The effect of long-term storage on the quality of sterilized processed cheese

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Revised: 13 August 2014 / Accepted: 17 August 2014 / Published online: 30 August 2014
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Abstract The aim of this work is to evaluate the effect three different storage temperatures (6, 23 and 40 °C) on the sterilized processed cheese quality during 24-month storage. Sterilized processed cheese (SPC) is a product with extended shelf life (up to 2 years). The samples of SPC were subjected to basic chemical analyses, i.e. pH-values, dry matter, fat, crude protein and ammonia content, and microbiological analyses, i.e. total number of microorganisms, number of coliforms, colony forming units of yeasts and/or moulds and spore-forming microorganisms. Furthermore, amino acid content (ion-exchange chromatography), protein profile (SDS-PAGE) and fat globules size (image analysis of microscopic technique) were monitored and sensory analysis (scale test and pair comparative test) was implemented, too. Increasing storage temperature and length evoked decrease of total amino acid content and protein nutrition value, increase of ammonia amount, protein changes, enlargement of fat globule size and deterioration of sensory properties of SPC. All the changes grew expressive with increasing storage temperature and time.

Keywords Sterilized processed cheese · Temperature and length of storage · Amino acids · Proteins · Fat globules · Sensory properties

Introduction

Sterilized processed cheese (SPC) represents a special group of processed cheese whose durability is prolonged by thermosterilization to minimally 24 months and which can be used in common life when refrigerator device is not available (Buňka et al. 2004). Originally, SPC was designed to be used in so-called combat rations, e.g. in armies of USA, Germany or the Czech Republic (STANAG 2937) and also to ensure boarding of the Integrated Rescue System bodies. SPC is a product that can be world-widely used. In the area of Middle Europe, SPC could be stored under ambient temperature (approx. 20–25 °C). Recently, these products are sold to Africa and/or Asia where storing under higher temperatures (approx. above 30 °C) could be expected. Unfortunately, there is a lack of information about changing of quality of SPC in literature available.

Processed cheese is produced by blending cheese in the presence of emulsifying salts and other dairy and non-dairy ingredients followed by heating and continuous mixing to form a homogenous product. Since processed cheese is not sterile, thermosterilization could be used to prolong product durability. Primarily, thermosterilization is necessary to guarantee microbiological quality and enzyme stability of the product. Nevertheless, even sterilized food is not completely stable and its long-term storage is connected with significant physico-chemical development, especially at elevated temperatures (Gliguem and Birlouez-Aragon 2005). Reactions proceeding during the storage affect all the basic components of processed cheese – proteins, fat and lactose. Maillard reaction complex (MR) ranks among the most important reactions

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including both proteins (amino compounds) and carbonyl compounds (e.g. lactose). MR causes nutritional quality deterioration; primarily in consequence of essential amino acid degradation and digestibility reduction (Pizzoferrato et al. 1998). Brown pigments formation, namely darkening, and also consistency affection are regarded considerable phenomena accompanying MR in processed cheese and generally foodstuffs (Kristensen et al. 2001). The extent of non-enzymatic browning during storage advances due to rising amount of reducing saccharides, especially lactose, due to higher storage temperature and due to increasing origination of oxidizing lipids (Gaucher et al. 2008; Kristensen et al. 2001; Schär and Bosset 2002). Strecker degradation giving rise to ammonia – marker of this reaction, racemization and oxidative reaction leading to nonutilizable products creation (Adamiec et al. 2001) are considered the most common destructive reactions of amino acids. Other compounds participating in processed cheese storage reactions are lipids that undergo oxidation resulting in release of volatile carbonyl compounds causing off-flavours. Oxidation process is mostly related to higher storage temperature (Kristensen and Skibsted 1999).

Many studies dealing with the changes during storage of UHT milk have been published over the recent years. According to these papers, mainly proteolysis, protein cross-linking, colour changes and volatile components formation occur during the storage of UHT milk at both ambient and elevated temperatures (Al-Saadi and Deeth 2008; Enright et al. 1999; Valero et al. 2001). Similar reactions could be expected in SPC, especially at elevated temperatures. Besides the above mentioned chemical or physico-chemical reactions which can take place during the storage, enzymatic reaction can occur, too. Haki and Rakshit (2003) stated that some thermostable enzymes could remain active at elevated temperature such as 100–200 °C held a few seconds. Studies that have dealt with the effect of storage at higher temperature on solid dairy products are published very rarely.

