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Evaluation of processed cheese viscoelastic properties during sterilization observed in situ

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ABSTRACT

Sterilized processed cheese is a specific dairy product with a prolonged shelf life intended for regular retail offer but also as food provisions for armies during peacetime, as well as during crisis and emergency situations, and for storage in state material reserves. Storage requirements are usually defined as ≤25°C for at least 24 mo. One of the ways to achieve such a shelf life is sterilization. Therefore, the aim of the work was to describe, for the first time in the available scientific literature, in situ changes in the viscoelastic properties of spreadable melt (34% wt/wt DM content, 45% wt/wt fat in DM content, and 14% wt/wt protein content) during an increase in temperature (target temperature 122°C), holding at sterilization temperature (20 min) and subsequent cooling (to ~30°C). While increasing to the target sterilization temperature, a significant decrease occurred in the storage and loss moduli values. Both moduli started to increase again during the target sterilization temperature period and during the whole cooling phase. The values of the storage and loss moduli were significantly higher at the end of the cooling of the sterilized product, and conversely, the phase angle value was lower compared with the melt before sterilization. As a result of sterilization, an increase occurred in the levels of markers of the Maillard reaction complex and lipid oxidation processes. The value of hardness, corrected stress, and elongational viscosity also increased compared with nonsterilized products. As a result of sterilization, the flavor worsened and sterilized processed cheeses showed darker (brownish) color. However, even after sterilization, the products were evaluated as acceptable for consumers and maintained their spreadability.

Key words: processed cheese, sterilization, rheology, heating, in situ study

INTRODUCTION

Sterilized processed cheese (**SPC**) is a specific dairy product with a prolonged shelf life. The beginnings of its production date back to the middle of the 20th century and are mainly connected with the provision of food for the armies of many countries during peacetime and also during crisis and emergency situations (Meyer and McIntire, 1953; Jaynes et al., 1961; Buňka et al., 2004). Sterilized processed cheeses are not only part of the combat rations and state reserves of many countries but also part of the regular retail offer for consumers who demand something other than canned meat only (Lazárková et al., 2010, 2011). A minimum shelf life of at least 24 mo at 25°C is required for these products (NATO, 2019).

The first stage of SPC production is usually identical to the production of conventional processed cheeses that could also be named as melt; the term "hot melt" is usually used for melt immediately after reaching the target temperature and holding it (generally in the range of 90–100°C); the term "cooled melt" is usually used for melt after cooling (Kapoor and Metzger, 2008). The hot melt (generally in the range of 70–80°C, because there is a delay between the end of production and filling the doses) is then dosed into suitable containers and sterilized in autoclaves under overpressure, generally at a level of 0.3 to 0.4 MPa. Due to the usual pH value of the melt (5.60–6.00), to ensure food safety, it is necessary to apply temperatures corresponding to the equivalent of 121.1°C (for z-values ~10°C; see the Materials and Methods section) for a holding time longer than 10 min to inactivate the present bacterial spores

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(for example, strains of the genera *Bacillus*, *Geobacillus,* and *Clostridium*). In most cases, the cooled melt (generally <10°C) is sterilized (generally in the range of 115–125°C) as the dairy equipped with manufacturing equipment and the facility with a suitable autoclave are usually located in different places (Lazárková et al., 2011; Buňková and Buňka, 2017; Černíková et al., 2022).

An essential parameter of processed cheeses, including SPC, is the consistency when the hardness of the sample increases due to sterilization. Processes in which the cooled melt is reheated are well described in the literature (e.g., Lee and Anema, 2009; Guinee and O'Kennedy, 2009), especially related to the monitoring of processed cheese meltability. Specifically, changes in the viscoelastic properties of processed cheese during heating have been monitored, generally in the range of 5 to 90°C. Mostly with increasing temperature, the authors recorded decreasing values of storage modulus (G', Pa) and loss modulus (G'', Pa) and, in contrast, an increase in phase angle (δ; degrees) or loss tangents ($\tan \delta$; dimensionless) in a critical part of the heating interval. However, in the work of Shirashoji et al. (2006) the values of δ (or tan δ) first increased, then stagnated or even slightly decreased. However, several publications point out that the actual changes in viscoelastic properties during heating of processed cheese depend on several parameters, such as DM and fat content; the presence of carbohydrates; the use of caseins, caseinates, and whey proteins; the composition and concentration of emulsifying salts; the application of hydrocolloids; and the temperature and holding time, as well as the pH value of the melt (Lee and Anema, 2009; Shirashoji et al., 2010; Sołowiej et al., 2015).

It follows from the reviews above that there are no available studies in the literature dealing with the behavior of processed cheese (melt) when heated >90°C, which is not only significant for SPC but also for several different industrial and gastronomic operations that use raw materials based on processed cheeses and analogs, for example as toppings for their products (e.g., pizza and pasta). Temperatures >100°C are commonly used in these cases. Furthermore, it is currently possible (for the purpose of improving food safety) to notice a trend of the target temperatures increasing already during the production of processed cheeses, when these temperatures can reach up to ~115°C and in the case of the continuous production of processed cheeses (UHT) even ~140°C (Lee et al., 2003; Kapoor and Metzger, 2008). However, in the available literature, no sufficient information was found on the behavior of the melt or processed cheeses at temperatures generally >100°C. In addition, in the case of SPC, a spreadable form of processed cheese is usually required, and the publications mainly deal with block-type processed cheeses or their analogs (e.g., Lee and Anema, 2009; Sołowiej et al., 2015).

