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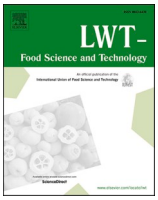

Evaluation of the viscoelastic properties of pork liver pâté during sterilisation observed *in situ*

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A R T I C L E I N F O

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A B S T R A C T

The first aim of the study was to observe *in situ* changes in the viscoelastic properties of pork liver pâté during an increase in temperature (the target temperature of 122 °C), holding it (10 min), and the subsequent cooling thereof using a dynamic oscillatory rheometry equipped with a pressure cell. At the same time, the samples were treated according to the same sterilisation mode in an autoclave. The second objective was to characterise the physical, chemical and microscopic properties of the sterilised pork liver pâté samples after 7 days of storage at 5 °C. A sharp decrease in the values of the storage (G'; Pa) and loss (G''; Pa) moduli up to ≈58 °C was followed by a stagnation of these parameters up to ≈70 °C and a further increase in G' and G'' up to ≈85 °C. After cooling the sterilised samples, the G' and G'' values were significantly higher than those of the original untreated sample. Knowledge about the course of the viscoelastic moduli development during sterilisation and subsequent cooling and the quality characteristics of final food products is crucial for the control of these processes during manufacturing and for information important for pipeline transport. In the future, selected hydrocolloids could be evaluated as possible substances for confirmation of their effect on the viscoelastic properties during sterilisation.

1. Introduction

Pork liver pâté is a globally popular emulsified meat product characterised by its taste, convenience, availability, and nutritional composition. It is a cooked meat product composed mainly of backfat, water or broth, raw liver, cooked muscle tissue, salt, and possibly a small number of other components such as additives and/or spices. Pasteurised liver pâtés with a minimum shelf life of several weeks can be found on the market. Regarding the pH value, bacterial spores (especially representatives of the genera *Bacillus*, *Geobacillus* and *Clostridium*) must be inactivated to prolong shelf life, which is achieved mainly by heat sterilisation (heating equivalent to 121.1 °C for a duration of 10 min for a z-value ≈10 °C). Products treated in this way can be stored for several years at ambient temperature (Cao et al., 2022; Delgado-Pando, Cofrades, Ruiz-Capillas, Triki, & Jiménez-Colmenero, 2012; Rezler, Krzywdzińska-Bartkowiak, & Piatek, 2021). Sterilised liver pâtés also form part of packaged food rations (combat rations), which are subject to a minimum shelf-life requirement of 24 months at 25 °C within the North Atlantic Treaty Organisation (NATO) (STANAG 2937, 2019; NATO Standard AMedP 1.11, 2019). The requirement to meet NATO standards is receiving increasing attention in this period of natural and anthropogenic turbulence in relation to the effective provision of food to troops operating in crisis situations or catering for humanitarian or military missions (Tulach & Foltin, 2019).

Liver spreads (more generally, emulsified meat products) are produced by milling the ingredients of the raw material composition in order to obtain a dispersion of water, fat, and protein, i.e. to obtain a stable oil-in-water emulsion. Muscle tissue proteins can be divided into

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three groups - myofibrillar, sarcoplasmic, and connective tissue proteins. Actin and myosin (myofibrillar proteins) and collagen (part of connective tissue) are typical fibrous proteins. Myofibrillar proteins play an important role in the desired structure of cooked meat products due to their ability to form three-dimensional gels. Liver proteins (mainly albumin, α-lipoprotein, oromucoid and others) are crucial for the formation of a stable pork liver pâté structure because they have emulsifying properties and promote the formation of a specific three-dimensional matrix that stabilises water and fat in the meat product system (Amako & Xiong, 2001; Delgado-Pando et al., 2012; Morris & Wilde, 1997; Retzler et al., 2021; Tiensa, 2017; Tornberg, 2005; Yilmaz, Vural, & Yadigari, 2017; Zhang et al., 2022).