The aim of this work is to evaluate the effect of three different storage temperatures (6, 23 and 40 °C) on the sterilized processed cheese quality, particularly on the amino acid content, fat globules size, protein profile and sensory properties, during 24-month storage. The results of our study could be used for the estimation of processed cheese quality changing under different conditions and contribute to improvement of product quality for consumers.

Materials and methods

All of the analyses were performed in half-year intervals, i.e. in months 0, 6, 12, 18 and 24 after production, except for amino acid content determination (months 0, 12 and 24) and SDS-PAGE analysis (month 24).

Samples of sterilized processed cheese

Storage experiment monitored SPC with 38 % w/w dry matter and 45 % w/w fat in dry matter, which was produced by Madeta, Inc., the Czech Republic. A mixture of a Dutch-type cheese with 55 % w/w dry matter and 45 % w/w fat in dry matter, butter, water, emulsifying salts (JOHA, Benckiser-Knapsack, Ladenburg, Germany) and whey powder (0.5 % w/w) was used for the processed cheese manufacturing.

Melting was accomplished at 92 °C using a Stephan TC/SK 400 batch-type industrial dairy plant equipment (Stephan Machinery, Hameln, Germany) and the product was packed into the laminated aluminium containers with seal lids. Processed cheese was sterilized in a Lubeca LW 5013 batch-type industrial autoclave (Lubeca Maschinenbau Scholz, Coesfeld, Germany) at 117 °C for 20 min. The products were cooled to 25 °C and divided into 3 parts after the sterilization. First part was stored for 2 years in a refrigerator at 6 ± 2 °C (SR), second part at ambient temperature (23 ± 2 °C, SA) and third part in a thermostat (40 ± 2 °C, ST). Processed cheese manufacturing was accomplished twice for statistical purposes.

Basic chemical analyses

The samples of SPC were characterized by determining their pH, dry matter, ash, fat and crude protein content. Values of pH were measured using a pH meter with glass electrode (GRYF 209S, Havlickuv Brod, the Czech Republic). Dry matter content was determined by gravimetric method according to the ISO Standard No. 5534 (2004). Ash content was detected after burning a sample in a muffle furnace at 550 °C for 5 h. Fat content was determined according to the van Gulik acid butyrometer method (Dimitreli and Thomareis 2007) and crude protein content was assessed according to the Kjeldahl method using factor 6.38 (Dimitreli and Thomareis 2007).

Microbiological analyses

Microbiological quality of SPC was controlled by assessment of the total number of microorganisms (CFU) according to the ISO Standard No. 4833 (2003), the number of coliforms according to the ISO Standard No. 4832 (2006), the colony forming units of yeasts and/or moulds according to the ISO Standard No. 6611 (2004) and spore-forming microorganisms according to Harrigan (1998). Further, a thermostat test was accomplished at 37 ± 1 °C for 10 days (Harrigan 1998). All media used for cultivation were obtained from HiMedia (Bombay, India).

Amino acid, ammonia, protein profile and fat globule size analyses

Total amino acid content (both free and bound) was assessed using ion-exchange liquid chromatography (IEC) as described by Buňka et al. (2009). Ammonia amount was provided by microdiffusive Conway method (Buňka et al. 2004). Protein profile was identified by SDS-PAGE (Lazárková et al. 2011). Fat globule size was assigned using image analysis of microscopic technique as described by Tremlová et al. (2006).

Sensory analysis

Sensory evaluation was accomplished using scale and pair comparative tests. A seven-point hedonic scale (1 – excellent, 4 – good, 7 – unacceptable) with the characterisation of each point was used for the assessment of appearance and colour, gloss, consistency, flavour and for overall evaluation. Moreover, three pair comparative tests were employed to confront firmness, shade and preference of the monitored cheese. Sensory panel consisted of 24 employees from the Faculty of Technology, Tomas Bata University in Zlin, trained according to the ISO Standard No. 8586 (2012).

