

Application of Different Sterilising Modes and the Effects on Processed Cheese Quality

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Abstract

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The aim of the present work is to evaluate the impacts of four different sterilising modes (110°C 100 min, 115°C 32 min, 120°C 10 min, and 125°C 3.2 min – with a constant lethal effect on microorganisms) on some chemical (pH, total and bio-available lysine, and ammonia content), microbiological, and sensory (shade and acceptability) properties of processed cheese depending on the lactose additions (0.0–2.0% w/w). All sterilising modes used were sufficient to inactivate the microorganism groups observed (total number of microorganisms, colony forming units of yeasts and/or moulds, number of spore-forming microorganisms). The falling sterilisation temperature kept for an adequately prolonged period of time caused darkening of the processed cheese and a decline of their acceptability. Consequently, greater losses of lysine and ammonia content increase occurred when the sterilisation temperature decreased. Compared to non-sterilised products, the smallest changes were detected in the cheese treated with temperatures 125°C for 3.2 min, and 120°C for 10 minutes. The decrease of the processed cheese quality was more apparent with the growing lactose concentration.

Keywords: processed cheese; sterilising modes; lysine; bio-available lysine; ammonia; sensory properties

Processed cheese is normally produced by comminuting, blending, and melting one or more natural cheeses and adding optional ingredients (dairy ingredients, vegetables, meats, stabilisers, colours, preservatives) into the smooth homogeneous blend with the aid of heat, mechanical shear, and emulsifying salts (GUINEE *et al.* 2004). The inclusion of skim milk powder or whey powder in the processed cheese (mainly for economic reasons) could influence the nutritional and sensory qual-

ity of the processed cheese, undoubtedly because of the higher amount of lactose. The presence of this disaccharide increases the susceptibility to browning reactions associated with nutritional consequences (BLEY *et al.* 1984).

Processed cheese, as a typical non-acid food, has a durability lasting several months. For prolonging the shelf life, it is possible to use sterilisation in hermetically closed containers. Sterilisation temperature usually ranges within the interval of

115°C to 125°C with the exposure time from 5 min to 30 min (MAFART *et al.* 2001). Such products can be used e.g. for Combat Rations of the armies of the NATO countries (e.g. USA, Germany, and the Czech Republic) and also for the Integrated Rescue System. Sterilisation temperature 117°C for 20 min is usually used for this purpose in the Czech Republic (BUŇKA *et al.* 2004).

As far as sterilisation is concerned, the combination of temperature and time is fundamental for the determination of the lethal effect on microorganisms (GAILLARD *et al.* 2005). Not only microorganisms but, of course, also the chemical substances present (e.g. proteins, lactose, fat or vitamins) are influenced by sterilisation. Among the most important reactions taking place during sterilisation definitely belongs the Maillard's reaction. It is a reaction of carbonyl compounds (mainly reducing saccharides) with amino compounds (usually amino acids) (FRIEDMAN 1996). Therefore, an increase in Maillard's reaction intensity can be assumed when using ingredients containing higher amounts of lactose (the already mentioned skim milk powder or whey powder) (BLEY *et al.* 1984; RUFÍAN-HENARES *et al.* 2006). Other interactions caused by thermo-sterilisation can include e.g. (i) reaction of bound asparagine and glutamine with lysine (free and/or bound) during which ammonia is released and an isopeptide bond (which cannot be broken down by enzymes in the human digestive tract) is formed; (ii) elimination of hydrogen sulphide from bound cysteine; (iii) Strecker's degradation of amino acids (oxidative decarboxylation) resulting in the formation of ammonia, carbon dioxide, and aldehyde; (iv) oxidation of lipids (FRIEDMAN 1996; GAUCHE-ROUN *et al.* 1999; ADAMIEC *et al.* 2001).

