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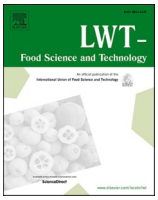

Critical view on sterilisation effect on processed cheese properties designed for feeding support in crisis and emergency situations

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ABSTRACT

This study aimed to design high-quality and safe spreadable processed cheese with a prolonged shelf life appropriate for logistic support in emergency/crisis situations, foreign missions, and/or state material reserves. Samples with dry matter content (DM) of 30–55 g/100 g and fat in dry matter content (FDM) of 30–50 g/100 g were produced, hermetically sealed and sterilised at 120 °C for 40 min. The microbiological, chemical, textural, viscoelastic, and organoleptic parameters of unsterilised and sterilised processed cheese were compared. The effect of the products' dry matter and fat content on the sterilised samples' properties was also evaluated. The sterilisation regime applied was sufficient to ensure the microbiological safety of processed cheese. A significant decrease in pH values and increased rate of the complex of Maillard reactions and lipid oxidation processes ($P > 0.05$) was detected due to sterilisation. These processes were more intense with increasing dry matter and fat content ($P > 0.05$). A deterioration in the appearance, colour, and aroma of processed cheese and an increase in the firmness of the products ($P > 0.05$) were also observed as a result of sterilisation. For the quality and safety of sterilised processed cheese, combinations of 40 g/100 g DM and 50 g/100 g FDM were recommended for spreadable products.

1. Introduction

In recent decades, the world has been buffeted by natural and anthropogenic emergencies with the potential for rapid escalation, the resolution of which requires advanced crisis management approaches. A crucial task of organisations sending emergency teams is to ensure the ability to provide logistical support to humanitarian and/or military missions. The appropriate readiness for crisis situations also includes available and appropriate tools for feeding of personnel (Tulach & Foltin, 2019). In the first days of deployment for soldiers, members of rescue forces, or humanitarian workers, special long-shelf-life food packages, called *combat rations*, can be used as part of the logistical support of the operation. The standardisation agreement STANAG 2937 (2019) and the follow-up standard NATO AMedP 1.11 (2019) require a minimum storage life of components in the packages of at least 24 months at 25 °C.

Due to limitations on the ability to control temperature during transport and subsequent storage, sterilisation at temperatures of about 120 °C with a holding time of several dozen minutes is a preferred approach. From a nutritional point of view, it is highly desirable to include dairy products in these combat rations and national material reserves, particularly as a source of essential nutrients that support the physical endurance of the personnel. However, the range of dairy products that are stable at temperatures ≥25 °C is very narrow and, in practice, limited to condensed milk, powdered dairy powders, and sterilised processed cheeses (Lazárková et al., 2021).

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Preservation by heating food sealed in hermetic packaging has been one of the most effective and widespread methods of extending storage life in recent decades. At the same time, the organoleptic, nutritional, and functional properties of the heat-treated food should be preserved as much as possible (Wang et al., 2020).

Sterilisation heating significantly causes changes in colour, consistency, flavour, and thermolabile biologically active substances. The latter reactions are mainly related to the course of the complex of Maillard reactions (CMR) (Silva, Oliveira, & Hendrickx, 1993). However, CMR can also lead to the formation of process contaminants, which have received much attention in recent years. Many of these compounds may be carcinogenic and mutagenic (especially heterocyclic aromatic amines, such as pyridoimidazoles, pyridoindoles, or tetraazafluoranthenes). Several factors influence the rate of the CMR process, including the use of different raw materials for food production (and thus different proportions of proteins, fats, and mono-, di- and polysaccharides in the system), the water content and the water activity value (a_W), the heat treatment method, the temperature applied and the holding time for this temperature, the rate of the gradient of temperature increase and subsequent cooling, and many others (Iriondo-DeHond et al., 2020; Li et al., 2021; Rannou, Laroque, Renault, Prost, & Sérot, 2016).

Modelling heat transport (inside the product) by conduction to various foods has been the subject of many publications. However, in the case of dairy products, there are only very few studies available on the effect of sterilisation on the properties of processed cheese with a very limited range of DM and FDM content (practically only 38–40 g/100 g and 45–50 g/100 g, respectively) (Buňka, Štětina, & Hrabě, 2008; Lazárková et al., 2010). However, processed cheeses with a much wider variation in DM and FDM can be found on the market (c. 35–50 g/100 g and 35–50 g/100 g, respectively). Therefore, this study aimed to produce processed cheeses (PC) with a DM content of 30–55 g/100 g and a FDM content of 30–50 g/100 g and subject them to sterilisation at 120 °C for 40 min in a hermetically sealed container. First, the time courses of actual temperatures were modelled in individual processed cheeses using advanced regression analysis methods to describe the dependence of the heat treatment process on the DM and FDM content of the product. Subsequently, after 30 days of storage, microbiological, chemical, textural, viscoelastic and organoleptic parameters of non-sterilised (NPC) and sterilised (SPC) processed cheese were compared. The effect of the DM and FDM content of the products on the properties of the samples, including the CMR rate and lipid oxidation, was also evaluated to indicate possible chemical hazards to human health. Based on the results obtained, the activity focused on optimising the combination of DM and FDM content of SPC and assessing its quality and safety and, thus, its usability for logistic support in emergency/crisis situations and storage in state material reserves.

2. Material and methods

2.1. Samples preparation

For the manufacture of the model PC samples with a DM content of 30–55 g/100 g (six different proportions scaled by 5 g/100 g) and a FDM content of 30–50 g/100 g (three levels differed by 10 g/100 g), the following materials were used: (i) Dutch type cheese (52 g/100 g DM content and 30 g/100 g FDM content; Lacrum Ltd., Velké Meziříčí, Czech Republic), butter (84,5 g/100 g DM content, 83,1 g/100 g fat content; Lacrum Ltd., Velké Meziříčí, Czech Republic), water and emulsifying salts (Fosfa plc, Břeclav, Czech Republic). The formulation of PC is presented in Table S1. The total amount of emulsifying salts was calculated as a constant ratio of emulsifying salts to protein and set at the level of ≈0.15 according to Guinee and O'Callaghan (2013). A PC with 55 g/100 g DM content and 30 g/100 g FDM was not produced because the raw materials used (especially Dutch type cheese and its DM and FDM contents) did not allow the manufacture of a product with the required parameters mentioned above. Therefore, 17 types of PC were prepared. A Niromix 5 (Nirosta Ltd., Chlumec nad Cidlinou, Czech Republic) with indirect heating was used for the manufacture of the PC. The target temperature of 90 °C was held for 3 min and the agitation speed was set at 1500 rpm (pressure ≈ 20 kPa).