Therefore, this work first aimed to observe in situ changes in the viscoelastic properties of the cooled melt during the increase in temperature, holding at the sterilization temperature and subsequent cooling. The intention, based on the design of the raw material composition (see the Material and Methods section, the subsection "Sample Preparation and Lethal Effect of Sterilization"), was to produce SPC with the character of spreadable processed cheese even after sterilization. The course of changes in the temperature of the cooled melt during the sterilization mode, which took place in the autoclave, was monitored using dataloggers in the product. On the basis of the latter mentioned data, the temperature development in time was then simulated using dynamic oscillatory rheometry. The second objective was to describe the changes in the microbiological, chemical, physical, and sensory properties of the samples as a result of sterilization, which were precisely monitored by the dataloggers during the sterilization mode. Changes were evaluated after 30 d of storage (comparing nonsterilized and sterilized processed cheese). None of the available publications report that such changes were related to the precisely known course of the sterilization mode.

A precise and exact description and understanding of changes in viscoelastic properties are essential for the design of a suitable raw material composition and sterilization mode for the production of commodities with an extended shelf life, which are usable in different climatic conditions when it is not possible to guarantee compliance with the cooling chain or, more generally, to maintain a suitable temperature. This is essential today when we are exposed to various natural or anthropogenic emergencies that require an advanced crisis management approach and an appropriate reaction, including logistic support (Tulach and Foltin, 2019).

MATERIALS AND METHODS

No human or animal subjects were used, so this analysis did not require approval by an Institutional Animal Care and Use Committee or Institutional Review Board.

Sample Preparation and Lethal Effect of Sterilization

For the manufacture of the samples with 34% wt/wt DM content, 45% wt/wt fat in DM content, and 14% wt/wt protein content, the following materials were used: Dutch-type cheese and butter (both obtained from Lacrum Ltd.), water, and emulsifying salts (Fosfa

Table 1. Formulation of the samples with target values: 34% wt/wt DM content, 45% wt/wt fat in DM content, and 14% wt/wt protein content

Raw material	Composition of raw material (% wt/wt)			Absolute amount (g)	Relative amount (rel. %)
	DM content	Protein content	Fat content		
Dutch-type cheese	52.12	31.26	15.6	682.0	45.38
Butter	84.50	0.00	83.1	148.0	9.85
Emulsifying salts[1]	95.00	0.00	0.0	32.0	2.13
Na_2HPO_4	95.00	0.00	0.0	12.5	39.00
NaH_2PO_4	95.00	0.00	0.0	5.8	18.00
$Na_4P_2O_7$	95.00	0.00	0.0	6.7	21.00
POLY20[2]	95.00	0.00	0.0	7.0	22.00
Water	0.00	0.00	0.0	641.0	42.65
Total				1,503.0	100.00

[1]Composition information was obtained from the manufacturer of emulsifying salts (Fosfa Plc.).
[2]Sodium salt of polyphosphate with the mean chain length n ~20.

Plc.). The formulation of samples (including the parameters of raw materials) is presented in Table 1. A Vorwerk Thermomix TM6 blender cooker (2-L capacity; Vorwerk and Co., Thermomix GmbH) with indirect heating was employed for the production of the hot melt [a similar device was used previously by Lee and Anema (2009) or Lazárková et al. (2010, 2011)]. The target temperature of 90°C was held for 3 min and the agitation rate was 3,100 rpm. The samples were designed to be spreadable, based on our previous studies (Lazárková et al., 2010, 2011; Jedounková et al., 2022).

The hot melt (immediately after production) was poured into the laminated aluminum containers (conical shape; inner dimensions of 26.8 mm in height, 81.1 mm in diameter at the top, and 68.9 mm in diameter at the bottom) with seal lids (the sealing was carried out using NovaSeal equipment, Nirosta Ltd.). The weight of the sample in one container was approximately 95 ± 2 g (therefore, 14 containers from each production batch were obtained). Subsequently, the samples were divided into 2 groups: 7 samples that were not sterilized (nonsterilized samples, **NPC**) and samples that were subsequently sterilized according to the conditions mentioned in the next paragraph (SPC; 7 samples from a batch). All NPC samples were cooled down and stored at 6 ± 1°C. Samples designated for sterilization were also cooled down to 6 ± 1°C and stored to the next day, when they were sterilized. The latter mentioned approach simulates the reality in praxis because the production of the hot melt, packing, and cooling (after cooling the so-called cooled melt was obtained) is carried out in the first day, the packed products are transported usually during evening or night to a plant equipped with appropriate autoclaves, and the next day, the cooled melts are sterilized (target temperature of 122°C; holding time of 20 min).

A laboratory autoclave (FEDEGARI FVA2/A1; Fedegari Autoclavi SpA, Albuzzano, Italy) with inner dimensions of 600 mm in height and 405 mm in diameter was used. The target temperature of 122°C and the holding time of 20 min was applied. To maintain a pressure equal to the pressure in the container during the first minutes of the cooling period, compressed air was fed into the retort. The final temperature after cooling in the autoclave was set at 50°C. The actual temperature in the container placed at the coldest point in the retort (based on the findings originated in the preliminary study) was recorded using data loggers Ellab TrackSense Pro (Ellab A/S) and evaluated by the ValSuite software (version 4.1; Ellab A/S). The NPC and SPC samples were stored at 6 ± 1°C, until analyses (30 d after manufacturing). The hot melt was produced twice for repetition, verification, and confirmation of the results.

For a numerical presentation of the lethal effect of a combination of sterilizing temperature and holding time, the sterility value at the coldest point (F_0) was used. The results are expressed in minutes of a heat treatment at a constant reference temperature (generally T_{ref} = 121.1°C) or as any equivalent heat treatment that would cause the same extent of destruction, calculated according to Lazárková et al. (2011) for the slope index of thermal death time curve in °C (generally set at 10°C).