Statistical analysis

Results of basic chemical analyses (dry matter, ash, fat and crude protein content and pH values), fat globule size analysis and determination of ammonia and amino acid content were statistically evaluated using a parametrical test comparing mean values of two independent assortments (Student *t*-test). The data from SDS-PAGE were subjected to cluster analysis using Euclidean distance measure and linking method based on average between groups. Results of sensory analysis were estimated by the Wilcoxon test and the test of binomial distribution parameter. The Unistat 5.5 software (Unistat Ltd., London, UK) was used for statistical evaluation. The level of significance was set at 95 %.

Results and discussion

Microbiological and basic chemical analyses

Microbiological analyses did not show presence of any monitored microorganism, even in thermostat test; hereby we can conclude that applied sterilizing process (117 °C, 20 min) was sufficient for inactivation of microflora and that SPC samples remained sterile even after 2-year storage. This finding agrees with published information on combined effect of temperature and time of sterilization at 110–135 °C for 5–30 min (Mafart et al. 2001).

Basic chemical analyses confirmed expectation that SPC did not show any significant differences ($P \geq 0.05$) in dry matter (35.70–37.59 % w/w), ash (3.86–4.32 % w/w), fat (17.0–18.0 % w/w) and crude protein (15.03–17.43 % w/w) content within the whole storage. Therefore, these parameters were not affected either by temperature or by length of storage. The pH-values of SPC declined ($P < 0.05$) gradually during storage; total decrease was about 0.2–0.3. The highest rate and extent of pH decline during the storage was demonstrated by ST, followed by SA and SR. This pH-values reduction can be attributed to formation of acids in MR, dephosphorylation of caseins, protein-protein reactions resulting in proton release, breakdown of lactose and changes in the calcium-phosphorus equilibrium (Al-Saadi and Deeth 2008; Gaucher et al. 2008). The drop of pH during storage of UHT milk at 40 °C was observed also by Gaucher et al. (2008).

Amino acid and ammonia content analyses

Results of amino acid analysis are presented in Table 1. Storage length influenced decrease of glutamic acid, tyrosine and histidine content. Threonine, lysine, arginine and methionine showed lower concentrations during storage at 23 and 40 °C, serine and proline only at 40 °C and cysteine at 23 °C ($P < 0.05$). The decline of phenylalanine and asparagic acid amount was observed only after 1-year storage. On the contrary, alanine, isoleucine and leucine did not reduce their content by 24 months ($P \geq 0.05$). Higher storage temperature affected adversely glutamic acid, tyrosine, arginine and methionine levels. Concentration of asparagic acid, serine, proline, valine and isoleucine dropped only at the highest storage temperature. In the case of threonine, leucine, phenylalanine, histidine and lysine content fall caused by temperature was detected after 2 years of storage ($P < 0.05$). Glutamic acid (together with glutamine), proline, leucine and lysine ranked among the most abundant amino acids.

Concerning evaluation of total amino acid content (data not shown), definite decrease caused by both storage temperature and length can be concluded. While the amino acid decrement during the storage in refrigerator (SR) reached 2 % after 1-year and 4.5 % after 2-year storage, it grew to 7.5 % and almost to 12 % in the case of the thermostat treated samples (ST).

Further, essential amino acid indexes (EAAI) were calculated (data not shown). They exhibited slight decline owing to both storage temperature and length. During 24-month storage EAAI dropped by 4, 6 and 12 % in SR, SA and ST, respectively. This implies some, though not very considerable, protein nutrition value decrease.