The course of the above-mentioned reactions influences both the sensory quality and the nutritive value of the processed cheese. Amino acid losses can occur during sterilisation (BUŇKA *et al.* 2004). However, these do not have to imply the direct destruction of amino acids. Also, the formation of bonds that do not cleave easily in the human digestive tract can be assumed. The substances bound in this way become less available in the human body. Lysine, for example, undergoes these reactions, in particular in the presence of reducing saccharides (e.g. lactose) (FERRER *et al.* 2000). However, the common analysis of amino acids includes this problematically available lysine in the total lysine content in the sample, which

causes overestimation of its content from the nutritional point of view (TORBATIJENAD *et al.* 2005). There are some derivatisation methods that enable to determine only bio-available lysine in the amino acid analysis as supported, for example, by the work of RUFÍAN-HENARES *et al.* (2006). Lysine is an essential amino acid usually used as an indicator of the potential biological value of the food protein. The knowledge of the amounts of unmodified (bio-available) and modified (blocked) lysine facilitates the evaluation of the effects of treatment on protein quality (ALBALÁ-HURTADO *et al.* 1997).

The lethal effect of the sterilisation temperature and time is usually expressed numerically by the *F*-value. *F*-value is defined in minutes as the time of the heat treatment at a constant reference temperature (generally 121.1°C) or as any equivalent heat treatment which would cause the same destruction ratio (SKJÖLDEBRAND & OHLSSON 1993; MOHAMED 2003; GAILLARD *et al.* 2005). According to BYLUND (1995), it is recommended to use rather a higher sterilisation temperature kept for adequately shorter periods of time (to ensure high nutritional quality of the sterilised products). This rule is followed, for example, during the production of UHT milk and UHT cream, whose shelf life can be extended by UHT treatment (approximately 130–145°C for a few seconds) (CONTARINI *et al.* 1997). However, it appears that no published study exists on the application of the above mentioned rule to other dairy products, e.g. processed cheese.

The scope of this study is to analyse the effects of different modes of sterilisation (with a constant lethal effect on microorganisms) on the quality of processed cheese with various lactose additions.

MATERIALS AND METHODS

Processed cheese (38% w/w dry matter and 50% w/w fat in dry matter) was prepared with the addition of lactose (0.0, 0.5, 1.0, 1.5, and 2.0% w/w). The ingredients used for the processed cheese preparation were Dutch-type natural cheese (Eidamska cihla; 30% w/w fat in dry matter and 50% w/w dry matter), butter (82% w/w fat and 84% w/w dry matter), water, and emulsifying agents (JOHA, Benckiser-Knapsack, Ladenburg, Germany). The laboratory equipment used for the processed cheese production is described in the works of BUŇKA

et al. (2007) and LEE *et al.* (2004). The addition of lactose was compensated for by changing the amounts of butter and water to achieve a constant dry matter and fat contents. The melting temperature was 90°C for 1 min and the total processing time was approximately 10 minutes. After melting, the cheese was filled into laminated aluminium containers with seal lids. A small part of each batch was cooled to $6 \pm 2^\circ\text{C}$ (non-sterilised processed cheese) and other four parts were sterilised (autoclave SVV2AKV, Pacovské strojírný, Pacov, Slovak Republic) (sterilised processed cheese). For the sterilisation, four combinations of temperature and time (sterilising modes) were applied: 110°C for 100 min (A), 115°C for 32 min (B), 120°C for 10 min (C), and 125°C for 3.2 min (D). All modes possessed a constant lethal effect on microorganisms ($F = 7.78$); calculated according to SKJÖLDEBRAND and OHLSSON (1993). Thus, 25 types of samples were obtained: four groups of the sterilised cheese differing in the sterilising mode (A–D), and a group of non-sterilised products; each group contained 5 samples with the lactose concentration ranging from 0.0–2.0% w/w. Each sample type was prepared three times and all analyses were performed after 1 month-storage at $6 \pm 2^\circ\text{C}$.