The hot melt (immediately after production) was poured into the laminated aluminium containers with seal lids (the sealing was carried out using the equipment NovaSeal – Nirosta Ltd., Chlumec nad Cidlinou, Czech Republic). The weight of the sample in the container was approximately 95 ± 2 g. Subsequently, the samples were divided into two groups: samples that were not sterilised (NPC) and samples that were sterilised according to the following conditions (SPC). The NPC were left to cool and stored at 6 ± 1 °C. The samples designated for sterilisation were cooled to 25 °C and sterilised at the same day the melt was manufactured.

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. A temperature of 120 °C and a holding time of 40 min were 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 and then the samples were cooled down to 25 ± 1 °C. The actual temperature in the container placed at the coldest point in the retort was recorded using Ellab TrackSense Pro datalogger (Ellab A/S, Hilleroed, Denmark) and evaluated by the ValSuite software (Ellab A/S, Hilleroed, Denmark). NPC and SPC samples were stored at 6 ± 1 °C and 25 ± 1 °C, respectively, until analyses (30 days after manufacturing). Each PC was produced three times; therefore, 102 lots in total were made (17 types of samples × 3 repetitions × 2 heat treatments [NPC and SPC]).

For a numerical presentation of the lethal effect of a combination of sterilising temperature and holding time, the sterility value at the coldest point (F_0) was used. The F_0-value was expressed in minutes of a heat treatment at a constant reference temperature (T_{ref} = 121.1 °C) using the z-value ($z = 10$ °C). The F_0-values were calculated according to Lazárková et al. (2011) using t_1 as the sterilisation time (minutes) when the temperature in the product first reached the temperature of 100 °C and t_2 as the sterilisation time (minutes) when the temperature in the product last reached the temperature of 100 °C.

2.2. Modelling of temperature-to-time curves and statistical analysis

The effect of the DM and FDM content of processed cheese on the actual temperature course (at the coldest point in the retort) of the individual products was assessed using advanced regression analysis methods. The empirical curves of the statistical relationship observed between the actual temperature T_c (°C) and sterilisation time t (minutes) are of an 'S shape' (see Fig. S1 below). This curve is suitable for modelling the dynamics of a wide range of time-dependent variables, e.g., for describing growth models (Richards, 1959) *or a generalised stock-production model (*Pella & Tomlinson, 1969).

In this paper, the 'S shaped' time curve of a special shape, namely the generalised logistic curve (Richards, 1959), is used to describe the actual temperature T_c (°C) as a function of the sterilisation time t. *This time curve is given by formula*

$$T_c = \frac{\beta_3}{(1 + e^{-\beta_2 t})^{\beta_1}}\ , \tag{1}$$

where β_1, β_2 and β_3 are the parameters determining the function (1) waveform. This process can be approximately assessed concerning the values of these parameters using: (i) The range over which the actual temperature values vary. For increasing values of the sterilisation time t, the values of the actual temperature Tc approach the value of the parameter β_3; (ii) Using the inflexion point of the function (1), where the convex waveform becomes concave. Therefore, the inflexion point

position allows us to approximately conclude what the curve (1) shape is, whether the actual temperature T_c increases rapidly with respect to the sterilisation time t or, on the contrary, is more gradual. The inflexion point of the function (1) corresponds to the value of the sterilisation time

$$t_{inf} = \beta_2^{-1} \ln \beta_1 \quad (2)$$

and the value of the actual temperature

$$T_{inf} = \beta_3 \left(1 + \beta_1^{-1}\right)^{-\beta_1} \quad (3)$$

Thus, for a fixed value of β_2, as the value of β_1 parameter increases, the value of t_{inf} (sterilisation time; equation (2)) increases and the value of T_{inf} (actual temperature; equation (3)) decreases. On the contrary, when β_1 is fixed and β_2 increases, the value of t_{inf} decreases and the value of T_{inf} does not depend on the value of β_2 parameter; (iii) when the sterilisation time t is in the vicinity of $t = t_{inf,}$ the actual temperature increase T_c per unit time is the highest.

Based on the datasets, the parameters β_1, β_2 and β_3 were estimated by using the nonlinear least squares method, and the standard deviation of the estimation was also determined. Next, the coordinates of the inflexion point t_{inf} and T_{inf} were estimated using the estimations of the parameters β_1, β_2 and β_3. The calculation was performed using MATLAB (version 2020b, The Math Works, Inc., Natick, Mass., USA), and the *fitnlm* function was used to estimate the parameters.

The results obtained from the analyses carried out after 30 days of storage and described in subsections 2.3–2.8 were evaluated using the Kruskal-Wallis and Wilcoxon tests ($\alpha = 0.05$). The chi-square test was applied for comparison of the fat droplet size of the NPC and SPC models. The correlation analysis was also carried out using the Spearman correlation coefficient. Unistat® 6.5 software (Unistat, London, UK) was used for statistical analysis.

2.3. Microbiological analysis

The total number of aerobic and/or facultative anaerobic mesophilic microorganisms was determined according to ISO 4833–1:2013 (ISO, 2013), the number of coliforms according to ISO 4832:2006 (ISO, 2006a), the number of aerobic and anaerobic spore-forming microorganisms according to Harrigan (1998) and the number of yeasts and/or moulds according to ISO 21527–1:2008 (ISO, 2008).

2.4. Chemical analysis

The DM, fat, and protein content were determined according to ISO 5534:2004 (ISO, 2004a), ISO 1735:2004 (ISO, 2004b) and ISO 8968–1:2014 (ISO, 2014), respectively. pH was measured using a pH meter equipped with a glass tip electrode (pH Spear, Eutech Instruments, Oakton, Malaysia) in the samples.