Microbiological Analysis

The total number of aerobic and facultative anaerobic mesophilic microorganisms was determined according to ISO 4833–1:2013 (ISO, 2013), coliforms number according to ISO 4832:2006 (ISO, 2006a), number of aerobic and anaerobic spore-forming microorganisms according to Harrigan (1998), and number of yeasts

and molds according to ISO 21527–1:2008 (ISO, 2008). The analysis was performed 12 times.

Chemical Analysis

After 30 d of storage, the DM, fat, and protein contents were determined according to ISO 5534:2004 (ISO, 2004), ISO 23319:2022 (ISO, 2022), and ISO 8968–1:2014 (ISO, 2014), respectively. The pH of samples was measured using a pH meter equipped with a glass-tip electrode (pH Spear, Eutech Instruments). The analysis was performed 12 times.

The ammonia content was determined by the microdiffusion method of Conway, as described by Buňka et al. (2004). Lipid oxidation was evaluated by the 2-thiobarbituric acid method developed by Kristensen and Skibsted (1999) as "TBARS-value." Results were expressed as absorbance units per milligram of sample ($\lambda = 532$ nm for red pigment, and $\lambda = 450$ nm for yellow pigment). The analysis was performed 12 times.

Rheological Analyses

The development of viscoelastic properties during the sterilization of the cooled melt was actually first studied in situ by means of rotational viscometry using a Physica MCR502 modular rheometer (Anton Paar), which was equipped with a pressure cell. Detailed knowledge of the rheological behavior is crucial to control and guide the process of the cooled melt sterilization. A bob/cup measuring system (CC 25/Pr; Anton Paar) was used inside the pressure cell with gas-pressurization mode (nitrogen). The pressure cell was interconnected with a C-ETD300 Peltier unit (Anton Paar) to control the temperature appropriately. To evaluate the course of the sterilization process, a constant strain ($\gamma = 0.03$) and frequency ($f = 0.1$ Hz) were set with a reading of 1 point/s at a constant pressure (0.36 MPa) during the entire process with the following temperature profile: (1) increase in temperature from 25°C to a sterilization temperature of 122°C (linear heating rate of 4°C·min^{-1}; i.e., ~24 min), (2) holding at the sterilization temperature (20 min), and (3) cooling to ~30°C (linear heating rate of −2°C·min^{-1}; i.e., ~46 min). The described temperature profile was based on values monitored using data loggers recording the real process of sterilization of the cooled melt in an autoclave. Primarily, the storage (G') and loss (G'') moduli were recorded, tan δ was calculated as G''/G' and δ as arctangents of G''/G'. For our purposes, G' characterizes the elastic portion of the viscoelastic behavior and practically describes solid-state behavior of the sample, and G'' represents the viscous portion of the viscoelastic behavior and practically explains the liquid-state behavior of the sample. The values of tan δ and δ, respectively, characterize the ratio of the 2 portions of the viscoelastic behavior. With the increase of G' and the decrease of tan δ and δ, the rise of the gel strength of the sample should be expected and vice versa (Winter and Chambon, 1986; Sołowiej et al., 2015; Černíková et al., 2022).

To compare the viscoelastic properties of NPC and SPC (after 30 d of storage), frequency sweep tests were performed. A parallel-plate measuring system with a diameter of 25 mm was used, while the plate gap was set to 1 mm. The temperature during the measurement was set to 25°C and was controlled using a water-cooled Peltier system (Physica H-PTD 200). The frequency sweeps were performed using a frequency ranging from 0.05 Hz to 10.00 Hz with the applied constant shear stress of 5 Pa (within viscoelastic region). The analysis was performed 6 times.

For the evaluation of the sample viscoelastic properties, the Winter's critical gel theory was implemented. According to Equation [1], the complex modulus (G^*, Pa; obtained as the complex sum of G' and G'') can be expressed as follows (Winter and Chambon, 1986):

$$G^*(f) = A_F \cdot f^{\frac{1}{q}}, \qquad [1]$$

where A_F (Pa·s$^{1/z}$) is the gel strength, f is the frequency (Hz) and q corresponds to the interaction factor.

Textural Analyses

The textural properties of NPC and SPC (after 30 d of storage) were evaluated using a texture analyzer TA.XTplus (Stable Micro Systems Ltd.) equipped with a 20 mm in diameter cylindrical aluminum probe. The analysis was performed by penetration into the sample (strain 25% and trigger force 5 g; deformation rate was 2 mm·s^{-1}) at 20 ± 1°C (the measurement was carried out within the containers). From the force and time curves, we obtained the hardness (the maximum force observed during penetration; N); cohesiveness (strength of the internal bonds of samples calculated as the positive force area of the second peak to that of the first peak; unitless); and relative adhesiveness (relative strength of adhesiveness between the cheese and the probe surface calculated as a ratio of the absolute value of the negative force area to the positive force area of the first peak; unitless) for NPC and SPC. The analysis was performed 12 times.

The force versus time data were converted to a corrected stress, Hencky strain, elongational viscosity, and Hencky strain rate using the following equations:

$$\sigma_C = \frac{F(t)H(t)}{A_0 H_0}, \quad [2]$$

$$\varepsilon_H = \ln\left(\frac{H_0}{H_{(t)}}\right), \quad [3]$$

$$\eta_E = \frac{2F(t)H(t)}{\pi r^2 v}, \quad [4]$$

$$\dot{\varepsilon_H} = \frac{v}{2H_{(t)}}, \quad [5]$$

where σ_C is the corrected momentary stress (Pa), ε_H the dimensionless momentary Hencky strain, η_E the elongational viscosity (Pa·s), $\dot{\varepsilon_H}$ the Hencky strain rate (biaxial extensional strain rate, s^{-1}), $F(t)$ the momentary force at time t (s), H_0 the initial cylindrical sample height (m), $H(t)$ the height (m) of the deformed sample at the time t (s), A_0 the cross-sectional area of the original sample (m^2), v the velocity (deformation rate, $m \cdot s^{-1}$), and r is the radius (m) of the sample (ISO, 2006b; Chatziantoniou et al., 2019).