The determinations of pH, dry matter, ash, fat, and crude protein contents were carried out for the sterilised and non-sterilised processed cheeses characterisation. The pH value was measured with a pH-meter GRYF209S with a combined glass electrode (GryfHB, Havlíčkův Brod, Czech Republic). Dry matter content was assessed according to ISO 5534:2004 – Cheese and processed cheese – Determination of the total solids content, fat content acidobutyrometrically, and crude protein content according to the Kjeldahl method (using multiplying factor 6.38). Each sample was analysed at least six times.

Microbiological analysis of the sterilised and non-sterilised samples was performed by specifying the total number of microorganisms (ISO 4833:2003 – Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of microorganisms – Colony-count technique at 30°C), the colony forming units of yeasts and/or moulds (ISO 6611:2004 – Milk and milk products – Enumeration of colony-forming units of yeasts and/or moulds – Colony-count techniques at 25°C), and the numbers of aerobic and anaerobic spore-forming microorganisms. Also, the sterilised processed

cheese was treated with thermostat test (incubation of the samples at $37 \pm 1^\circ\text{C}$ for 10 days followed by the determination of the total number of microorganisms). All media used for cultivation were obtained from HiMedia (Bombay, India).

For the total lysine assessment, 100 mg samples were weighed into screw-capped test tubes with teflon caps. Fifteen milliliters of 6 mol/l HCl were added to the tubes. The tubes were subsequently purged with argon and closed. Then the tubes were placed into a thermoblock (Labicom, Olomouc, Czech Republic) and hydrolysed at 110°C for 23 hours. After hydrolysis, HCl was evaporated and the ropy residue was diluted with the loading buffer (sodium citrate buffer, pH 2.2) in a volumetric flask. All samples were analysed at least twelve times on different days. The released lysine was determined by ion-exchange chromatography with sodium citrate buffers and ninhydrine detection (Amino Acid Analyser AAA400, Ingos, Prague, Czech Republic). All reagents for the amino acids determination were obtained from Ingos (Prague, Czech Republic). Lysine concentration was expressed as $\text{g} \times 16 \text{ g/N}$.

Bio-available lysine was determined according to the Carpenter method modified by BOOTH (1971). Briefly, 100 mg samples of the processed cheese were weighed and transferred to the bottom of screw-capped test tubes with teflon caps. One milliliter of 8% (w/v) NaHCO_3 (LachNer, Neratovice, Czech Republic) was added per tube, which were left to stand for 10 minutes. Then, 1.5 ml of 3% (v/v) FDNB-solution (1-fluoro-2,4-dinitrobenzene in ethanol; obtained from Sigma Aldrich, St. Louis, USA) was added into each tube and the tubes were shaken for two hours. Ethanol was subsequently evaporated in a boiling water bath. When the mixture cooled down, 15 ml of 6 mol/l HCl were put into the tubes; the following hydrolysis and subsequent analysis of lysine content were the same as in the above-mentioned protocol. All samples were processed at least twice on different days. The bio-available lysine amount (rel. %) was calculated as the ratio of the difference between the total lysine content and lysine content after the reaction with FDNB-solution to the total lysine content.

Ammonia content was determined according to the Conway method as described by BUŇKA *et al.* (2004).

The samples of both the sterilised and non-sterilised processed cheeses were assessed by a panel

of 16 employees from the Department of Food Engineering, Faculty of Technology, Tomáš Baťa University, Zlin, Czech Republic, trained according to the ISO 8586-1:1993 – Sensory analysis – General guidance for the selection, training and monitoring of assessors – Part 1: Selected assessors. The cheese acceptability was evaluated using a seven-point hedonic scale (1 – excellent, 4 – good, 7 – unacceptable). The effects of different modes of sterilisation and lactose additions on the shade of the processed cheeses were analysed by 10 ranking tests according to the ISO 8587:1993 – Sensory analysis – Ranking test. The assessors were in charge of ranking 5 samples according to their shades (1 – the brightest sample, 5 – the darkest sample). At first, the samples subjected to different heat treatments were ranked (non-sterilised and four modes of sterilisation) separately for a constant lactose addition (5 tests in total). Then, the samples with different lactose concentrations (0–2.0% w/w) were ranked, separately for the same heat treatment (non-sterilised and four modes of sterilisation – 5 tests in total). The values of total ranking are presented. In this case, the higher the value of the total ranking, the darker the shade of the sample.