The ammonia content was determined by the method of Conway (Buňka, Hrabě, & Kráčmar, 2004). Lipid oxidation was evaluated using the 2-thiobarbituric acid method as thiobarbituric acid reactive substances (TBARS) value (Kristensen & Skibsted, 1999). The results were expressed as absorbance units per milligram of sample ($\lambda = 532$ nm for the red pigment and $\lambda = 450$ nm for the yellow pigment).

2.5. Rheological and texture analyses

Viscoelastic properties were determined using a dynamic oscillatory shear rheometer (Rheostress 1, Haake, Bremen, Germany) as described by Pluta-Kubica et al. (2021). The following modifications were applied: (i) samples were measured at a frequency ranging from 0.05 to 10.00 Hz, and (ii) the shear stress amplitude (5 Pa) was selected in the linear region of viscoelasticity in the samples with 30 g/100 g DM content and 40 and 50 g/100 g FDM content.

The textural properties of the model PC samples were evaluated using a TA.XTplus texture analyser (Stable Micro Systems Ltd., Godalming, UK) equipped with a 20 mm diameter cylindrical aluminium probe. Analysis was carried out by penetration into the sample (strain 25% and trigger force 5 g; deformation rate was 2 mm/s) at 20 ± 1 °C (the measurement was carried out within the container). From the force/time curves, the hardness value was obtained as the maximum force observed during penetration (N). Force versus time data were converted to an elongational viscosity and Hencky strain rate according to ISO/TS 17996:2006 (ISO, 2006).

2.6. Instrumental colour analysis

The HunterLab UltraScan® Pro Colour Measurement Spectrophotometer (Hunter Associates Laboratory, Inc., Reston, VA, USA) was used for instrumental colour evaluation. The CIE Lab colour scale ($L^*a^*b^*$) was used, with the illuminant D65 (standard daylight) and 10° angle. Parameters L* (luminosity; 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 reflectance mode, with specular reflection excluded, and using white (C6299 HunterLab Colour Standard) and grey (C6299G HunterLab Colour Standard) reference plates. A 10 mm quartz cuvette was used for the readings.

2.7. Scanning electron microscopy and analysis of the milk fat droplets size

The processed cheese for scanning electron microscopy (SEM) was processed as follows: samples of 35 × 25 × 2 mm were fixed in cacodylate buffer (0.2 mol/l) in glutaraldehyde (3.0ml/100 ml). After fixation, the samples were washed 3 times in cacodylate buffer (0.2 mol/l) for 15 min. The samples were resized to 25 × 2 × 2 mm and further fixed in osmium tetroxide (1.0 g/100 ml). After fixation, the samples were washed 3 times in cacodylate buffer (0.2 mol/l) for 15 min. It was followed by dehydration with an alcohol series (30ml/100 ml, 50ml/100 ml, 70ml/100 ml, 80ml/100 ml, 90ml/100 ml, 96ml/100 ml, 100ml/100 ml) at 15-min intervals. The dehydrated samples were fractured in liquid nitrogen and subsequently dried at an Emitech K850 critical point (Quorum Technologies, Laughton, United Kingdom). Samples were coated with 10 nm gold Q150R ES (Quorum Technologies, Laughton, United Kingdom) and imaged in 6 frames using SEM MIRA3 (Tescan plc, Brno, Czech Republic). Image processing and analysis were performed using a modified method according to Impoco, Carrato, Caccamo, Tuminello, and Licitra (2007). The fat globules were selected by thresholding at a 16-bit grayscale. Binary operations included open 3 × 3, fill hole, smooth 2 × 2, linear close 1 × 3. In the images, the morphological parameters (Feret diameter 90; μm; mean ± standard error) of the fat globules were measured using NIS Element software in 5.2 (Laboratory Imagine, Prague, Czech Republic).

2.8. Sensory analysis

Sensory evaluation was carried out by a panel of 12 selected assessors and experts trained according to ISO 8586:2012 (ISO, 2012). The following scales were used for the assessment of NPC and SPC: a nine-point scale (1-excellent, 5-good, 9-unacceptable) for appearance, consistency and flavour; a nine-point scale (1-soft, 4-medium, 9-extra hard) for hardness; and a nine-point scale (1-negligible, 5-medium, 9-excessive) for off-flavour. The samples were served in random order and at a controlled temperature of 20 ± 2 °C in a sensory laboratory equipped with sensory booths (under normal light conditions) according to ISO 8589 (ISO, 2007). Water was provided to rinse the mouth between the evaluation of the PC samples tested to avoid carryover effects.

3. Results

3.1. Analysis of temperature vs. time data dependence in the autoclave

The observed empirical curves of the actual temperature to the time of heat treatment were interpolated with the model function (1), the parameters β_1, β_2 and β_3 and the standard error of their estimation was estimated. The results are shown in Table 1. Next, for each curve, its inflexion points were determined, the coordinate of the inflexion point corresponding to the sterilisation time was denoted as t_{inf}, the coordinate corresponding to the actual temperature was denoted as T_{inf}, and the calculated values of these two coordinates are also shown in Table 1. A graphical representation of the empirical and estimated model curves for selected sterilised processed cheeses is presented in Fig. S1 in the Supplementary files. There is perfect agreement between the empirical data curves and the model curves.

Finally, the observed model curves were characterised using inflexion points. The results are shown in Table 1 and graphically in Fig. S1 (Supplementary files). The use of the inflexion point to describe the shape of the regression function, together with the graphical representation of the individual regression functions (both empirical and model), leads to the conclusion that for most DM contents, the sterilisation time with the highest rate of increase in sterilisation temperature in a container (t_{inf}) was slightly lower for samples with 50 g/100 g FDM compared to other FDMs (Table 1).

For all SPCs (regardless of DM and FDM content), sterilisation heating at 120 °C for a holding time of 40 min resulted in sterility values (F_0) above 10 min ($P < 0.05$; data not shown). Samples with an FDM content of 50 g/100 g typically showed a lower value of F_0 ($P < 0.05$) compared to PC with a lower FDM content (but still at $F_0 > 10$ min). This finding is consistent with the results obtained using the analysis of the time curves and the coordinates of their inflexion points (in particular, the values t_{inf}, see above).