One of the most important processes occurring during heat treatment is the denaturation of proteins leading to their aggregation, the formation of protein-protein bonds, and the formation of a three-dimensional system-gel (Amako & Xiong, 2001; Tornberg, 2005). Temperatures used in pasteurisation and/or sterilisation lead to conformational changes in the secondary and tertiary structures of proteins, which are collectively referred to as denaturation processes. Using differential scanning calorimetry (DSC), three temperature ranges have been identified in many publications where denaturation occurs for different groups of proteins: 54–58 °C for myosin; 65–75 °C for collagen (a range of 53–63 °C was also published for collagen) and sarcoplasmic; 78–83 °C for actin. However, the course of denaturation and the formation of the gel stabilising the water and fat present depend on the specific composition of the liver pâté and the manufacturing process. The latter processes can be monitored, for example, by rheological methods by measuring the storage (G'; Pa) and loss (G''; Pa) moduli during heat treatment (Amako & Xiong, 2001; Brunton, Lyng, Zhang, & Jacquier, 2006; Tornberg, 2005; Yilmaz et al., 2017).

Most of the available literature is devoted to monitoring the evolution of the storage and loss moduli of meat batters (MB; heat-untreated raw materials after mixing) and during heating in the range of 5–90 °C, ensuring pasteurisation effect but without inactivation of bacterial spores. The authors generally described that heating to 55–60 °C causes a slight decrease or stagnation of the storage and loss moduli. Further increases in temperature (generally >55 °C) typically increased the stiffness of the product, mainly due to continued denaturation of proteins and their aggregation leading to the formation of a three-dimensional matrix (gel). However, the specific temperatures in each production run depended on the raw material composition and the process parameters (Brunton et al., 2006; Delgado-Pando et al., 2012; Li et al., 2015; Wei, Li, Wang, Kang, & Ma, 2019). Moreover, most publications are mainly dedicated to different types of sausages, the scenario for liver pâtés is rarely published.

Considering the typical pH value (generally pH > 5.0), temperatures above 100 °C should be used to prolong the shelf life of liver pâtés. There are very few publications in the literature on the development of the consistency of heat-treated products at temperatures above 100 °C. Zhang, Xue, Xu, Li, and Xue (2013) and Zhang, Zhang, and Wang (2016) published a comparison of the stiffness of products (surimi gels from Alaska Pollock (*Theragra chalcogramma*)) treated at temperatures of 100–120 °C, but without continuous monitoring of the evolution of the storage and loss moduli during heating. The authors concluded that products treated at lower temperatures are stiffer than those heated to higher sterilisation temperatures. A unique publication on the continuous evolution of liver pâté stiffness during sterilisation heating is that of Rezler et al. (2021). The authors reported that in the temperature range of 100–120 °C, the storage and loss moduli decreased, attributing this mainly to the formation of non-disulphide covalent bonds, which weakened the gel formed at temperatures up to 100 °C, and to the partial destruction of ionic bonds and hydrophobic interactions between protein chains. However, it was not stated whether the heating was under overpressure or atmospheric pressure, which may also affect the consistency of meat products (Wei et al., 2019).

The consistency of the product also changes during product cooling. Likewise, there are an inadequate number of publications in the available literature on the cooling of meat products and the development of the storage and loss moduli. Savadkoohi, Shamsi, Hoogenkamp, Javadi, and Farahnaky (2013) and Westphalen, Briggs, and Lonergan (2006) reported that the stiffness of the system increases during cooling, mainly due to the further aggregation of proteins, the formation of disulphide bridges, hydrophobic interactions, hydrogen bridges, and the crystallization of fat.