Instrumental Color Analysis

The ColorFlex EZ (Hunter Associates Laboratory Inc.) was used for the instrumental color evaluation of NPC and SPC (after 30 d of storage). The CIELAB color scale ($L^*a^*b^*$) was used, with the illuminant D65 (standard daylight) and 10° angle. Parameters L^* (lightness or brightness; 0 indicates black; 100 indicates white), a^* (greenness to redness), and b^* (blueness to yellowness) were determined according to the International Commission on Illumination. The apparatus was calibrated in the reflectance mode, with specular reflection excluded and using white (A41–1014–635 Rev. B; Hunterlab ColorFlex CZ; Hunter Associates Laboratory Inc.) and black (A41–1017–037 Rev A; Hunterlab ColorFlex CZ; Hunter Associates Laboratory Inc.) reference plates. A 25-mm glass sample cup was used for the readings. The analysis was performed 12 times.

Sensory Analysis

Sensory evaluation of NPC and SPC (after 30 d of storage) was carried out by a panel consisting of 12 selected assessors or experts trained according to ISO 8586:2012 (ISO, 2012). During the training of all assessors (including experts), different processed cheese samples (including SPC) were used. The assessment used a 9-point scale to evaluate both NPC and SPC on a variety of parameters: appearance, consistency, and flavor (1-excellent, 5-good, 9-unacceptable); hardness (1-soft, 5-medium, 9-extra hard); and for off-flavor (1-negligible, 5-medium, 9-excessive) . The samples were served in random order and at controlled temperature of 20 ± 2 °C in a sensory laboratory equipped with sensory booths (under normal light condition) in accordance with ISO 8589:2007 (ISO, 2007). Water was provided for mouth rinsing between the tested samples evaluation to avoid carryover effects. The analysis was performed 24 times.

Statistical Analysis

The obtained results were evaluated using Wilcoxon tests (the significance level was $P = 0.05$). Unistat 6.5 software (Unistat) and Microsoft Excel (Microsoft Corporation) were used for the statistical analysis.

RESULTS AND DISCUSSION

Changes of the Cooled Melt Viscoelastic Properties During Sterilization

Figure 1 shows in situ changes in viscoelastic properties of the cooled melt (G' and G'', part A; δ, part B) during (1) heating from a temperature of 25°C to the target sterilization temperature (122°C) lasting 25 min, (2) holding at the target sterilization temperature (20 min), and (3) subsequent cooling to ~30°C. Table 2 shows the values of G', G'', and tan δ for selected temperatures of the cooled melt during the sterilization and cooling processes. During heating, a significant decrease occurred in G' and G'' ($P < 0.05$). At the same time, it follows from Figure 1 that the rate of decrease of the parameters mentioned above changed during heating. Primarily, a steep decrease of both viscoelastic moduli was recorded in the first ~8 min. (up to ~53°C; $P < 0.05$), which in the case of G'' continued until ~11.5 min (up to ~67°C; $P < 0.05$; Figure 1A). In the case of δ (Figure 1B), a very slight increase occurred in the first ~3 min (up to ~31°C; $P \geq 0.05$), followed by a significant decrease to ~11.5 min (up to ~67°C; $P < 0.05$), from which it follows that in the period of heating approximately at 3 to 11.5 min, the value of G'' decreased faster than G' ($P < 0.05$). On the contrary, in the following ~4.5 min (up to ~85°C), a steep increase in δ values was recorded (Figure 1B; $P < 0.05$), which also results from the course of the dependence of G' and G'' values on time (Figure 1A). From the ~16th to ~21st min of heating (up to ~105°C), the G' value decreased ($P < 0.05$) and subsequently, until the ~25th min (up to ~122°C, the beginning of the target steril-

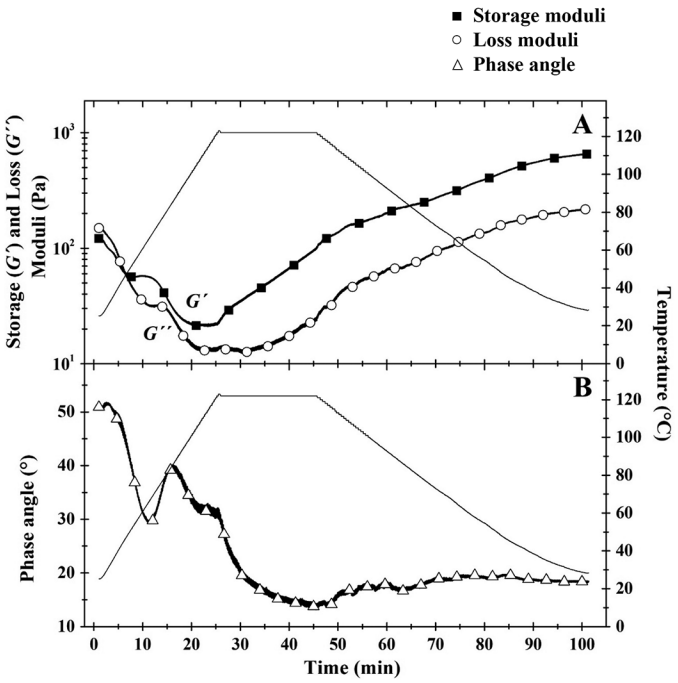

Figure 1. The development of storage and loss moduli (A) and phase angle (B) of the cooled melt in time during heating, sterilization process, and subsequent cooling direct in the rheometer equipped with the pressure cell (3.6 bar). The progress of temperature was presented as a line without any symbols (f = 0.1 Hz; n = 6) in both parts (A and B).