3.2. Results of microbiology analysis

For all NPC samples stored at 6 °C for 30 days, the total number of aerobic and/or facultative anaerobic mesophilic microorganisms was recorded at 1.31–2.95 log cfu/g (colony forming units per gram of the tested sample; data not shown). The number of aerobic and anaerobic spore-forming microorganisms ranged from 1.12 to 2.37 log cfu/g (data not shown). Coliforms and yeasts and/or moulds were not detected in any NPC samples. The SPC samples did not show the presence of any of the five indicator microorganism groups specified in section 2.3 (below the detection limit). Therefore, the designed sterilisation temperature of 120 °C with a 40-min holding time ensured the practical sterility of the processed cheese under the given conditions, regardless of the DM and FDM content.

3.3. Results of chemical analysis

Table S2 (Supplementary files) shows the measured values of DM, fat content, and protein content of the model samples. PCs were made with DM, fat and protein content that were not significantly different ($P \geq 0.05$) from the calculated raw material composition (see Table S1). The DM, fat, and protein contents did not change significantly with sterilising heating ($P \geq 0.05$).

The pH values of PC samples (Fig. S2 in Supplementary files) decreased with increasing DM content ($P < 0.05$) and conversely increased with increasing FDM content ($P < 0.05$). Sterilisation heating

Table 1
Estimations of parameters β_1, β_2 and β_3, generalised logistic curve (according to Equation (1)) including its coordinates of inflexion point (t_{inf}, T_{inf}) for sterilised processed cheese (SPC) with dry matter content 30–55 g/100 g and fat in dry matter content between 30 and 50 g/100 g (n = 3).

Dry matter Content (g/100 g)	Fat in dry matter content (g/100 g)	Parameters			Inflexion point (coordinates)	
		β_1	β_2	β_3	Actual sterilisation time t_{inf} (min; x-axis)	Actual temperature T_{inf} (°C; y-axis)
30	30	3.361 ± 0.001	0.149 ± 0.001	119.360 ± 0.007	8.136	49.736
	40	2.797 ± 0.002	0.127 ± 0.001	119.178 ± 0.013	8.099	50.687
	50	3.992 ± 0.006	0.117 ± 0.001	119.487 ± 0.036	11.832	48.951
35	30	3.361 ± 0.001	0.149 ± 0.001	119.360 ± 0.007	8.136	49.736
	40	3.214 ± 0.002	0.123 ± 0.001	120.177 ± 0.013	9.492	50.315
	50	2.737 ± 0.004	0.175 ± 0.001	119.004 ± 0.028	5.754	50.744
40	30	3.466 ± 0.002	0.132 ± 0.001	118.165 ± 0.013	9.417	49.081
	40	3.418 ± 0.002	0.133 ± 0.001	119.825 ± 0.012	9.241	49.842
	50	3.506 ± 0.006	0.140 ± 0.001	119.776 ± 0.034	8.961	49.692
45	30	3.522 ± 0.003	0.141 ± 0.001	117.967 ± 0.018	8.929	48.919
	40	3.208 ± 0.003	0.114 ± 0.001	118.656 ± 0.021	10.225	49.688
	50	3.657 ± 0.004	0.146 ± 0.001	119.238 ± 0.020	8.881	49.260
50	30	2.185 ± 0.003	0.149 ± 0.001	116.584 ± 0.032	5.246	51.174
	40	3.377 ± 0.006	0.193 ± 0.001	119.453 ± 0.026	6.306	49.750
	50	4.314 ± 0.009	0.131 ± 0.001	118.053 ± 0.038	11.159	48.027
55	40	3.061 ± 0.008	0.130 ± 0.001	117.223 ± 0.054	8.606	49.341
	50	3.296 ± 0.003	0.141 ± 0.001	118.097 ± 0.019	8.459	49.311

at 120 °C for a holding time of 40 min caused a decrease in pH values in the interval of 0.10–0.20 (Fig. 1; $P < 0.05$).

With increasing DM content, both ammonium content (Fig. S2 in Supplementary files; $P < 0.05$) and TBARS value (Fig. S2 in Supplementary files, $P < 0.05$) increased in NPCs. For samples with increasing FDM (with constant DM content), decreasing ammonium content ($P < 0.05$) but increasing TBARS value levels ($P < 0.05$) were observed. As a result of sterilisation, both the ammonia content and the TBARS value increased significantly ($P < 0.05$), the change rate increasing significantly ($P < 0.05$) with increasing DM content in PCs.

3.4. Results of rheological and texture analyses

The results of the G* (Pa) and δ (°) for a reference frequency of 1 Hz are shown in Fig. 1. The course of the G' and G" moduli for selected samples and a frequency range (f) of 0.1–10.0 Hz are shown in Fig. S3 (Supplementary files). The G* values (Fig. 1) increased significantly with increasing DM content (at constant FDM; $P < 0.05$) and, conversely, decreased with increasing FDM content (at constant DM content; $P < 0.05$). The firmness of the sample (G* values) increased significantly ($P < 0.05$) after applying the sterilising heating. The δ values showed (Fig. 1) that with increasing DM content and decreasing FDM content, the samples exhibited a more solid-like behaviour than liquid-like behaviour ($P < 0.05$). A decrease in δ values was also observed for SPC samples compared to NPCs ($P < 0.05$; products with the corresponding DM content and the FDM content were always evaluated).

Firmness values (F_{max}; N; Fig. 1) increased significantly with increasing DM content ($P < 0.05$) and, conversely, decreased with decreasing FDM content ($P < 0.05$). PC sterilisation increased the firmness of the products studied ($P < 0.05$) for all combinations of DM content and FDM studied.

The results of a deeper analysis of the stress curve (*force vs. time curves*) until F_{max} is reached are shown in Fig. 2, which presents the dependence of the elongational viscosity (Pas) on Hencky strain rate (s^{-1}). A sterilisation temperature of 120 °C for a 40-min holding time led to a significant increase in the elongational viscosity (for illustration, see parts A of Fig. 2; $P < 0.05$). An initial sharp increase in the elongational viscosity is evident, corresponding to the transient flow regimes, followed by an approximately linear part corresponding to the squeezing flow regime. The linear parts of the samples' curves remained almost horizontal, with the elongational viscosity becoming practically independent of Hencky strain rate. In Fig. 2, parts B–D, it is clear that with decreasing DM content and increasing FDM content, elongational viscosity decrease significantly ($P < 0.05$).