Therefore, this work was first carried out to observe *in situ* changes in the viscoelastic properties of pork liver pâté during an increase in temperature (target temperature of 122 °C), holding it at the sterilisation temperature (10 min), and subsequent cooling thereof using dynamic oscillatory rheometry. At the same time, the untreated samples (from the same batch as the material for the *in-situ* study) were treated according to the same sterilisation mode in an autoclave. The course of changes in the temperature of the samples during the sterilisation mode, which took place in the autoclave, was monitored using dataloggers in the product. The second objective was to characterise the physical, chemical, and microscopic properties of the sterilised pork liver pâté, under precisely monitoring by the dataloggers during the sterilisation mode. The products were evaluated after 7 days of storage at 5 °C. The temperature of 5 °C is not typical for sterilised samples, on the other hand, we would like to maximally maintain the properties of the sterilised pork liver pâté without the effect of elevated temperature. No similar study was found describing *in situ* changes in the viscoelastic properties of pork liver pâté during the sterilisation process under overpressure (the normal condition in the autoclave). At the same time, none of the available research papers report the wide range of pork liver pâté properties, where the course of sterilisation was precisely monitored.

2. Material and methods

2.1. Pork liver pâté preparation and lethal effect of sterilisation

For the manufacture of pork liver pâtés with 28.0 g/100g dry matter content, 16.5 g/100g w/w fat content and 8.5 g/100g protein content, the following raw materials were used: lean meat from pork shoulder, pork liver, pork fatback (Kostelecké uzeniny, Plc., Kostelec, Czech Republic; individually packaging weighted 1–2 kg), broth and salt (containing 0.55 g/100g of sodium nitrite, MASO-PROFIT Ltd., Prague, Czech Republic). The formulation of the samples (including the parameters of the raw materials applied) is presented in Table 1. First, the raw liver (small cuts of 3 × 3 cm) was homogenized with salt using a Vorwerk Thermomix TM 6 blender cooker (2L capacity; Vorwerk & Co Thermomix GmbH, Wuppertal, Germany) for 5 min and stored at (5 ± 1) °C. Pork shoulder and fatback (small cuts of 3 × 3 cm without tendons and visible fat) were boiled in water until tender. Then, they were ground with a Vorwerk Thermomix TM 6 blender cooker and mixed with the broth obtained during the cooking of the meat (about 5 min). The temperature of the cut mass was about 60 °C. Then the cut mass was indirectly cooled using drift ice at 45–48 °C, the homogenized liver was added, and the mixing was continued for 10 min until a fine structure was obtained. The final temperature ranged from 63 to 65 °C and the mass was dosed into the laminated aluminum containers (conical shape; inner dimensions of 26.8 mm in height, 81.1 mm in diameter at the top

Table 1
Formulation of the samples with target values: 28.0 g/100g dry matter content, 16.5 g/100g fat content and 8.5 g/100g protein content.

Raw material	Absolute amount (g)	Relative amount (rel. %)
Pork shoulder	400.0	26.2
Pork liver	400.0	26.2
Pork fatback	200.0	13.1
Broth	500.0	32.8
Salt	25.5	1.7

and 68.9 mm in diameter at the bottom) 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 one container was approx. (95 ± 2) g. Subsequently (in the same day), the samples were sterilised according to the conditions which are mentioned in the next paragraph (LP).

A laboratory autoclave (FEDEGARI FVA2/A1; Fedegari Autoclavi SpA, Albuzzano, Italy) was used with inner dimensions of 600 mm in height and 405 mm in diameter. The target temperature of 122 °C and the holding time of 10 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. The actual temperature in the container placed at the coldest point in the retort (based on the findings originating in a preliminary study) was recorded using data-loggers Ellab TrackSense Pro (Ellab A/S, Hilleroed, Denmark) and evaluated by the ValSuite software (Ellab A/S, Hilleroed, Denmark). The LP samples were stored at (5 ± 1) °C, until analyses (7 days after manufacturing). The products were manufactured three times for repetition.

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).