The results of basic chemical and microbiological analyses and sensory analysis using the hedonic scale were statistically processed by the use of the Kruskal-Wallis and the Wilcoxon tests. The data from the ranking test were subjected to the Friedman test. Unistat version 5.5 was used for statistical evaluation.

RESULTS AND DISCUSSION

The microbiological analysis of non-sterilised cheese showed the following results: total number of microorganisms (aerobic and facultatively anaerobic bacteria) 4.1×10^4 CFU/g, aerobic spore-forming microorganisms 2.9×10^4 CFU/g, anaerobic spore-forming microorganisms 2.1×10^4 CFU/g, and moulds and/or yeasts 7.2×10^3 CFU/g. All of the sterilising modes tested ($F = 7.78$) ensured inactivation of the microflora observed; no microorganisms were found in the sterilised processed cheese. The F -value used is equivalent to the commercially applied treatment of 117°C for 20 min (BUŇKA *et al.* 2004).

The basic chemical analysis did not reveal any effect of either lactose concentration or the sterilising mode ($P \geq 0.05$) on dry matter content (range 37.60–38.12% w/w) and fat content (18.7–19.1% w/w). This is important to ensure the comparability of the samples (LEE *et al.* 2004; DIMITRELI & THOMAREIS 2007). Crude protein amount was not affected by sterilisation ($P \geq 0.05$), in contrast to the rising lactose concentration which caused the crude protein content decline ($P < 0.05$). The decline of the crude protein content (range 11.77 to 13.15 % w/w) with increasing lactose addition was necessary for maintaining constant dry matter and fat contents.

The values of pH in the non-sterilised processed cheese ranged within the interval 5.99–6.08. Sterilisation caused a significant decrease of pH by 0.1–0.2 ($P < 0.05$). The possible reasons for

Table 1. Total lysine content (in g \times 16 g/N) in sterilised and non-sterilised processed cheese with lactose addition 0.0–2.0% w/w*

Lactose content (% w/w)	Temperature and time of sterilisation				
	None	110°C 100 min (A)	115°C 32 min (B)	120°C 10 min (C)	125°C 3.2 min (C)
0.0	7.84 \pm 0.23 ^a A	6.90 \pm 0.14 ^a B	7.27 \pm 0.09 ^a C	7.23 \pm 0.14 ^{a,b} C	7.56 \pm 0.07 ^a A
0.5	7.65 \pm 0.05 ^a A	6.84 \pm 0.10 ^a B	7.34 \pm 0.22 ^a C	7.42 \pm 0.07 ^a C	7.51 \pm 0.16 ^a A,C
1.0	7.81 \pm 0.13 ^a A	6.98 \pm 0.27 ^a B,C	6.86 \pm 0.08 ^b B	7.27 \pm 0.13 ^{a,b} C	7.57 \pm 0.13 ^a A
1.5	7.63 \pm 0.12 ^a A	6.72 \pm 0.10 ^a B	7.02 \pm 0.03 ^c C	7.45 \pm 0.09 ^a A	7.55 \pm 0.04 ^a A
2.0	7.61 \pm 0.14 ^a A	6.50 \pm 0.06 ^b B	6.77 \pm 0.15 ^b C	7.25 \pm 0.06 ^b D	7.38 \pm 0.25 ^a A,D

*Lysine content is presented by a mean \pm SD ($n = 12$). Means within a column (effect of lactose content) followed by no common superscript letter differ ($P < 0.05$). Means within a row (effect of temperature and time of sterilisation) followed by no common capital letter differ ($P < 0.05$)