ization temperature holding time), it stagnated (Figure 1A; Table 2; $P \geq 0.05$). A decrease in the level of G'' values was recorded ~1.5 min later (corresponding to a temperature of ~6°C higher), then G'' also started to stagnate. The values δ decreased throughout the ~16th to 25th min interval of heating (Figure 1B).

As shown in Figure 1 and Table 2, during the holding time of the target sterilization temperature of 122°C (20 min), a significant increase in G' and G'' was recorded, and on the contrary, a decrease in the δ ($P < 0.05$) parameter, although the beginning of a significant increase in G'' values was delayed by ~6.5 min. The last-mentioned observation was logically the reason why the rate of decrease for δ was higher ($P < 0.05$; the slope indexes are not shown) in the first ~6.5 min than in the remaining minutes of the target sterilization temperature holding (next ~13.5 min; Figure 1B).

During the subsequent cooling to the target temperature of ~30°C during ~55 min., consistent significant increases in both G' and G'' were already recorded (Figure 1A; Table 2; $P < 0.05$). First, the values δ increased for ~7.5 min of cooling (from the end of the target sterilization temperature holding time; up to ~108°C; $P < 0.05$), then they practically stabilized and slightly oscillated around δ ~18.5° until the end of measurement (Figure 1B).

If at the beginning of the measurement, the values of G' ~120 Pa and G'' ~160 Pa (Table 2) and δ > 45° (Figure 1B) were recorded in a cooled melt, meaning the sample showed rather liquid-like behavior, then at the end of in situ monitored sterilization mode (including cooling), the levels of G' and G'' were more than 3 times higher and the value of the parameter tan δ was simultaneously much lower (Table 2; $P < 0.05$). Thus, the product showed rather solid-like behavior.

A direct comparison of the development of the viscoelastic properties of the cooled melt during the applied sterilization mode monitored in situ with the available literature is not possible, as adequate references have not been found. Only the development of rheological parameters when heated to ~90°C can be compared

Table 2. Results of the viscoelastic parameters at the selected temperature during heating, sterilization process, and subsequent cooling of the cooled melt sample in the pressure cell of the rheometer (f = 0.1 Hz; the results were expressed as mean ± SD; n = 6)

	Parameter		
Temperature (°C)	Storage modulus (G', Pa)	Loss modulus (G'', Pa)	Loss tangent (tan δ, dimensionless)
25	119.1 ± 8.3[a]	159.3 ± 11.0[a]	1.311 ± 0.095[a]
100[1]	23.5 ± 1.4[b]	15.8 ± 0.9[b]	0.671 ± 0.047[b]
122[2]	21.4 ± 1.6[b]	13.9 ± 1.1[b]	0.654 ± 0.048[b]
122[3]	94.2 ± 5.8[c]	24.3 ± 1.5[c]	0.242 ± 0.018[c]
100[4]	175.7 ± 9.8[d]	48.6 ± 2.7[d]	0.285 ± 0.022[d]
30	660.2 ± 41.6[e]	215.6 ± 13.6[e]	0.320 ± 0.024[e]

[a–e]Means within a column (the difference between the selected temperature) differ ($P < 0.05$) by letter.

[1]Temperature in the sterilization time first reached the temperature of 100°C.

[2]Temperature in the sterilization time first reached the target temperature of 122°C.

[3]Temperature in the sterilization time finally reached the target temperature of 122°C.

[4]Temperature in the sterilization time finally reached the temperature of 100°C.

with the literature, and still mostly limited, because in the available sources, a block-type processed cheese with a much higher DM content was studied. The decrease in G' and G'' values as a result of increasing the temperature of the cooled melt up to ~90°C is in agreement with the majority of available literature sources, although some authors have found that from a certain temperature (usually ~65–75°C) stagnation of G' can occur, possibly even a slight increase in its value (Shirashoji et al., 2006, 2016; Guinee and O'Kennedy, 2009). During the cooking stage, several different physicochemical reactions are likely occurring in the casein matrix that aid dispersion, including reduced contact between casein molecules due to weakening strength of certain types of interaction (including hydrogen bonding). Decrease in the G' and G'' is also associated with the increase in thermal motion of molecules, the decrease in ordering of water molecules (increased entropy in the system), and generally, the decrease in hydrogen bonding with increasing temperature (Shirashoji et al., 2006, 2016; Lee and Anema, 2009). The decrease in G' a G'' as a result of increasing the temperature of the system is also attributed to liquefaction of the fat phase (Guinee and O'Kennedy, 2009).

On the contrary, the development of the values of δ observed by us in the first minutes of heating was different compared with most available sources, although in some cases (e.g., Guinee and O'Kennedy, 2009; Lee and Anema, 2009) a practical stagnation of the level of δ was recorded, approximately when temperatures rise from 30 to 50°C. In the article of Guinee and O'Kennedy, (2009), even a decrease in δ values was observed from 50°C. Differences in the development of changes of δ during heating compared with the abovementioned publications can also be caused by, for example, the properties of the raw material composition (including pH values) and the process parameters used, especially agitation speed during heating or target temperature (Bowland and Foegeding, 1999; Lee et al., 2003; Sołowiej et al., 2015).