2.2. Chemical analysis

Standard AOAC methods (Association of Official Analytical Chemists, 2000) were used to investigate the proximate composition of LP. The moisture content was determined gravimetrically by oven-drying to constant weight at (103 ± 2) °C following the standard AOAC method, 950.46 B. The protein and fat contents were measured according to the AOAC methods, 981.10 and 960.69, respectively. The same approach was used in work of Polášek et al. (2021). The water activity (a_W) was carried out according to ISO 18787:2017 (ISO/TSStandardNo. 18787, 2017) using the WATERLAB aw ANALYSER (Steroglass S.r.l., Perugia, Italy). The analyses were performed nine times.

2.3. Rheological analyses

The development of viscoelastic properties during the sterilisation of the mass was studied *in situ* by means of rotational viscometry using a Physica MCR302e modular rheometer (Anton Paar, Austria), which was equipped with a pressure cell. Detailed knowledge of the rheological behaviour is crucial to control and guide the process of pork liver pâté sterilisation. A bob/cup measuring system (CC 25/PR) was used inside the pressure cell in gas pressurization mode (nitrogen). The pressure cell was interconnected with a C-PTD200 Peltier unit to properly control the temperature. To evaluate the course of the sterilisation process, a constant strain ($\gamma = 0.03$) and frequency (f = 0.1 Hz) were set with a reading of 1 point·s^{-1} at a constant pressure during the entire process (p = 0.36 MPa) with the following temperature profile: (i) increase in temperature from 25 °C to a sterilisation temperature of 122 °C (linear heating rate of 30 min i.e. ≈3.2 °C·min^{-1}), (ii) holding at the sterilisation temperature (10 min), (iii) cooling to ≈25 °C (linear heating rate of 45 min i.e. ≈ −2.1 °C·min^{-1}). The described temperature profile was based on values monitored using data loggers recording the real process of sterilisation of the sample in an autoclave. Primarily, the storage (G'; Pa) and loss (G''; Pa) moduli were recorded, tan δ (dimensionless) was calculated as G''/G' and δ (°) as arctangents of G''/G'.

For the characterisation of the viscoelastic properties of the LP (after 7 days of storage), frequency sweep tests were performed. A profiled parallel-plate measuring system with a diameter of 50 mm (PP50/P2 with INSET 50 mm PROFILED) was used, while the plate gap was set at 2 mm. The temperature during the measurement was set to 25 °C and was controlled using a water-cooled Peltier system (Physica PT 100). The frequency sweeps were performed using a frequency ranging from 0.1 to 10.0 Hz with an applied constant strain of $\gamma = 0.03$ (within the viscoelastic region). The analyses were performed nine 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 & Chambon, 1986):

$$G^*(f) = A_F \bullet f^{\frac{1}{q}} \quad (1)$$

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

2.4. Textural analyses

The textural properties of the LP (after 7 days of storage) were evaluated using a texture analyzer TA.XTplus (Stable Micro Systems Ltd., Godaming, UK) equipped with a 20 mm in diameter cylindrical aluminium 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 (25 ± 1) °C (the measurement was carried out within the containers). From the force/time curves, the hardness (the maximum force observed during penetration; N); cohesiveness (the 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 adhesiveness (the strength of adhesiveness between the product and the probe surface calculated as the absolute value of the negative force area; N·s) were obtained for the LP.

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 elongational viscosity (Pa·s), $\dot{\varepsilon_H}$ 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·s^{-1}) and r is the radius of the sample (m) (ISO/TS 17996:2006; ISO/TSStandard No. 17996, 2006b). The analyses were performed nine times.

2.5. Differential scanning calorimetry

After 7 days of storage, the DSC analysis of the LP was performed on DSC 250 Discovery (TA Instruments, USA) with Tzero patented technology. To avoid exothermal crystallization loop at temperature around −20 °C which is typical for high water uptake samples, Tzero patented technology (heat flow mode T1) was applied for the cooling and heating cycle. Each sample (13.0 ± 0.5) mg was filled in Tzero aluminium pin hole hermetic pans (with hole 1 mm), and an empty aluminium pan was used as a reference. The thermogram was obtained by scanning from

25 °C to −50 °C using a cooling ramp of 5 °C·min^{-1}, subsequently isothermal step at −50 °C for 1 min, and following a heating cycle in the temperature range from −50 °C to +50 °C at a heating rate of 10 °C·min^{-1}. The measurement was carried out under nitrogen atmosphere at a flow rate of 50 ml min^{-1}.