Results of ammonia content determination are shown in Fig. 1. During the 24-month storage at 23 and 40 °C, the ammonia content rise occurred ($P < 0.05$). Ammonia amount

Table 1 Amino acid content (g/16gN) in sterilized processed cheese depending on temperature and length of storage

Amino acid	Storage temperature (°C)	Storage length (months)		
		0	12	24
Asparagic acid	6	6,25±0,249 ^a	6,02±0,319 ^b A	5,92±0,216 ^b A
	23	6,25±0,249 ^a	6,00±0,130 ^b A	5,94±0,298 ^b A
	40	6,25±0,249 ^a	5,75±0,142 ^b B	5,65±0,354 ^b B
Threonine	6	3,19±0,144 ^a	3,18±0,204 ^a A	3,12±0,061 ^a A
	23	3,19±0,144 ^a	3,06±0,139 ^b B	2,94±0,086 ^c B
	40	3,19±0,144 ^a	3,10±0,080 ^b B	2,67±0,119 ^c C
Serine	6	4,72±0,211 ^a	4,73±0,280 ^a A	4,54±0,175 ^b A
	23	4,72±0,211 ^a	4,52±0,201 ^b B	4,46±0,198 ^b A
	40	4,72±0,211 ^a	4,53±0,173 ^b B	3,82±0,117 ^c B
Glutamic acid	6	19,88±0,979 ^a	19,05±0,638 ^b A	18,54±0,355 ^c A
	23	19,88±0,979 ^a	18,27±0,441 ^b B	17,95±0,274 ^c B
	40	19,88±0,979 ^a	17,23±0,342 ^b C	17,08±0,223 ^b C
Proline	6	9,72±0,376 ^a	9,54±0,352 ^{a,b} A	9,46±0,301 ^b A
	23	9,72±0,376 ^a	9,50±0,301 ^b A	9,38±0,215 ^b A
	40	9,72±0,376 ^a	9,22±0,224 ^b B	9,02±0,359 ^c B
Glycine	6	1,57±0,052 ^{a,b}	1,58±0,034 ^a A	1,54±0,023 ^b A
	23	1,57±0,052 ^{a,b}	1,59±0,033 ^a A	1,56±0,015 ^b A
	40	1,57±0,052 ^a	1,51±0,020 ^b B	1,55±0,039 ^{a,b} A
Alanine	6	2,37±0,074 ^a	2,43±0,141 ^a A	2,26±0,067 ^b A
	23	2,37±0,074 ^a	2,32±0,082 ^a B	2,26±0,045 ^b A
	40	2,37±0,074 ^a	2,39±0,035 ^a A	2,19±0,064 ^b B
Valine	6	5,62±0,143 ^a	5,46±0,111 ^b A	5,43±0,063 ^b A
	23	5,62±0,143 ^a	5,51±0,154 ^b A	5,40±0,154 ^c A
	40	5,62±0,143 ^a	5,39±0,118 ^b B	5,19±0,153 ^c B
Izoleucine	6	4,11±0,085 ^a	4,12±0,083 ^a A	3,96±0,099 ^b A
	23	4,11±0,085 ^a	4,10±0,126 ^a A,B	3,94±0,151 ^b A
	40	4,11±0,085 ^a	4,07±0,073 ^a B	3,78±0,124 ^b B
Leucine	6	8,18±0,108 ^a	8,20±0,342 ^a A	7,99±0,173 ^b A
	23	8,18±0,108 ^a	8,14±0,391 ^a A	7,89±0,104 ^b B
	40	8,18±0,108 ^a	8,09±0,366 ^a A	7,72±0,219 ^b C
Tyrozine	6	5,07±0,059 ^a	4,95±0,117 ^b A	4,82±0,121 ^c A
	23	5,07±0,059 ^a	4,82±0,113 ^b B	4,72±0,140 ^c B
	40	5,07±0,059 ^a	4,75±0,094 ^b C	4,51±0,168 ^c C
Phenylalanine	6	4,49±0,051 ^a	4,30±0,270 ^b A	4,28±0,108 ^b A
	23	4,49±0,051 ^a	4,22±0,124 ^b A	4,19±0,132 ^b B
	40	4,49±0,051 ^a	4,06±0,318 ^b B	3,95±0,073 ^b C
Histidine	6	2,75±0,067 ^a	2,66±0,137 ^b A	2,52±0,037 ^c A
	23	2,75±0,067 ^a	2,69±0,098 ^b A	2,43±0,032 ^c B
	40	2,75±0,067 ^a	2,43±0,100 ^b B	2,10±0,047 ^c C
Lyzine	6	6,75±0,144 ^a	6,70±0,180 ^a A	6,52±0,104 ^b A
	23	6,75±0,144 ^a	6,64±0,117 ^b A	6,45±0,066 ^c B
	40	6,75±0,144 ^a	6,00±0,097 ^b B	5,89±0,053 ^c C
Arginine	6	3,65±0,066 ^a	3,54±0,202 ^b A	3,48±0,147 ^b A
	23	3,65±0,066 ^a	3,37±0,120 ^b B	3,27±0,096 ^c B
	40	3,65±0,066 ^a	3,21±0,105 ^b C	3,08±0,114 ^c C
Cysteine	6	0,43±0,015 ^a	0,39±0,023 ^b A	0,37±0,027 ^b A
	23	0,43±0,015 ^a	0,39±0,016 ^b A	0,36±0,031 ^c A