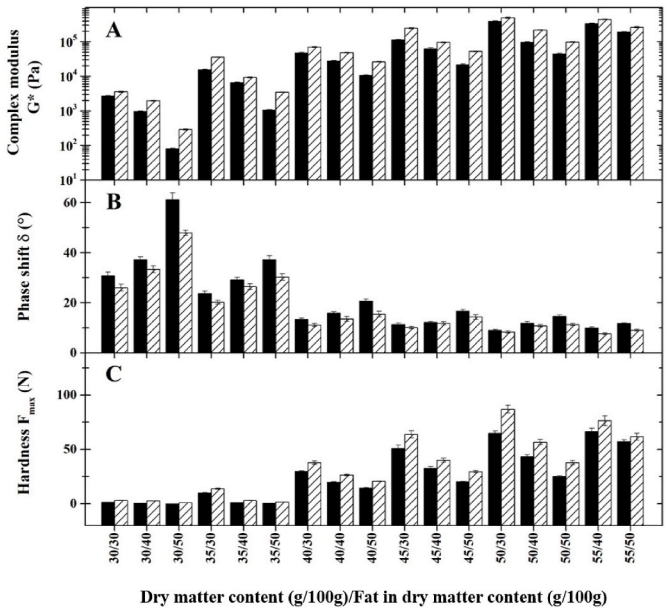

Fig. 1. Results of the complex modulus G* (Pa), the phase shift δ (°) and the hardness F_{max} (N) in non-sterilised processed cheese (NPC; black columns) and sterilised processed cheese (SPC; cross-hatched columns) with dry matter content of 30–55 g/100 g and fat in dry matter content 30–50 g/100 g. The results are expressed as means ± standard deviation (n = 27).

3.5. Results of instrumental colour analysis and analysis of the milk fat droplets size

Fig. 3 showed that luminosity did not differ significantly for individual NPC samples with 30 and 40 g/100 g FDM content, regardless of their DM content ($P \geq 0.05$). NPCs with 50 g/100 g FDM showed significantly lower luminosity compared to products with lower values of FDM ($P < 0.05$). In the case of chromaticity on the green-red and blue-yellow axes, the results (Fig. 3) showed that there was a significant shift to the red and yellow regions, respectively, as the DM content and FDM increased ($P < 0.05$). The applied sterilising heating caused a significant decrease in luminosity ($P < 0.05$) and a shift of chromaticity to the red or yellow regions ($P < 0.05$). These changes due to heat treatment showed an increasing change rate ($P < 0.05$) with increasing DM content of PCs (samples with constant FDM were compared).

By comparing projections (Feret's diameters 90; μm) of fat globules, its size was found to increase with increasing FDM content ($P < 0.05$; data not shown) and, conversely, to decrease significantly with increasing DM content ($P < 0.05$; data not shown). Applied sterilising heating did not significantly affect the size of the fat globules for any variation in DM content or FDM ($P \geq 0.05$). Fig. S4 (Supplementary files) shows the size and distribution of fat globules in selected sterilised processed cheeses and includes the values of Feret's diameters 90 (μm).

3.6. Results of sensory analysis

The sensory analysis results are presented in Table S3 (Supplementary files). Appearance and flavour were rated excellent or very fine for most NPCs regardless of DM content and FDM content ($P \geq 0.05$). As the samples were presented to the assessors as spreadable processed cheese, the consistency of the NPCs with a DM content of 30 g/100 g was evaluated as poor or very poor, mainly due to high fluidity ($P < 0.05$). Samples with 50 and 55 g/100 g of DM content ($P < 0.05$) were evaluated to be poor to unacceptable NPCs due to high firmness. For an FDM content of 30 g/100 g, the samples with DM contents of 40 and 45 g/100 g were evaluated to be less good or poor. On the contrary, NPCs ($P < 0.05$) with 40 g/100 g of DM content and 50 g/100 g of FDM content (very fine), 35 g/100 g of DM content and 40 g/100 g of FDM content (fine) and 50 g/100 g FDM content (very good) were better evaluated. All PCs showed an increase in firmness as a result of sterilisation ($P < 0.05$), which was evaluated to result in worse consistency in most of the SPC samples compared to the corresponding NPCs ($P < 0.05$). The SPC sample with 40 g/100 g of DM content and 50 g/100 g of FDM content was considered the best in terms of consistency. The applied sterilising heating also caused a deterioration in flavour ($P < 0.05$), mainly due to the off-flavour occurrence.

4. Discussion

The applied sterilisation heating of 120 °C for a 40-min holding time led to the sterility value $F_0 > 10$, provides a good prerequisite for the long-term storage life of products even at temperatures ≈25 °C (Harrigan, 1998; Wang et al., 2020). Practical sterility was also confirmed by microbiological analysis, where no spores of aerobic and anaerobic bacteria, moulds, or yeasts were detected after application of the sterilising treatment. Therefore, applied heating can be recommended for industrial application and to obtain (from a microbiological point of