The DSC instrument was calibrated by an indium standard. The thermal behaviour of the pork liver pâté samples was evaluated by parameters of the temperature peaks: T_{oc} – onset crystallization temperature; T_{pc} – exothermal crystallization peak temperature; T_{om} – onset melting temperature; and T_{pm} – endothermal melting peak temperature. Relevant enthalpies, i.e., freezing enthalpy (ΔH_c) and melting fusion enthalpy (ΔH_{fus}) were calculated as the integral area below thermogram peaks and expressed in normalized values (J·g^{-1}) (Lapčíková, Lapčík, Valenta, Majar, & Ondroušková, 2021).

To separate non-freezable and freezable water in pork liver pâtés samples, DSC peaks detected during the heating cycle were used to determine the freezable water content ($W_{fs;}$ rel.%). The freezable water content was expressed as the ratio of the emulsion melting enthalpy to the endothermic enthalpy of pure water by the following equation:

$$W_{fs} = \frac{\Delta H_s}{\Delta H_{H_20}} \bullet 100 \quad (6)$$

where W_{fs} is freezable free and freezable bound water content, ΔH_s is total enthalpy of freezable water in the sample, $\Delta H_{H2O} = 333.5$ J g^{-1} represents melting enthalpy of pure water (Yang & Mather; Guan, Xu, & Huang, 2011). The analysis was performed nine times.

2.6. Scanning electron microscopy

The LP (after 7 days of storage) for scanning electron microscopy (SEM) were processed as follows: samples of 35 × 25 × 2 mm were fixed in cacodylate buffer (0.2 mol l^{-1}) in glutaraldehyde (3.0 % v/v). After fixation, the samples were washed 3 times in cacodylate buffer (0.2 mol l^{-1}) for 15 min. The samples were resized to 3 × 3 × 3 mm and further fixed in osmium tetroxide (1.0 % w/v). After fixation, the samples were washed 3 times in cacodylate buffer (0.2 mol l^{-1}) for 15 min. It was followed by dehydration with an alcohol series (30 %, 50 %, 70 %, 80 %, 90 %, 96 %, 100 %; v/v) at 30-min intervals. 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) in different resolution (the final magnification is burned in microphotographs). Image processing and analysis were performed using a modified method according to Impoco, Carrato, Caccamo, Tuminello, and Licitra (2007).

2.7. Statistical analysis

The obtained results were evaluated using Wilcoxon tests (the significance level was 0.05). Unistat® 6.5 software (Unistat, London, UK) and Microsoft Excel (Microsoft Corporation, Santa Rosa, CA, USA) were used for the statistical analysis.

3. Results and discussion

Fig. 1 shows *in situ* changes in the viscoelastic properties of the meat batter (G' and G'' - part A and δ - part B) during (i) heating from 25 °C to the target sterilisation temperature of 122 °C; (ii) holding at 122 °C (10 min); and (iii) cooling to 25 °C. Table 2 shows the values of G', G'' and tan δ for the selected temperatures during heating, holding and cooling of the meat batter. The storage modulus (G') values were statistically significantly higher ($P < 0.05$) than the loss modulus (G'') levels in the range of temperatures studied, as evidenced by the low phase angle values (δ). The same phenomenon, i.e. higher values of G' compared to