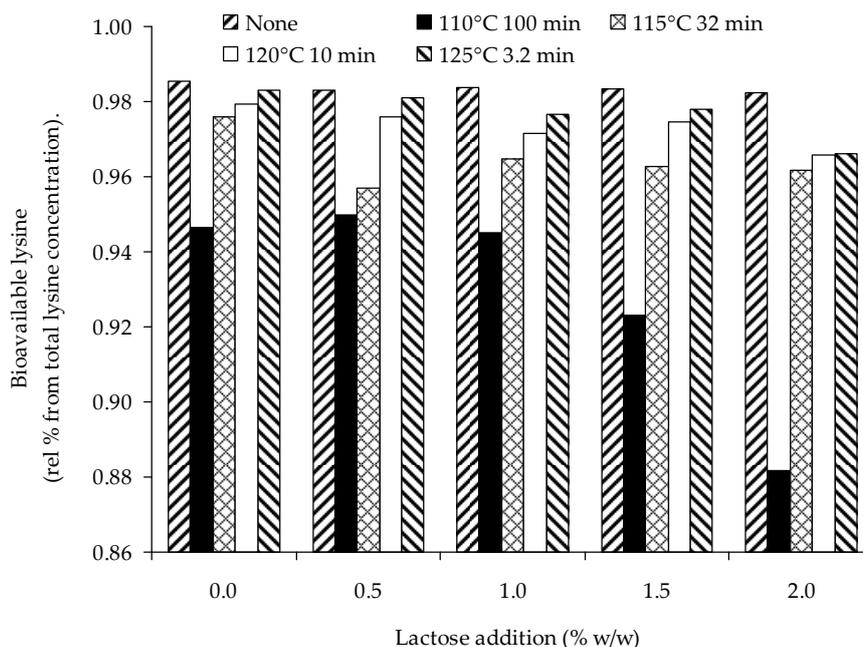


Figure 1. The effect of temperature and time of sterilisation on relative amount of bio-available lysine (in comparison with total lysine content = 100 %) in non-sterilised and sterilised processed cheese with lactose addition 0.0–2.0% w/w

this decrease can be found in the hydrolysis of the polyphosphate emulsifying agent (MOLINS 1991). Lactose addition did not affect pH of the processed cheese ($P \geq 0.05$).

In Table 1, the results of total lysine content analysis are shown. It can be stated that all cheeses sterilised with modes A and B and most samples treated with mode C showed significantly lower lysine contents ($P < 0.05$) as compared to the non-sterilised products (the average losses reached approximately 10% for modes A and B and 5% for C). All of the products treated with the mode D did not significantly differ from the non-sterilised processed cheese in lysine content ($P \geq 0.05$). Sterilising mode D caused average losses of 3% in comparison with the non-sterilised processed cheese. The majority of cheeses treated with modes A and B showed lower concentrations of lysine than the products sterilised with modes C and D ($P < 0.05$). On the other hand, in most cases, no significant differences in lysine content occurred between the products treated with modes C and D ($P \geq 0.05$). No definite dependence was found of the lysine amount on increasing lactose addition (Table 1).

Also, the content of bio-available lysine was determined; the results are demonstrated in Figure 1. In the non-sterilised processed cheese, the proportion of bio-available lysine was more than

98% of its total content in the product, regardless of the concentration of lactose. In the majority of the products tested, the sterilisation heat caused a decrease in bio-available lysine content. The most noticeable decrease in bio-available lysine amount (in comparison with non-sterilised products) was observed in the products treated with sterilisation mode A. While with the lactose addition of up to 1.0% w/w the loss of bio-available lysine ranged from 30 to 4% (in comparison with the non-sterilised products), at the concentrations of 1.5% and 2.0% the decrease reached 6% and 10%, respectively. The majority of the products treated with the other sterilisation modes (B–D) showed a loss of bio-available lysine below 2%. The additions of lactose to this sterilised processed cheese (B–D) caused only slight losses of bio-available lysine in comparison with the products without the lactose addition. Thus, these results confirm the statement of TORBATINEJAD *et al.* (2005) about the importance of evaluating not only the total content of amino acids, but also their bio-availability for the human body in order to obtain objective information about the amino acids content and its potential for the human nutrition.