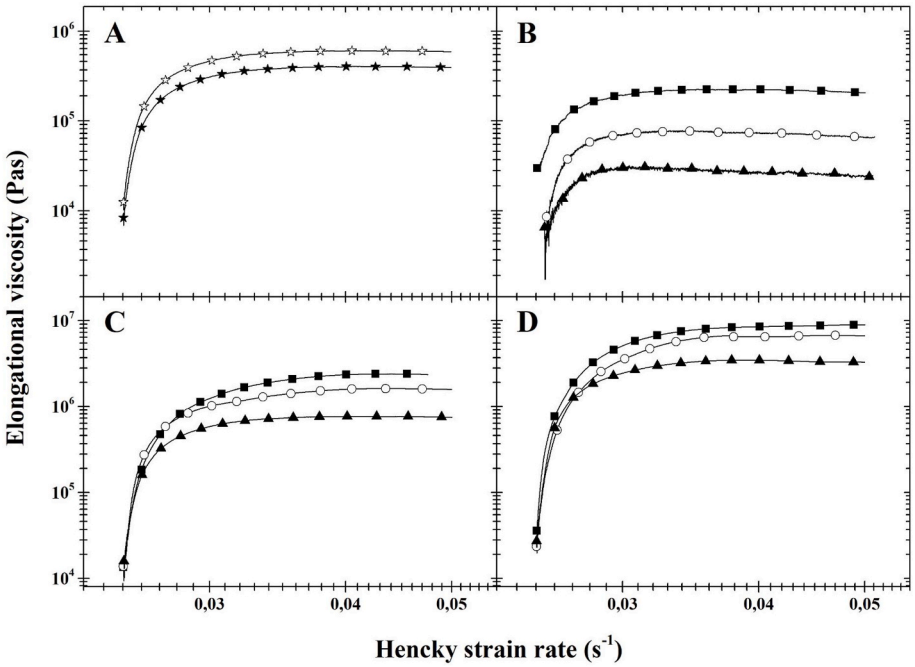

Fig. 2. The dependence of elongational viscosity (Pa·s) on Hencky strain rate (s^{-1}) of non-sterilised (★) and sterilised (☆) processed cheese samples with 35 g/100 g dry matter (DM) content and 50 g/100 g fat in dry matter (FDM) content (Part A). The dependence of corrected stress (Pa) on Hencky strain (dimensionless) of sterilised processed cheese with 30 g/100 g FDM content (■), 40 g/100 g FDM content (◯) and 50 g/100 g FDM content (▲): Part B – samples with 30 g/100 g DM content; Part C – samples with 4 g/100 g DM content; and Part D – samples with 50 g/100 g DM content (n = 27).

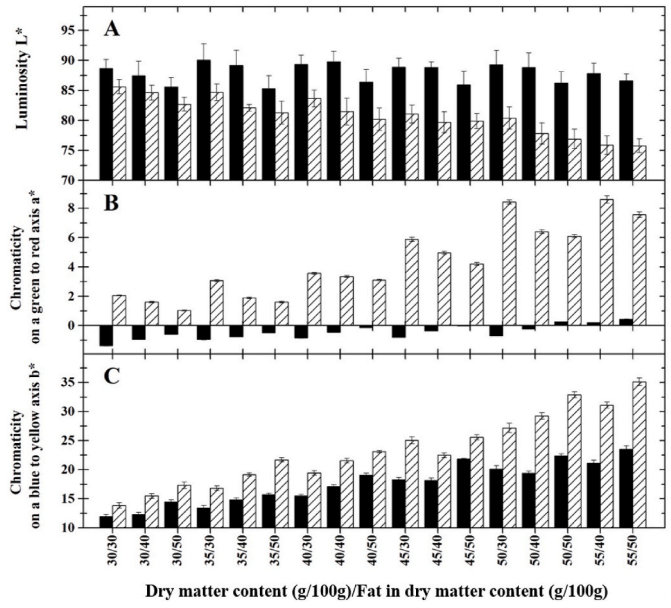

Fig. 3. Results of Luminosity (L*), chromaticity on a green to red axis (a*) and chromaticity on a blue to yellow axis (b*) in non-sterilised processed cheese (NPC; black columns) and sterilised processed cheese (SPC; cross-hatched columns) with dry matter content of 30–55 g/100 g and fat in dry matter content of 30–50 g/100 g. The results are expressed as means ± standard deviation (n = 27).

view) health-safe SPC in a wide range of DM content (30–55 g/100 g) and FDM content (30–50 g/100 g).

The shape of the regression function, simplified by the inflexion point, corresponds well to the graphical waveform of the observed processes and allows for a simple assessment of the influence of the DM content and FDM values on the current sterilisation temperature in the container as a function of sterilisation time. Analysis of the measured parameters and the estimated inflexion points concludes that the values of the DM content and the corresponding FDM content influence the temperature increase in each case. Thus, the analysis of the results shows that the t_{inf} parameter is a good characteristic for comparing the progress of thermal processes of this type and can be recommended for further usage.

PC sterilisation resulted in an increase in ammonia concentration and TBARS value, which corresponds to the findings of Buňka et al. (2004), who analysed sterilised processed cheese with 40 g/100 g DM and 45 g/100 g FDM and Lazárková et al. (2011), who tested sterilised processed cheese with 38 g/100 g DM and 50 g/100 g FDM. The ammonia content is a significant marker of the progress of CMR that involves, among others, Strecker degradation of amino acids, the product of which is ammonia (Iriondo-DeHond et al., 2020; Li et al., 2021). Increasing TBARS value indicates the amount of secondary lipid oxidation products and describes the rate of oxidation reactions in food (Kristensen, Hansen, Arndal, Trinderup, & Skibsted, 2001; Kristensen & Skibsted, 1999). A higher protein and fat content may provide higher concentrations of precursors (especially amino acids and fatty acids) for the CMR as well as lipid oxidation processes (Friedman, 1996; Kristensen & Skibsted, 1999).

The results clearly showed that sterilisation significantly affected the organoleptic quality of the PC due to several physical processes and, especially, chemical reactions (Friedman, 1996; Lazárková et al., 2021).

The course of the chemical reactions, markers of which were tested, significantly affected the appearance and colour of the PC. As a result of sterilisation, the luminosity of PC decreased, and the chromaticity shifted from green to red and from blue to yellow, corresponding to the findings of Buňka et al. (2008), who tested sterilised processed cheese with 38 g/100 g DM and 45 g/100 g FDM or Wang et al. (2020), who analysed sterilised coconut milk. The intensity of luminosity decrease and the chromaticity shift from green to red and from blue to yellow increased significantly with increasing protein and fat content in the PC matrix. The latter phenomena can be explained by CMR and lipid oxidation reactions, as evidenced by the high correlation coefficients between the change in markers of these interactions and the evolution of PC colour characteristics (0.8923–0.9305; $P < 0.05$). Browning of sterilised processed cheese during storage was also observed in the work of Fan et al. (2014) who proposed the use of vitamin E, citric acid and L-Cysteine as a browning inhibitor. Changes in the instrumentally determined colour profile of the SPC were confirmed by sensory evaluation. All SPCs have been reported to have a deterioration in

appearance compared to the corresponding NPC, which can negatively affect the acceptability of the products by consumers. In terms of appearance, SPC with a DM content of 35 and 40 g/100 g and FDM of 50 g/100 g were considered the best.