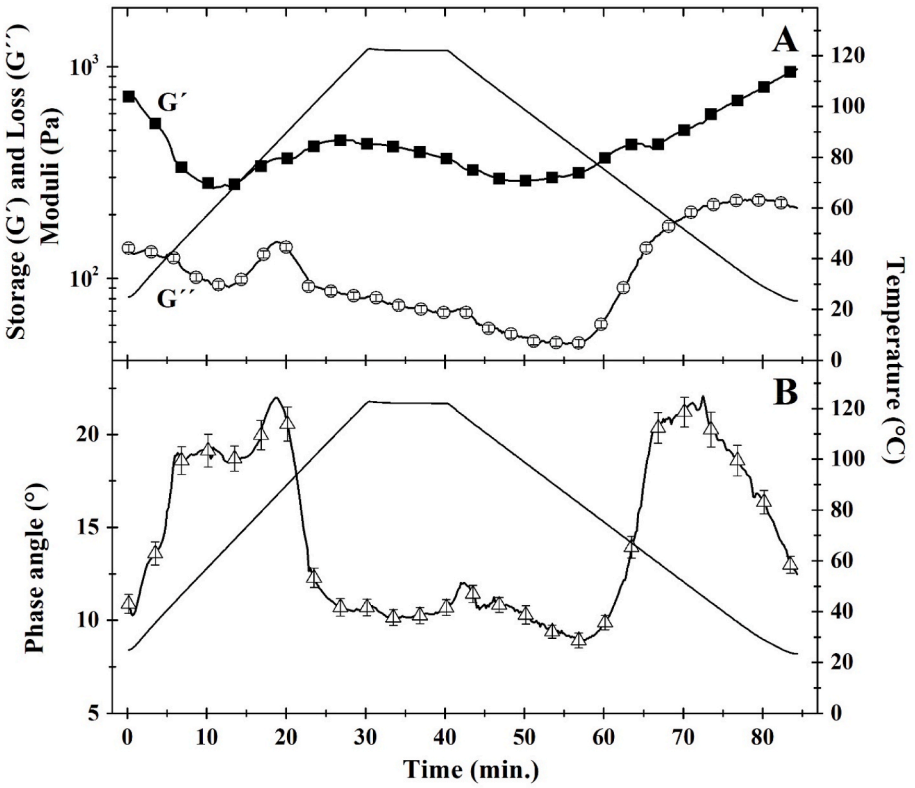

Fig. 1. The development of storage (G'; Pa; ■) and loss (G''; Pa; ◯) moduli (part A) and phase angle (δ; °; △; part B) of the meat batter over time during heating, sterilisation and cooling (time is presented in minutes on the x-axis) directly in the rheometer equipped with a pressure cell (f = 0.1 Hz; p = 0.36 MPa). The progress of the temperature is presented as a line without any symbols (n = 9) in both parts (A and B).

Table 2
Results of the viscoelastic parameters at the selected temperatures during heating, sterilisation process and subsequent cooling of the meat batter in the pressure cell of the rheometer (f = 0.1 Hz; the results were expressed as mean ± standard deviation; n = 9).

Temperature (°C)	Parameters [a]		
±	Storage modulus (*G'*; Pa)	Loss modulus (*G''*; Pa)	Loss tangent (tan *δ*; dimensionless)
25	720.5 ± 27.6 [a]	138.6 ± 5.7 [a]	0.192 ± 0.009 [a]
100 [b]	414.1 ± 16.2 [b]	91.9 ± 3.7 [b]	0.222 ± 0.010 [b]
122 [c]	431.7 ± 17.0 [b]	81.5 ± 3.3 [c]	0.189 ± 0.008 [c]
122 [d]	365.3 ± 14.9 [c]	68.9 ± 2.8 [d]	0.189 ± 0.009 [c]
100 [e]	287.8 ± 11.6 [d]	53.1 ± 2.2 [e]	0.185 ± 0.009 [c]
25	898.5 ± 37.0 [e]	225.9 ± 9.1 [f]	0.251 ± 0.010 [d]

[a] the means within a column (the difference between the selected temperature) followed by different superscript letters differ (P < 0.05).
[b] the temperature in the sterilisation time first reached the temperature of 100 °C.
[c] the temperature in the sterilisation time first reached the target temperature of 122 °C.
[d] the temperature in the sterilisation time last reached the target temperature of 122 °C.
[e] the temperature in the sterilisation time last reached the temperature of 100 °C.