During sterilization and subsequent cooling, an additional "creaming" process may also occur, as some types of protein-protein interactions (especially fibrillogenic caseins) may occur in processed cheese, including advanced protein-protein associations due to exposed nonpolar groups. Caseins could reassociate or repolymerize and form new networks, e.g., via hydrophobic and electrostatic interactions, hydrogen bonds, formation of emulsifying salts–calcium complexes that can help crosslinking caseins in the system, and some other types of interaction (Lee et al., 2003; Shirashoji et al., 2006; Vollmer et al., 2021).

The course of complex Maillard reactions (**CMR**), when new bonds between proteins can form, could also contribute to the increase in SPC hardness during sterilization and subsequent cooling (Friedman, 1996; Bertrand et al., 2015; Li et al., 2021). An example of such an interaction can be the formation of an isopeptide bond between proteins through the ε-amino group of lysine, which was demonstrated in SPC, for example, by Lazárková et al. (2010).

Comparison of Nonsterilized and Sterilized Processed Cheese

The influence of different sterilization regimens on the quality of processed cheeses has already been described in the literature (e.g., Buňka et al., 2004; Lazárková et al., 2010, 2011). However, none of the available publications compares NPC and SPC, for which the course of temperature change during heating was precisely known, including an exact description of the development of viscoelastic properties. The results of selected NPC and SPC parameters after 30 d of storage at 6 ± 1°C are shown in Table 3. The applied sterilization mode leading to the value of $F_0 > 15$ ($P > 0.05$) was sufficient to inactivate the monitored indicator groups of microorganisms, especially spore-forming microorganisms, as it follows from the results of microbiological analysis (Table 3; $P < 0.05$). The indicator groups of microorganisms determined by the researchers represent a fundamental risk for canned foods and their inactivation is necessary to ensure food safety (Harrigan, 1998).

As a result of hermetically sealed aluminum packaging, no significant changes were seen in DM, fat in DM, or protein contents due to sterilization ($P \geq 0.05$; Table 3). However, a decrease in the pH value was recorded ($P < 0.05$; Table 3), which is primarily related to the interaction of calcium ions with hydrogen phosphate anions to form calcium phosphate and the release of hydrogen cations, possibly hydrolysis of polyphosphates and a change in their buffering capacity (Molins, 1991; Lazárková et al., 2011). As a result of the applied sterilization mode, a significant increase occurred in ammonia content and TBARS-value ($P < 0.05$; Table 3), from which an intensive course of CMR, including the Strecker degradation of amino acids and lipid oxidation, can be concluded (Kristensen and Skibsted, 1999; Buňka et al., 2004; Bertrand et al., 2015). The last-mentioned reactions influenced changes in other properties of processed cheese, especially in color, rheological, and textural properties, and therefore sensory quality. Table 3 clearly shows that the sterilization

Table 3. Results of the analysis of the nonsterilized and sterilized processed cheese samples after 30 d of storage (6 ± 1°C) measured at 25°C (results were expressed as mean ± SD, or range of observed values; n varies with the parameter investigated)

Parameters	Processed cheese[1]	
	Nonsterilized	Sterilized
DM content[2] (% wt/wt)	34.09 ± 0.45[a]	33.94 ± 0.52[a]
Fat content[2] (% wt/wt)	15.3 ± 0.8[a]	15.3 ± 0.8[a]
Fat in DM content (% wt/wt; calculated)	44.94	44.98
Protein content[2] (% wt/wt)	13.97 ± 0.76[a]	14.10 ± 0.77[a]
pH value[2]	5.81 ± 0.02[a]	5.94 ± 0.01[b]
Ammonia content[2] (mg/100 g)	25.9 ± 1.7[a]	95.1 ± 8.4[b]
TBARS-value[2,3] (arbitrary units/mg)	15.3 ± 0.9[a]	60.8 ± 5.2[b]
Total number of aerobic and facultative anaerobic mesophilic microorganisms[4] (log cfu/g)	1.77–2.95[a]	ND[b]
Number of aerobic spore-forming microorganisms[4] (log cfu/g)	1.31–2.38[a]	ND[b]
Number of anaerobic spore-forming microorganisms[4] (log cfu/g)	1.16–1.95[a]	ND[b]
Coliforms number[4] (log cfu/g)	ND	ND
Number of yeasts and molds[4] (log cfu/g)	ND	ND
Hardness[2] (N)	0.78 ± 0.06[a]	1.60 ± 0.11[b]
Cohesiveness[2] (unitless)	0.540 ± 0.041[a]	0.541 ± 0.037[a]
Relative adhesiveness[2] (unitless)	0.442 ± 0.028[a]	0.352 ± 0.022[b]
Storage modulus in 1 Hz[5] (Pa)	2,001.3 ± 147.1[a]	4,749.8 ± 350.2[b]
Loss modulus in 1 Hz[5] (Pa)	1,370.9 ± 98.2[a]	1,980.3 ± 144.5[b]
Phase angle in 1 Hz[5] (degrees)	34.6 ± 2.4[a]	22.9 ± 1.6[b]
Gel strength[5] (Pa·s$^{1/z}$)	2,257.2 ± 189.9[a]	5,079.4 ± 337.9[b]
Interaction factor[5] (unitless)	2.1 ± 0.1[a]	3.6 ± 0.3[b]
Lightness[2]	93.6 ± 0.2[a]	87.4 ± 0.3[b]
Chromaticity on a green to red axis[2]	−0.20 ± 0.01[a]	6.42 ± 0.58[b]
Chromaticity on a blue to yellow axis[2]	11.2 ± 0.2[a]	16.6 ± 0.4[b]

[a,b]Means within a row differ ($P < 0.05$) by letter.