Increasing protein content and decreasing fat content resulted in a significant decrease in the dissipation of strain (mechanical) energy into heat, which was reflected, for example, by a shift in the transient flow regime to a squeezing flow regime (when analysing the response of samples under large-scale strains) or a decrease in the value of δ values. It is probably the result of a more compact network due to the multiplication of interactions between the protein chains present. The lower fat content (acting as a lubricating agent) results in the formation of smaller fat globules in the final PC matrix, less intensively disrupting the protein matrix continuity (Guinee & O'Callaghan, 2013; Pluta-Kubica et al., 2021). The slightly lower pH, which was closer to the isoelectric point of the caseins present, probably contributed to the more intense crosslinking of the PC with a higher protein content and a lower fat content (Guinee & O'Callaghan, 2013; Pluta-Kubica et al., 2021).

Regardless of the DM content and FDM content, the sterilisation of PC resulted in an increase in the product firmness, which was demonstrated both by the application of instrumental small- and large-scale strains and by sensory analysis. Similar conclusions were also reached by Buňka et al. (2008) for SPC with a DM content of ≈40 g/100 g and an FDM content of ≈45 g/100 g. The increase in the gel strength of the studied matrix due to sterilisation can be explained by the aggregation processes of caseins at higher temperatures, resulting in a multiplication of interactions between proteins within the casein network. The CMR, during which new bonds between proteins may be formed (e.g., isopeptide bonds through the ε-amino group of lysine), may have contributed to the increase in the intensity of binding between proteins (Friedman, 1996; Lazárková et al., 2010; Li et al., 2021; Wang et al., 2020). From the point of view of organoleptic properties, SPC with 40 g/100 g of DM content and 50 g/100 g of FDM content was found to be the best in terms of consistency, and it can be recommended for further testing or practical usage. However, for this FDM, further experiments should also consider the lipid oxidation process or the possibilities to eliminate these undesirable interactions to the maximum extent.

Sterilisation resulted in a deterioration of flavour at all levels of DM content and FDM content tested. According to Friedman (1996), Kristensen and Skibsted (1999), Lazárková et al. (2010) and Wang et al. (2020), the occurrence of off-odours and off-flavours can be considered as a consequence of the course of the CMR and lipid oxidation reactions described above. The latter interactions can result in the formation of many organoleptic active substances with also possible adverse health effects on consumers (e.g. carbonyl compounds, heterocyclic compounds, etc.).

Taking into account the organoleptic quality of the SPC, its biological and, in particular, chemical safety, the results of this study suggest that a product with a combination of 40 g/100 g of DM content and 50 g/100 g of FDM is optimal. SPC with designed parameters could contribute to enhance the logistic support of soldiers and/or members of military, rescue, and/or humanitarian teams in foreign missions and could be appropriate and useful also for state material reserves.

CRediT authorship contribution statement

Alena Jedounková: Methodology, Investigation, Formal analysis, Writing – original draft. **Zuzana Lazárková:** Methodology, Investigation, Writing – review & editing, (including final approval of the version to be submitted). **Lucie Hampelová:** Methodology, Data curation, Formal analysis, Writing – review & editing, (including final approval of the version to be submitted). **Vendula Kůrová:** Methodology, Writing – review & editing, (including final approval of the version to be submitted). **Matej Pospiech:** Methodology, Investigation, Writing – review & editing, (including final approval of the version to be submitted). **Leona Buňková:** Methodology, Investigation, Writing – review & editing, (including final approval of the version to be submitted). **Pavel Foltin:** Methodology, Writing – review & editing, (including final approval of the version to be submitted). **Richardos Nikolaos Salek:** Methodology, Investigation, Writing – review & editing, (including final approval of the version to be submitted). **Jiří Malíšek:** Methodology, Writing – review & editing, (including final approval of the version to be submitted). **Jaroslav Michálek:** Methodology, Data curation, Formal analysis, Writing – review & editing, (including final approval of the version to be submitted). **František Buňka:** Conceptualization, and design of the study, Data curation, Formal analysis, Visualization, Supervision, Project administration, Writing – original draft.

Declaration of competing interest

None.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2022.114135.

References

Buňka, F., Hrabě, J., & Kráčmar, S. (2004). The effect of sterilisation on amino acid contents in processed cheese. *International Dairy Journal, 14*, 829–831. https://doi.org/10.1016/j.idairyj.2004.02.008

Buňka, F., Štětina, J., & Hrabě, J. (2008). The effect of storage temperature and time on the consistency and colour of sterilized processed cheese. *European Food Research and Technology, 228*, 223–229. https://doi.org/10.1007/s00217-008-0926-7

Fan, J., Zhang, J., Li, H., Jiang, S., Gao, Y., & Zhang, L. (2014). Study of methods of inhibiting browning of stored sterilized processed cheese. *Journal of Chinese Institute of Food Science and Technology, 14*(1), 204–212.

Friedman, M. (1996). Food browning and its prevention: An overview. *Journal of Agricultural and Food Chemistry, 44*(3), 632–653. https://doi.org/10.1021/jf950394r

Guinee, T. P., & O'Callaghan, D. J. (2013). Effect of increasing the protein-in-water ratio and reducing fat content on the chemical and physical properties of processed cheese product. *Journal of Dairy Science, 96*, 6830–6839. https://doi.org/10.3168/jds.2013-6685

Harrigan, W. F. (1998). *Laboratory methods in food microbiology* (3rd ed.). London, UK: Academic Press.

Impoco, G., Carrato, S., Caccamo, M., Tuminello, L., & Licitra, G. (2007). Quantitative analysis of cheese microstructure using SEM ImageryL. Puccio (Ed.). *Communications to SIMAI Congress, 2*, 1–10. https://doi.org/10.1685/CSC06096

Iriondo-DeHond, A., Elizondo, A. S., Iriondo-DeHond, M., Ríos, M. B., Mufari, R., Mendiola, J. A., et al. (2020). Assessment of healthy and harmful maillard reaction products in a novel coffee cascara beverage: Melanoidins and acrylamide. *Foods, 9*, 620. https://doi.org/10.3390/foods9050620

ISO (International Organization for Standardization). ISO/TS Standard No. ISO 8589. (2007). *Sensory analysis — general guidance for the design of test rooms*. Geneva, Switzerland: ISO.