Table 1 (continued)

Amino acid	Storage temperature (°C)	Storage length (months)		
		0	12	24
Methionine	40	0,43±0,015 ^a	0,36±0,027 ^{bA}	0,33±0,048 ^{bA}
	6	3,16±0,037 ^a	3,11±0,128 ^{a,bA}	3,06±0,067 ^{bA}
	23	3,16±0,037 ^a	2,99±0,057 ^{bB}	2,84±0,109 ^{cB}
	40	3,16±0,037 ^a	2,92±0,132 ^{bC}	2,61±0,115 ^{cC}

Amino acid content is presented as mean±SD ($n=20$). Means within a row (effect of storage length) with the same superscript do not differ ($P\geq 0.05$). Means within a column (effect of storage temperature) with various capital letters differ ($P<0.05$)

increased more than double in the case of samples stored in thermostat (ST). There was no definite growing trend in ammonia concentration in cheese stored in refrigerator (SR). Storage temperature affected ammonia amount in SPC even more markedly. Ammonia concentration increase ($P<0.05$) caused by rising temperature was observed in all samples. Growth of ammonia levels by three quarters (compared to SR) was determined after 2-year storage of ST. Increase of ammonia content is related to the above described amino acid detriment during SPC storage since ammonia is one of the amino acid degradation products (Efigênia et al. 1997). This rise could be particularly attributed to Maillard reaction complex and further reactions mentioned in the “Introduction” chapter (Adamiec et al. 2001; Pizzoferrato et al. 1998).

SDS-PAGE analysis

Dendrogram of SPC stored for 24 months is depicted in Fig. 2 (based on cluster analysis described in the part

“Statistical analysis”). Seventeen proteins with molecular weight in the range 3.7–28.0 kDa were determined in SR (the electrophoregram was not shown). Thirteen proteins (3.9–28.2 kDa) were detected in SA whose protein profiles were relatively analogous to those of cheese stored in refrigerator (see similar clusters of SA and SR in Fig. 2). The samples of SPC stored in thermostat (ST) at stressed temperature mostly degraded. This statement is supported with finding a low number of proteins (5 proteins with molecular weight 9.6–28.6 kDa). The diversity of protein profile of ST is confirmed with totally different cluster compared to other clusters of SPC (see Fig. 2). These results are in good agreement with those of Al-Saadi and Deeth (2008) who observed that the electrophoretic patterns of UHT milk samples stored at 5 and 20 °C were different from those stored at 37 and 45 °C. Proteolytic reactions of proteins were more extensive with rising storage temperature which also corresponds with findings of Al-Saadi and Deeth (2008) who stated that changes in

Fig. 1 Ammonia content (mg/kg) in sterilized processed cheese depending on temperature and length of storage. The values are the means with the standard errors of the means, shown by vertical bars. Samples of sterilized processed cheese were stored at 6 °C (SR), 23 °C (SA) and 40 °C (ST) and analysed immediately after production (0) and further in half-year intervals (6, 12, 18 and 24)

Fig. 2 Dendrogram of protein profile results of sterilized processed cheese based on cluster analysis of electrophoregram originated using SDS-PAGE analysis. Samples of sterilized processed cheese were stored at 6 °C (SR), 23 °C (SA) and 40 °C (ST) and analysed after 24 months. The distance (y-axis) is dimensionless

electrophoretic patterns of the UHT milk increased with storage temperature and time.