The effect of the sterilising mode on the ammonia content in the processed cheese without lactose addition is presented in Figure 2. The products treated with all sterilising modes revealed a sta-

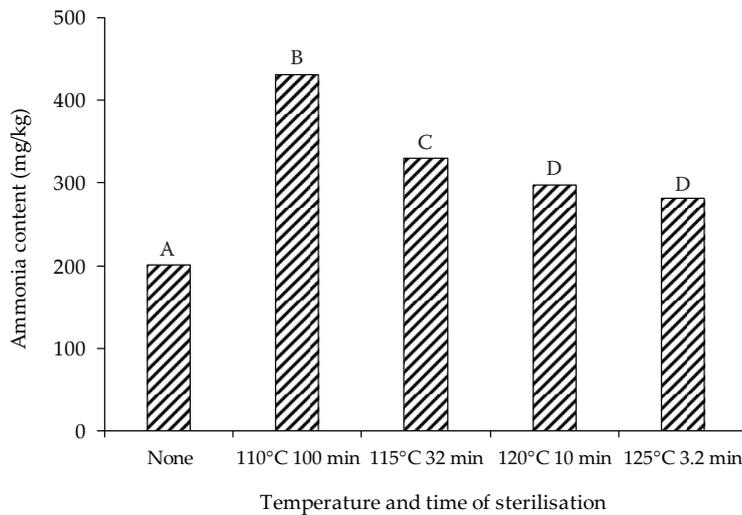


Figure 2. The effect of temperature and time of sterilisation on ammonia content in non-sterilised and sterilised processed cheese without lactose addition. The ammonia contents with no common capital letter differ ($P \leq 0.05$)

tistically significant growth of ammonia content ($P < 0.05$) in comparison with the non-sterilised cheese. Decreasing sterilising temperature and an adequately prolonged time of sterilisation caused a significant increase of ammonia content ($P < 0.05$). The differences in the ammonia amount were not significant ($P \geq 0.05$) only between the products treated with modes C and D. An elevated ammonia content generally corresponds with the reduced lysine amount observed. The reason could be found in Maillard's reaction or other interactions (e.g. Strecker's degradation of amino acids) (FRIEDMAN

1996; GAUCHERON *et al.* 1999; ADAMIEC *et al.* 2001). With the rising lactose concentration, no increase in the ammonia amount was observed ($P \geq 0.05$; data not shown).

The evaluation of the effects of different sterilisation temperatures and times on the shade of the processed cheese (evaluated by means of the ranking test) at the individual concentrations of lactose tested (0–2.0 % w/w) is illustrated in Figure 3. The shade of the processed cheese treated with mode D did not differ significantly from the shade of the non-sterilised samples ($P \geq 0.05$),