G'', was also observed by Delgado-Pando et al. (2012), who suggested that the explanation is in the formation of a firm and elastic gel, which shows appreciable evidence of the presence of an emulsion structure (viscous component).

In the first heating phase (up to ≈58 °C), a sharp decrease in the storage and loss moduli of pork liver pâté was observed with a concomitant increase in the phase angle (P < 0.05). At these temperatures, dimerization of myosin head molecules occurs, as does aggregation and the formation of three-dimensional structures, whereby hydrophobic grubs interact between individual myosin chains (Tornberg, 2005; Zhao, Zhou, & Zhang, 2019). On the other hand, the latter mentioned interaction was overlapped by interactions of the fat present. During the aforementioned temperature changes, the fatback overcomes its melting point and imparts a more viscous character to the three-dimensional matrix, which is evident from the slower decrease in G'' or the increasing values of the phase angle (δ; P < 0.05). A similar phenomenon was observed in the works of Rezler et al. (2021) and Savadkoohi et al. (2013). According to Li et al. (2015) and Wei et al. (2019), processes leading to the disruption of previously formed three-dimensional structures associated with denaturation of part of the myosin tails can also be expected at temperatures above 50 °C.

In the temperature range ≈58–70 °C, the values of the storage and loss moduli were practically stagnant (P ≥ 0.05). However, heating above 70 °C led to a statistically significant increase (P < 0.05) in the storage modulus (G'), practically up to the target temperature of 122 °C. In the case of the loss modulus (G''), an increase was observed up to ≈85 °C, after which its values continuously decreased with a simultaneous decrease in the phase angle (δ; P < 0.05). In this temperature range, an increase in the stiffness of the studied matrix was also observed by a number of other authors, such as Brunton et al. (2006), Rezler et al. (2021), Savadkoohi et al. (2013), and Tornberg (2005). At temperatures up to 100 °C, the increase in stiffness of the studied matrix is mainly associated with denaturation of the proteins present and their aggregation, the massive formation of hydrophobic interactions and sulfhydryl-disulfide interactions between individual proteins, with weak binding interactions such as hydrogen bridges likely also playing a contributing factor. Pork liver proteins and the involvement of its denatured globulins in the three-dimensional structure also play a significant role here (Brunton et al., 2006; Delgado-Padano et al., 2012; Li et al., 2015; Rezler et al., 2021; Savadkoohi et al., 2013; Tornberg, 2005; Wei et al., 2019).

Fig. 1 and Table 2 further show that the holding at 122 °C and the subsequent cooling to ≈100 °C was accompanied by a decrease in the storage and loss moduli (P < 0.05), as well as a slight decrease in the phase angle. The literature suggests that this is due to a decrease in the intensity of hydrophobic interactions, ionic bonds, hydrogen, and disulfide bridges between individual muscle tissue proteins. The tail portion of myosin is in the native stage in a α-helical arrangement, but thermal heating, especially above 100 °C, changes the secondary structure into a β-sheet or random coil, which contributes to the reduction of the strength of the resulting gel. As the temperature increases, the proportion of random coil of myosin increases, thus decreasing the strength of the resulting three-dimensional structure (Rezler et al., 2021; Zhang et al., 2013, 2016).

With further cooling of the product (<100 °C; see Fig. 1 and Table 2), the storage and loss moduli increased. At temperatures ≈80-50 °C, the increase in loss modulus was more intense than that of the storage modulus, leading to an increase in phase angle values. The rationale can be found in the continued aggregation of the proteins present and the formation of additional disulphide, and hydrogen bridges, hydrophobic interactions and other ionic bonds. The reverse recrystallisation of the fat present, which commonly occurs at temperatures <80 °C, also contributes to the increase in stiffness (Brunton et al., 2006; Rezler et al., 2021; Westphalen, 2006; Zhao et al., 2019).