Fat globule size analysis

Results of image analysis expressed as rel. % of fat globule size distribution are shown in Fig. 3; it is evident that fat globules smaller than $100 \mu\text{m}^2$ and bigger than $5,000 \mu\text{m}^2$ occurred rarely in SPC (0.2–4.7 rel.%). The most abundant fat globule size in SPC was in the range $500\text{--}1,000 \mu\text{m}^2$ (44.4–57.1 rel.%). Rising amount of fat globule size in the interval $1,000\text{--}5,000 \mu\text{m}^2$ (9.1, 11.5, 16.4 and 23.0 rel.% in S0, SR6, SS6 and ST6, respectively) was observed with increasing

storage temperature during the first 6 months of storage ($P < 0.05$). After 1 year of storage both SR and SA showed similar fat globule size ($P \geq 0.05$), whereas ST displayed bigger fat globules ($P < 0.05$). Starting with 18th storage month no marked changes in fat globule size were detected ($P \geq 0.05$).

Fat globule interior consists of triacylglycerols while milk fat globule membrane (MFGM) is composed of a complex mixture of proteins, glycoproteins, enzymes, phospholipids, cholesterol and other minor components. MFGM acts as a natural emulsifying agent preventing the coalescence of fat globules. However, it was stated that MFGM could be ruptured during thermal and mechanical treatment hereby resulting in aggregation and fusing of fat globules, thus

Fig. 3 Fat globule size distribution (rel. %) of sterilized processed cheese depending on temperature and length of storage. Samples of sterilized processed cheese were stored at 6 °C (SR), 23 °C (SA) and 40 °C (ST)

and analysed immediately after production (0) and further in half-year intervals (6, 12, 18 and 24)

causing enlargement of fat globule size (Impoco et al. 2012). Similar clustering of fat globules caused by MFGM composition changes was described during storage of UHT milk at 30 °C for 1 year (Yamuchi et al. 1982). These findings are in good agreement with observed increase of fat globule size in SPC stored in thermostat in our study.

Sensory analysis

Results of sensory analysis of SPC with the use of a seven-point hedonic scale are presented in Table 2. All of the descriptors, namely appearance and colour, gloss, consistency, flavour and overall evaluation of SR did not deteriorate significantly ($P \geq 0.05$) until the 2nd year of storage. However, even after 2 years, these samples were evaluated as “good” or better. In the samples SA quality impairment was noticed already after 6 months of storage ($P < 0.05$), whereas after 12th month of storage no quality deterioration of most descriptors was detected ($P \geq 0.05$); samples were assessed as “good” or “less good”. Most extensive changes were registered in ST during storage. Even after 6 months, these samples showed “unsatisfactory” level of most sensory descriptors. Further quality decline occurred after 12 months of storage; samples were evaluated as “unacceptable”. Hence, sensory analysis of ST samples was terminated after 12-month storage.

Storage temperature significantly affected sensory quality of SPC. With rising temperature, most descriptors showed quality impairment ($P < 0.05$) which is illustrated by pair comparative test results. It was found out that samples stored at lower temperature were preferred and samples stored at higher

temperature were evaluated as darker in all cases ($P < 0.05$). Presumably, darkening of the samples induced downgrade in appearance and colour descriptors. Colour changes of SPC can be supposedly attributed to reactions of nitrogen compounds, especially MR, whose intensive development is, inter alia, caused by use of whey powder in processed cheese production (Kristensen et al. 2001; Pizzoferrato et al. 1998; Schär and Bosset 2002). Browning of UHT milk stored at 37, 45 and 40 °C was reported by Al-Saadi and Deeth (2008) and Gaucher et al. (2008), respectively. Furthermore, flavour of processed cheese was significantly influenced. Storage length exerted slight impact only on samples stored at 23 and 40 °C. Contrary to the above, storage temperature influenced flavour deterioration much more markedly. Decreased sensory acceptability of UHT milk stored at ambient temperature was observed by Enright et al. (1999) and Valero et al. (2001). According to Gaucheron et al. (1999) there is a connection between MR and colour and flavour changes. Evaluation of processed cheese consistency (firmness) brought rather ambiguous results. All samples kept at higher temperature were described as tougher after 6 months ($P < 0.05$). After 12 months, samples stored at 40 °C were still tougher ($P < 0.05$) than SR and SA samples. However, firmness of cheeses stored at 6 and 23 °C did not differ significantly when compared ($P \geq 0.05$). Initial increase in firmness of cheese stored at higher temperature could be likely evoked by subsequent protein cross-linking developed due to hydrolysis of emulsifying salts and successive release of calcium ions (Schär and Bosset 2002). Incidence of two phenomena can be probably expected in longer time horizon. First, new protein bonds are formed (e.g. due to the above mentioned cross-

