvos stabilizatoriai. Pirmoji priemonė – dvigubosios V-A-V emulsijos su įkapsuliuotomis burokėlių sultimis. Jos buvo panaudotos mėsos sistemose siekiant pakeisti gyvūninius riebalus augaliniais ir suteikti mėsai raudoną spalvą. Sukurtos dvigubosios emulsijos pasižymėjo dideliu burokėlių sulčių įkapsuliavimo efektyvumu ir dideliu atsparumu aplinkos ir technologinių veiksnių poveikiui.

Antroji priemonė – liofilizuoti salierai, porai ir pastarnokai, kurie mėsos sistemose gali būti naudojami kaip netiesioginis nitrato šaltinis. Naudojant liofilizuotų daržovių priedus buvo gautos tokios pat šaltai rūkytų dešrų savybės, kaip ir su pridėtiniu nitritu. Parengta prototipinė šaltai rūkytų dešrų su liofilizuotais salierais technologija, leidžianti pakeisti nitritą išlaikant tas pačias gaminio technologines savybes, saugą ir kokybę.

Ginamieji disertacijos teiginiai

- Pagrindinis veiksnys, lėmęs dvigubųjų emulsijų su įkapsuliuotomis burokėlių sultimis stabilumą, yra osmosinio slėgio skirtumų tarpfazyje sukeltas klampos padidėjimas.
- Dviguboji emulsija su įkapsuliuotomis burokėlių sultimis gali būti naudojama mėsos gaminiuose siekiant pakeisti gyvūninius riebalus augaliniais ir išsaugoti raudoną mėsos spalvą.
- Nitratai iš liofilizuotų salierų gali būti panaudojami vietoje pridėtinio nitrito ar nitrato išlaikant tas pačias šaltai rūkytų dešrų technologines ir funkcines savybes.

IŠVADOS

1. Dvigubųjų V-A-V emulsijų stabilumas priklausė nuo A-V tarpfaziui stabilizuoti naudoto stambiamolekulio junginio rūšies, koncentracijos ir laikymo trukmės. Tiek išrūgų baltymų izoliatu (IBI), tiek karboksimetilceliulioze (KMC) stabilizuotos emulsijos išliko stabilios ~10 parų, tačiau ilgėjant laikymo trukmei prasidėjo destabilizacijos procesai. Nors vidutinis aliejaus lašelių dydis (d_{32} ~ 30 μm) panašus abiejų tipų emulsijose, statistiškai reikšmingai ($p < 0,05$) didesnis stabilumas nustatytas emulsijose, kurių A-V tarpfazyje buvo KMC. Nustatytos didesnės klampos konstantos emulsijose su KMC (2,095–4,381 $Pa \cdot s^n$) parodė, kad modifikuotas gamtinis

emulsiklis KMC padidino tolydinės fazės klampą, dėl to aliejaus lašelių judėjimas sulėtėjo ir taip buvo išvengta potencialių jų sąveikų.

2. Nustatyta, kad dviguboji V-A-V emulsija su įkapsuliuotomis burokėlių sultimis, kuriai gaminti naudota 6 % poliglicerolio poliricinoleato (PGPR) ir 0,5 % IBI, pasižymėjo dideliu gravitaciniu bei terminiu stabilumu ir įkapsuliavimo efektyvumu (100 %). Dėl osmosinio slėgio skirtumo emulsijų klampa laikymo metu padidėjo nuo 0,03 iki 2,9 Pa·s. Modifikuoto membraninio emulsavimo metu aliejaus lašelių dydis sumažėjo nuo ~32 iki 21,19–24,26 μm, priklausomai nuo naudoto slėgio. Didinant praleidimų skaičių aliejaus lašelių dydis mažėjo iki trečiojo praleidimo, toliau didinant praleidimų skaičių aliejaus lašeliai didėjo, o įkapsuliavimo efektyvumas ir emulsijų stabilumas mažėjo. Didinant burokėlių sulčių koncentraciją vidinėje vandens fazėje nuo 0 iki 2 % paviršiaus įtempis sumažėjo nuo 22,2 iki 12,7 mN/m.