[1]ND = not detected.

[2]n = 12.

[3]Lipid oxidation was evaluated by the 2-thiobarbituric acid method developed by Kristensen and Skibsted (1999) as "TBARS-value."

[4]Interval of determined numbers of indicator groups of microorganisms (n = 12).

[5]n = 6; the values were obtained from the frequency test.

process led to a significant decrease in lightness (L^*; $P < 0.05$) and a further shift in chromaticity to red (a^*) or yellow (b^*) area ($P < 0.05$). The changes described above significantly ($P < 0.05$) influenced the appearance of SPC (see Table 4).

As shown in the in situ study presented in the section "Changes of the Cooled Melt Viscoelastic Properties During Sterilization," a substantial increase was seen in the hardness of sample at the end of sterilization or sample cooling. In the area of large-scale strains (Table 3), as a result of sterilization, there was an increase in hardness ($P < 0.05$) and, conversely, a decrease in relative adhesiveness ($P < 0.05$), but the value of cohesiveness remained practically unchanged ($P \geq 0.05$). From Figures 2 and 3 it further follows that the sterilization mode led to a significant increase in the values of corrected stress (σ_C; $P < 0.05$) and elongational viscosity (η_E; $P < 0.05$), along nearly the entire monitored range of Hencky strain and Hencky strain rate, respectively. An initial sharp increase in elongational viscosity is evident, which corresponds to the transient flow regimens, followed by an approximately linear part, corresponding to the squeezing flow regimen. The linear parts of the curves for samples, remained almost horizontal, with elongational viscosity becoming almost independent of Hencky strain rate. It is also obvious that the point of transient flow regimen to squeezing flow regimen were due to sterilization moved into higher levels of Hencky strain rates (Figure 3; $P < 0.05$). The application of models expressing the dependence of corrected stress on Hencky strain and elongational viscosity on Hencky strain rate

Table 4. Sensory analysis (appearance, consistency, hardness, flavor, and off-flavor) of nonsterilized and sterilized processed cheese after 30 d of storage (6 ± 1°C), with results expressed as median (n = 24)

Parameters	Processed cheese	
	Nonsterilized	Sterilized
Appearance[1]	1[a]	6[b]
Consistency[1]	6[a]	4[b]
Hardness[2]	3[a]	5[b]
Flavor[1]	2[a]	6[b]
Off-flavor[3]	1[a]	4[b]

[a,b]Means within a row differ ($P < 0.05$) by letter.

[1]A 9-point scale (1-excellent, 5-good, 9-unacceptable) was used.

[2]A 9-point scale (1-soft, 5-medium, 9-extrahard) was used.

[3]A 9-point scale (1-negligible, 5-medium, 9-excessive) was used.

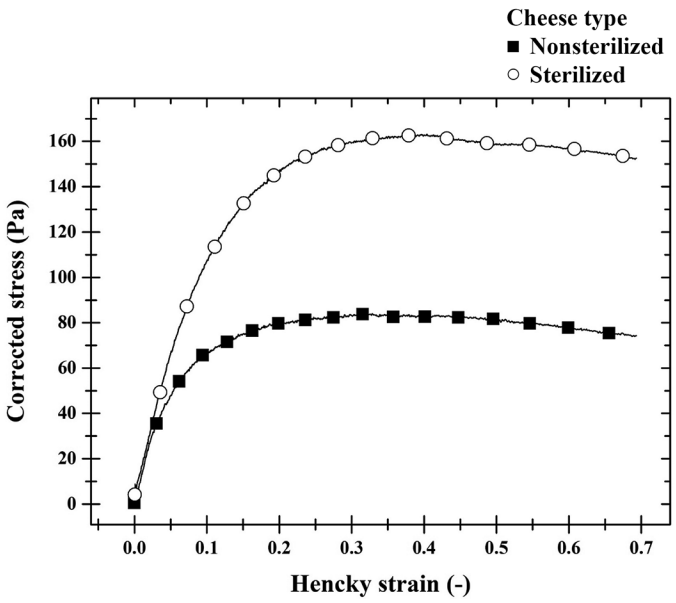

Figure 2. The dependence of corrected stress on Hencky strain (dimensionless) of nonsterilized and sterilized processed cheese samples after 30 d of storage measured at 25°C (6 ± 1°C; n = 6).

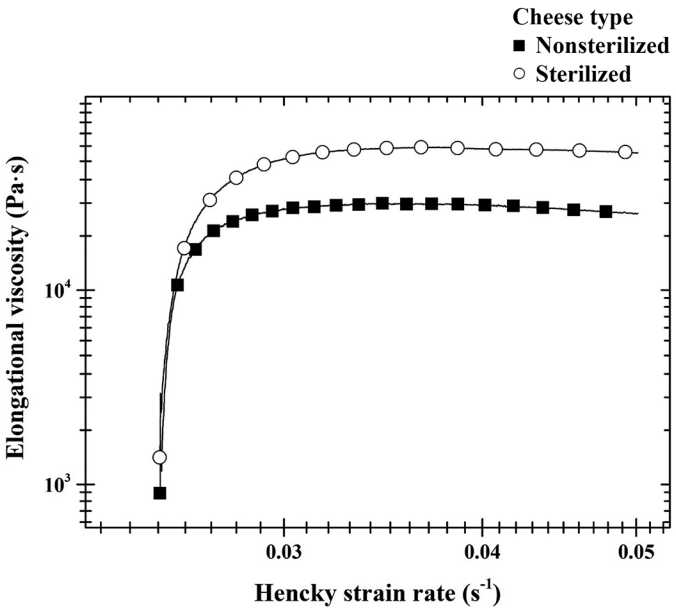

Figure 3. The dependence of elongational viscosity on Hencky strain rate of nonsterilized and sterilized processed cheese samples after 30 d of storage measured at 25°C (6 ± 1°C; n = 6).

was not found for SPC (and including the comparison with NPC) in the available literature.