Table 2 Results of sensory analysis of sterilized processed cheese depending on temperature and length of storage

Descriptor	Storage temperature (°C)	Storage length (months)				
		0	6	12	18	24
Appearance and colour	6	3 ^a	3 ^b A	3 ^b A	4 ^c A	4 ^c A
	23	3 ^a	4 ^b B	5 ^c B	5 ^c B	5 ^c B
	40	3 ^a	6 ^b C	6 ^b C	ND	ND
Gloss	6	3 ^a	3 ^a A	4 ^{a,b} A	4 ^b A	4 ^b A
	23	3 ^a	4 ^b B	5 ^c B	5 ^c B	5 ^c B
	40	3 ^a	5 ^b C	6 ^c C	ND	ND
Consistency	6	3 ^a	3 ^a A	4 ^b A	4 ^b A	4 ^b A
	23	3 ^a	4 ^b B	4 ^b A	4 ^b A	4 ^b A
	40	3 ^a	5 ^b C	6 ^b B	ND	ND
Flavour	6	3 ^a	3 ^b A	3 ^b A	4 ^c A	4 ^c A
	23	3 ^a	4 ^b B	5 ^c B	5 ^c B	5 ^c B
	40	3 ^a	6 ^b C	7 ^c C	ND	ND
Overall evaluation	6	3 ^a	3 ^b A	3 ^b A	4 ^c A	4 ^c A
	23	3 ^a	4 ^b B	5 ^c B	5 ^c B	5 ^c B
	40	3 ^a	6 ^b C	7 ^c C	ND	ND

Results of sensory analysis are presented as medians. Medians within a row (effect of storage length) with the same superscript do not differ ($P \geq 0.05$). Medians within a column (effect of storage temperature) with various capital letters differ ($P < 0.05$). ND not determined

linking) leading to firmness enhancement. On the other hand, protein hydrolysis, e.g. owing to activity of residual thermostable proteases, can occur. Providing that this process prevails over new bond formation, either at higher temperature or sooner than at lower temperature, firmness decline can be observed. Likewise, protein degradation hypothesis is supported by deterioration of SPC flavour during storage and corresponds with the findings of Buňka et al. (2008). Declined sensory quality of SPC stored at elevated temperature corresponds with observed increase of ammonia content and protein changes.

Conclusion

The effect of storage temperature and length on processed cheese quality was monitored in this study. Storage conditions exerted impact on protein changes in SPC. Amino acid content declined only moderately due to both storage temperature and length. Nevertheless, storage length showed slightly greater influence than temperature, which was considerable only in the case of thermostat storage. Hence, storage participated in protein nutrition value decrease, especially in cheese stored at the highest temperature for 24 months. Nutrition value of SPC remained satisfactory during storage though. Protein changes were also proved by protein profile analysis using SDS-PAGE. Besides protein profile, fat globule size was also affected by storage temperature and length. Destructive protein reactions reflected adversely in organoleptic quality of SPC, particularly colour and flavour. Storage at 40 °C emerged to be entirely improper, since all the monitored descriptors deteriorated significantly. Refrigerator storage appears to be the most suitable method for long-term storage of SPC; nevertheless, acceptable products were obtained also during storage at ambient temperature even if slight quality decline can be expected compared to storage at 6 °C.

Acknowledgments This work was supported by The National Agency for Agriculture Research, project No. QJ1210300, The Complex Sustainable Systems programme and the Internal Grant project of Tomas Bata University in Zlin No. IGA/FT/2013/010 funded from the resources for specific university research.

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