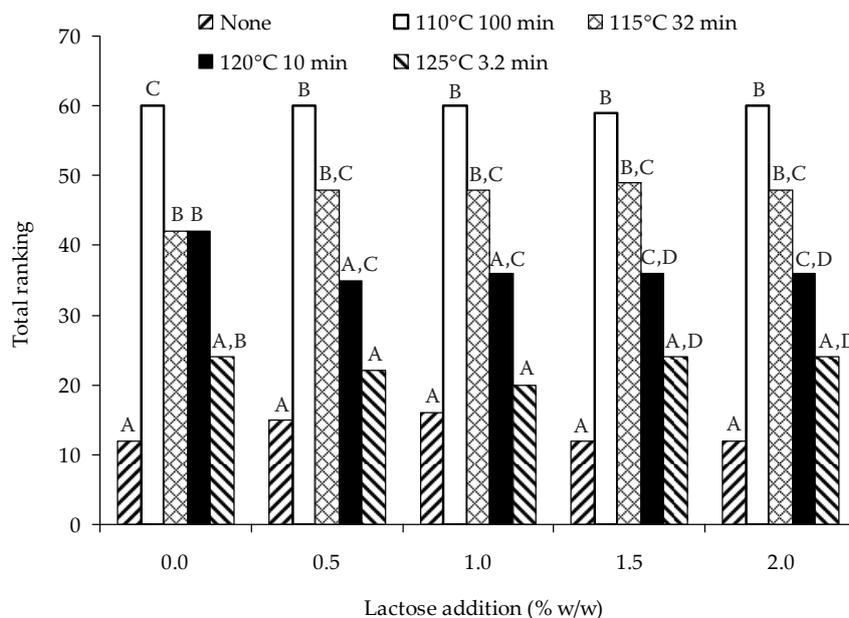


Figure 3. The effect of temperature and time of sterilisation on the shade of processed cheese with a different lactose addition evaluated by means of sensory analysis. Total ranking within the lactose content followed by no common capital letter differ ($P < 0.05$); samples with different lactose addition were evaluated separately

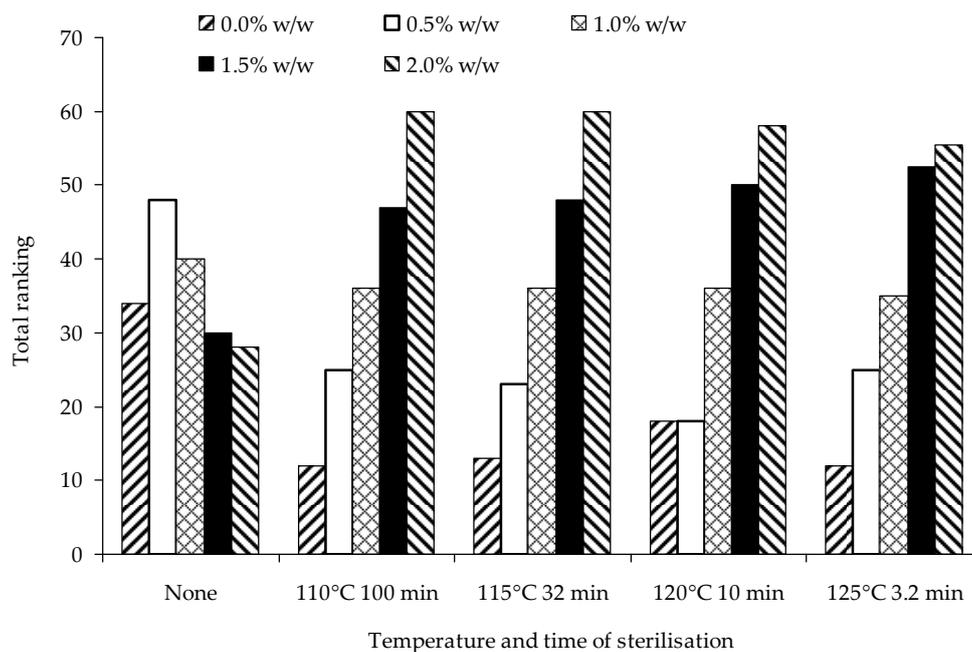


Figure 4. The effect of lactose addition on the shade of processed cheese with different temperature and time of sterilisation evaluated by means of sensory analysis. Total ranking within the temperature and time of sterilisation followed by no common capital letter differ ($P < 0.05$); samples with different temperature and time of sterilisation were evaluated separately

regardless of the lactose concentration. On the other hand, the products sterilised according to A and B had a significantly darker shade ($P < 0.05$) than the non-sterilised processed cheese, and in the case of all lactose additions (0.5–2.0% w/w) they were also darker ($P < 0.05$) than the products treated with mode D. The effect of sterilising mode C on the shade of the processed cheese was strongly dependent on the concentration of lactose. In the processed cheese with a lower concentration of lactose ($\leq 1.0\%$ w/w), the shade of the samples sterilised using mode C was similar to that of the non-sterilised products. With higher lactose concentrations tested ($> 1.0\%$ w/w), the processed cheeses treated with sterilisation mode C were significantly darker ($P < 0.05$) than the non-sterilised samples. The cheeses treated with mode C had a similar shade in comparison with the products sterilised using modes B and D ($P \geq 0.05$), regardless of the lactose concentration (0–2.0% w/w).