3. Dvigubosios emulsijos, mėsos sistemose panaudotos vietoje gyvūninių riebalų, pasižymėjo geromis vandens ir riebalų rišlumo savybėmis ir sumažino mėsos sistemų kietumą. Mėsos sistemose, kuriose buvo naudojamos dvigubosios emulsijos su įkapsuliuotomis burokėlių sultimis, raudona mėsos sistemų spalva (a*) buvo statistiškai reikšmingai ($p < 0,05$) ryškesnė (8,39–10,12) nei kontrolinių sistemų (2,19–2,4).

4. Nitratų redukcijos procesas fermentuojamose mėsos sistemose vyko lėtai ir nepriklausė nuo naudoto nitrato kilmės (pridėtas ar iš liofilizuotų daržovių). Mėsos sistemose su daržovių priedu nustatytos mažesnės pH vertės (4,77–5,02) ir didesnis susidariusios pieno rūgšties (0,9–1 g/100 g) bei laisvosios glutamo rūgšties (0,05–0,09 g/100 g) kiekis, tačiau toksikologiniu požiūriu pavojingiausio biogeninio amino – histamino – kiekis buvo žemiau jo aptikimo ribos (<5 mg/kg) visose mėsos sistemose. Koliforminių mikroorganizmų kiekis fermentacijai baigiantis mažėjo tik mėsos sistemose su daržovėmis.

5. Nustatyta, kad nitratų iš liofilizuotų daržovių panaudojimas mėsos sistemose jų fermentacijos metu lėmė mažesnį rudos spalvos metmioglobino kiekį (56,55–59,09 %), palyginti su sistemomis, kurių sudėtyje buvo pridėtas nitratas (61,66–65,32 %). Nitrozomioglobino kiekis statistiškai reikšmingai padidėjo pirmąją fermentacijos parą, t. y. tuo metu, kai vyko staigus nitratredukcinėmis savybėmis pasižyminčių stafilokokų augimas ir didžiausia nitratų redukcija, tačiau vėliau dėl galimai dalinės denatūracijos ar oksidacijos nitrozomioglobino kiekis sumažėjo iki 30–40 mg/kg ir

nebepriklausė nei nuo fermentacijai naudoto *Staphylococcus* rūšies, nei nuo nitrato kilmės (pridėtas ar iš liofilizuotų daržovių).

6. Nitratą ir nitritą pakeitus nitratais iš liofilizuotų daržovių šaltai rūkytose dešrose gautos mažesnės pH vertės (5,04–5,1). Liekamasis nitrato kiekis dešrose priklausė nuo nitrato kilmės ir nuo startinių kultūrų (*S. xylosus* ar *S. xylosus* kartu su *P. pentosaceus*), naudotų dešrų gamyboje. Bendrasis biogeninių aminų kiekis (<150 mg/kg) buvo mažesnis dešrose, fermentuotose startinių kultūrų mišiniu. Nors raudonos spalvos mioglobino formų kiekis ir kitimas buvo analogiškas visose dešrose, nepriklausomai nuo nitrato kilmės (pridėtas ar iš liofilizuotų salierų), tačiau mažesnes rausvumo (a*) vertes (8,51–9,32) dešrose su liofilizuotais salierais lėmė žalios spalvos pigmentai, esantys daržovėje. Juslinio vertinimo metu nustatyta, kad liofilizuotų salierų priedas neturėjo neigiamos įtakos dešrų savybėms, jos visos buvo įvertintos kaip priimtinos.

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Išleido Kauno technologijos universitetas, K. Donelaičio g. 73, 44249 Kaunas
Spausdino leidyklos „Technologija“ spaustuvė, Studentų g. 54, 51424 Kaunas