ISO (International Organization for Standardization). (2014). *Milk and milk products – determination of nitrogen content – Part 1: Kjeldahl principle and crude protein calculation*. Geneva, Switzerland: ISO. ISO/TS Standard No. ISO 8968-1.

ISO (International Organization for Standardization). (2004a). *Cheese and processed cheese – determination of the total solid content (reference method)*. Geneva, Switzerland: ISO. ISO Standard No. 5534.

ISO (International Organization for Standardization). (2004b). *Cheese and processed cheese products – determination of fat content – gravimetric method (Reference method)*. Geneva, Switzerland: ISO. ISO Standard No. 1735.

ISO (International Organization for Standardization). (2006a). *Microbiology of food and animal feeding stuffs – horizontal method for the enumeration of coliforms – colony-count technique*. Geneva, Switzerland: ISO. ISO/TS Standard No. 4832.

ISO (International Organization for Standardization). (2006b). *Cheese – determination of theological properties by uniaxial compression at constant displacement rate*. Geneva, Switzerland: ISO. ISO/TS Standard No. 17996.

ISO (International Organization for Standardization). (2008). ISO standard No. 21527-1. In *Microbiology of food and animal feeding stuffs – horizontal method for the enumeration of yeasts and moulds – Part 1: Colony count technique in products with water activity greater than 0* (Vol. 95). Geneva, Switzerland: ISO.

ISO (International Organization for Standardization). (2012). *Sensory analysis – general guidelines for the selection, training and monitoring of selected assessors and expert sensory assessors*. Geneva, Switzerland: ISO. ISO/TS Standard No. 8586.

ISO (International Organization for Standardization). (2013). *Microbiology of the food chain – horizontal method for the enumeration of microorganisms – Part 1: Colony count at 30 °C by the pour plate technique*. Geneva, Switzerland: ISO. ISO/TS Standard No. 4833-1.

Kristensen, D., Hansen, E., Arndal, A., Trinderup, R. A., & Skibsted, L. H. (2001). Influence of light and temperature on the colour and oxidative stability of processed cheese. *International Dairy Journal, 11*, 837–843. https://doi.org/10.1016/S0958-6946(01)00105-4

Kristensen, D., & Skibsted, L. H. (1999). Comparison of three methods based on electron spin resonance spectrometry for evaluation of oxidative stability of processed cheese. *Journal of Agricultural and Food Chemistry, 47*, 3099–3104. https://doi.org/10.1021/jf981396p

Lazárková, Z., Buňka, F., Buňková, L., Holáň, F., Kráčmar, S., & Hrabě, J. (2011). The effect of different heat sterilization regimes on the quality of canned processed cheese. *Journal of Food Process Engineering, 34*, 1860–1878. https://doi.org/10.1111/j.1745-4530.2009.00376.x

Lazárková, Z., Buňka, F., Buňková, L., Valášek, P., Kráčmar, S., & Hrabě, J. (2010). Application of different sterilizing modes and the effect on processed cheese quality. *Czech Journal of Food Sciences, 28*, 168–176. https://doi.org/10.17221/44/2008-CJFS

Lazárková, Z., Šopík, T., Talár, J., Purevdorj, K., Salek, R. N., Buňková, L., et al. (2021). Quality evaluation of white brined cheese stored in cans as affected by the storage temperature and time. *International Dairy Journal, 121*, Article 105105. https://doi.org/10.1016/j.idairyj.2021.105105

Li, Y., Wu, Y., Quan, W., Jia, X., He, Z., Wang, Z., et al. (2021). Quantitation of furosine, furfurals, and advanced glycation end products in milk treated with pasteurization and sterilization methods applicable in China. *Food Research International, 140*, Article 110088. https://doi.org/10.1016/j.foodres.2020.110088

NATO STANAG 2937. (2019). *Requirements of individual operational rations for military use* Version 5. .

NATO Standard AMedP-1.11. (2019). *Requirements of individual operational rations for military use*. Edition B Version 1.

Pella, J. S., & Tomlinson, P. K. (1969). A generalised stock-production model. *Inter-American Tropical Tuna Commission Bulletin, 13*, 421–496. https://aquadocs.org/handle/1834/21263.

Pluta-Kubica, A., Černíková, M., Dimitreli, G., Nebesářová, J., Exarhopoulos, S., Thomareis, A. S., et al. (2021). Influence of the melt holding time on fat droplet size and the viscoelastic properties of model spreadable processed cheeses with different compositions. *International Dairy Journal, 113*, Article 104880. https://doi.org/10.1016/j.idairyj.2020.104880

Rannou, C., Laroque, D., Renault, E., Prost, C., & Sérot, T. (2016). Mitigation strategies of acrylamide, furans, heterocyclic amines and browning during the Maillard reactions in foods. *Food Research International, 90*, 154–176. https://doi.org/10.1016/j.foodres.2016.10.037

Richards, F. J. (1959). A flexible growth function for empirical use. *Journal of Experimental Botany, 10*, 290–300. https://doi.org/10.1093/jxb/10.2.290

Silva, C. L. M., Oliveira, F. A. R., & Hendrickx, M. (1993). Modelling optimum processing conditions for the sterilization of prepacked foods. *Food Control, 4*(2), 67–78. https://doi.org/10.1016/0956-7135(93)90160-P

Tulach, P., & Foltin, P. (2019). Research methods in humanitarian logistics – current approaches and future trends. In D. Dujak (Ed.), *Business logistics in modern management. Osijek, Croatia: Ekonomski fakultet osijeku-fac economics osijek*.

Wang, W., Chen, H., Ke, D., Chen, W., Zhong, Q., Chen, W., et al. (2020). Effect of sterilization and storage on volatile compounds, sensory properties and physicochemical properties of coconut milk. *Microchemical Journal, 153*, Article 104532. https://doi.org/10.1016/j.microc.2019.104532