The evaluation of the effect of the lactose addition on the shade of the processed cheese (evaluated by means of the ranking test), in the individual combinations of the sterilisation temperature and exposure times (A–D), is presented in Figure 4. In the non-sterilised processed cheese, the concen-

tration of lactose (0–2.0% w/w) did not have any significant influence on the shade of the samples ($P \geq 0.05$). In the case of all sterilisation modes (A–D) realised, it was found out that the rising concentration of lactose in the samples resulted in the formation of a darker shade ($P < 0.05$). In all sterilisation modes tested (A–D), the products with the lactose addition $> 1.0\%$ w/w were significantly darker ($P < 0.05$) than those with the concentration of lactose $< 1.0\%$ w/w. Also, the processed cheese with the lactose addition 1.0% w/w was, in the majority of the sterilisation modes (A, B, and D), significantly darker ($P < 0.05$) than the products without lactose addition.

In addition, the acceptability of the non-sterilised and sterilised processed cheeses was assessed. All of the non-sterilised samples were evaluated as very good irrespective of the lactose concentration ($P \geq 0.05$). The cheese sterilised with mode D did not differ from the non-sterilised products in the majority of lactose concentrations tested ($P \geq 0.05$) and this cheese showed a good acceptability. In the case of the sterilising modes A and B, the acceptability of the processed cheese deteriorated significantly ($P < 0.05$) with the growing lactose concentration. Actually, the products with the highest lactose additions were evaluated as very poor

or unacceptable. Compared to the non-sterilised cheese and the products treated with the mode D, the cheeses sterilised with modes A and B showed a significant decrease in acceptability. The major part of the products treated with sterilising mode C with lactose addition $\leq 1\%$ w/w did not differ in acceptability ($P \geq 0.05$) from the cheese sterilised with the mode D and were evaluated as good in most cases. Nevertheless, with higher lactose concentrations the acceptability of this products deteriorated significantly ($P < 0.05$), as compared to the non-sterilised processed cheese.

As for the mildness/severity of the individual sterilisation modes and the intensity of their effects on the lysine amount and organoleptic characteristics of the processed cheese, the conclusions reached are very similar. Relatively mild heat treatments were the temperatures of 125°C and 120°C kept for 3.2 min and 10 min, respectively. On the other hand, the more severe treatments were the versions of 115°C and 110°C for 32 min and 100 min, respectively. These results are in agreement with the findings of BYLUND (1995) who stated that the lower the temperature of sterilisation (and adequately prolonged periods of the exposure time in order to maintain a constant lethal effect), the more intensive decline of the nutritive value and sensory quality take place in general. A similar conclusion is also in the work of CONTARINI *et al.* (1997).

These findings imply the recommendation not to include larger amounts of reducing saccharides into the mixture (raw materials) intended for the production of sterilised processed cheese. According to the results of this work, only the concentrations of lactose of up to 1.0% w/w in combination with higher sterilisation temperatures (and adequately with a shorter exposure time) are suitable for the sterilised processed cheese production since these modes provide good quality products.

CONCLUSION

The impact of different sterilising modes (110°C 100 min, 115°C 32 min, 120°C 10 min and 125°C 3.2 min) and different lactose additions (0.0, 0.5, 1.0, 1.5, and 2.0% w/w) on the quality of processed cheese were analysed. Generally, sterilisation caused the losses of total and bio-available lysine, ammonia content increase, formation of darker shade, and decrease of the processed cheese ac-

ceptability. These processes were more intense in the case of decreased temperature and prolonged periods of the exposure time. A further quality decrease was evoked by higher lactose concentrations (especially above 1% w/w). The sterilising modes of 125°C for 3.2 min and 120°C for 10 min can be recommended for practice, since the products obtained using these modes possessed characteristics resembling those of the non-sterilised processed cheese.

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