In the area of small-scale strains, a significant increase in the values of G' and G'' ($P < 0.05$) and a decrease in δ ($P < 0.05$) were recorded. The change in the nature of viscoelastic properties as a result of sterilization is also clearly visible from Figure 4, showing the dependence of G' and G'' on the frequency during the frequency sweep mode test. Changes observed in the area of small-scale strains are consistent with findings made during measurements in the area of large-scale strains. By applying the Winter's critical gel theory (according to Equation [1]), it was clearly demonstrated that there was a significant increase in gel strength (A_F; $P < 0.05$) and an increase in the interaction factor (q; $P < 0.05$; Table 3), indicating an increase in the number of interacting units in the systems, in this case especially protein-protein interactions (Bowland and Foegeding, 1999; Lee et al., 2003; Vollmer et al., 2021). A drop in pH probably also played a role here, when, as a result of sterilization, the caseins present slightly approached their isoelectric point, which could also support protein-protein interactions (Kapoor and Metzger, 2008). The course of CMR and lipid oxidation could also contribute to the increase in hardness, as mentioned in the section "Changes of the Cooled Melt Viscoelastic Properties During Sterilization."

The results shown in Table 4 describe how, as a result of sterilization, the development of chemical parameters, viscoelastic and textural properties, and changes in color evaluation were reflected in the sensory evaluation of SPC. The applied sterilization mode led to a significant deterioration of appearance and flavor, including intensification of off-flavor of tested samples

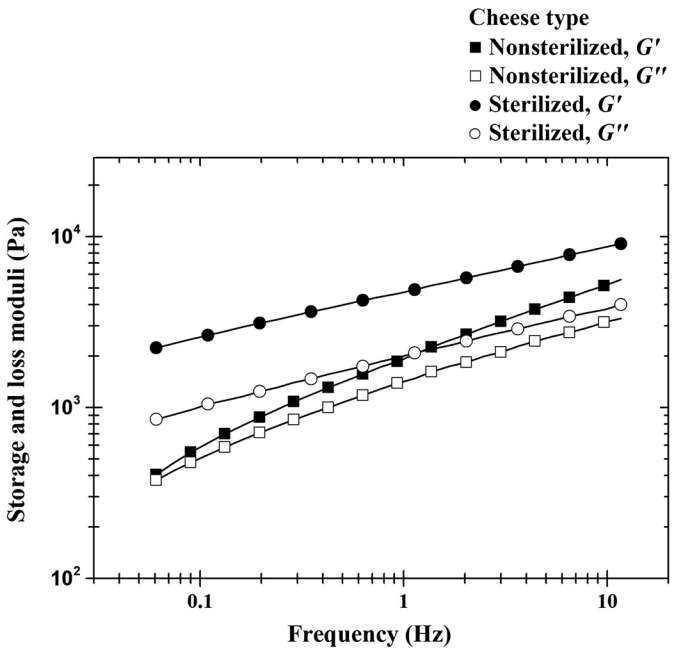

Figure 4. The dependence of storage (G') and loss (G'') moduli of nonsterilized and sterilized processed cheese samples on the frequency after 30 d of storage measured at 25°C (6 ± 1°C; n = 6).

($P < 0.05$). The formation of new aroma-active substances and the browning or accentuation of the pinkish color of cheeses as a result of CMR are described, for example, by Daly et al. (2012). These changes are in line with the observed changes in markers of ongoing CMR and lipid oxidation (ammonium content and TBARS-value) described in this study. The increase in hardness as a result of sterilization, detected by textural and rheological analysis, was also confirmed in sensory evaluation ($P < 0.05$; Table 4). However, these changes resulted in a significant improvement in consistency ($P < 0.05$). An increase in the hardness of samples as a result of sterilization was expected, and therefore DM content, fat in DM content, and whole raw material composition (Table 1) were designed in such a way that the NPC has more of a liquid-like behavior and the resulting SPC is easily spreadable when consumed, exhibiting the parameters of a material with solid-like behavior.

CONCLUSIONS

In in situ conditions, the course of changes in the viscoelastic properties of spreadable samples (34% wt/wt DM content, 45% wt/wt fat in DM content, and 14% wt/wt protein content) was described during the increase in temperature (target temperature 122°C), holding at the sterilization temperature (20 min), and subsequent cooling. It was proven that when the temperature increases, the storage (G') and loss (G'') moduli first significantly decrease, followed by a short stagnation of both moduli and then their continuous increase during the target sterilization temperature period and the entire cooling phase. The values of the storage (G') and loss (G'') moduli were significantly higher at the end of the cooling of the sterilized product and, conversely, the value of the phase angle (δ) was significantly lower compared with the state before heating the NPC. After 30 d of storage, NPC and SPC were compared. With the help of markers (ammonium content and TBARS-value), it was established that the course of the Maillard reaction complex and lipid oxidation took place. By instrumental and sensory analysis, SPC were evaluated as harder and darker compared with nonsterilized products. Despite the changes described above, the raw material composition and sterilization mode were designed in such a way that the resulting SPC were easy to spread and had an acceptable taste and appearance. The last-mentioned descriptor is key to the successful use of SPC during logistic support in crisis and emergency situations and in regular retail.

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