The manufactured sterilised pork liver pâtés were characterised after 7 days of storage at 5 °C. The latter temperature was chosen in order to minimize the changes during storage. However, a detailed characterisation of this type of products was not found in the available literature. The known properties of the sterilised pork liver pâtés could help to understand the course of changes in the viscoelastic properties observed during sterilisation (see above). The importance of this study is highlighted in order to manufacture products under very controlled conditions.

The applied sterilisation heating (the target temperature of 122 °C; the holding time 10 min) ensured the sterility value at the coldest point $F_0 = 12.94 \pm 0.87$ min (P < 0.05), which according to Harrigan (1998) and Lazárková et al. (2011) ensures sufficient sterilisation intervention inactivating the microorganisms and bacterial spores present. These results confirmed the above conclusions that the applied F_0-value is sufficient to ensure microbiologically stable canned products.

The chemical composition of LP (Table 3) confirmed that the products with planed dry matter, fat, and protein contents were manufactured. The value of water activity a_W and also the pH value of the sterilised pork liver pâté corresponds to the pH level of similar products published in the literature (e.g. Rezler et al., 2021; Śmiecińska, Gugolek, & Kowalska, 2022).

Fig. 2 shows that in the entire range of frequencies (f; Hz) studied, the G' level is statistically significantly higher (P < 0.05) than the G'' level, indicating the formation of a firm and elastic gel (Delgado-Pando et al.,

Table 3
Results of the analysis of sterilised pork liver pâté samples after 7 days of storage at 5 °C (the results were expressed as mean ± standard deviation; n = 9).

Parameters	Sterilised pork live pâté
Dry matter content (g/100g)	28.31 ± 0.19
Fat content (g/100g)	16.61 ± 0.49
Protein content (g/100g)	8.59 ± 0.27
pH-value	6.17 ± 0.02
Water activity (a_W)	0.967 ± 0.004
Hardness (N)	11.48 ± 0.26
Cohesiveness (unitless)	0.601 ± 0.039
Adhesiveness (N·s)	0.12 ± 0.03
Storage modulus at 1 Hz (Pa) [a]	13054 ± 665.2
Loss modulus at 1 Hz (Pa) [a]	3551 ± 98.1
Phase angle at 1 Hz (°)[a]	15.1 ± 0.4
Gel strength ($Pa{\cdot}s^{1/z}$) [a]	3601.7 ± 178.5
Interaction factor (unitless) [a]	7.65 ± 0.39

[a] The values were obtained from the frequency test.

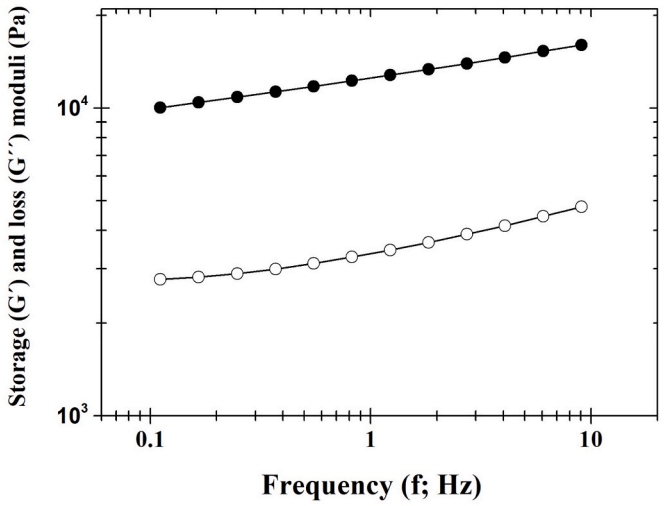

Fig. 2. The dependence of storage (G'; full symbols) and loss (G''; open symbols) moduli (Pa) of the sterilised pork liver pâté (●◯) on the frequency (f; Hz) after 7 days of storage (5 °C) measured at 25 °C (n = 9).

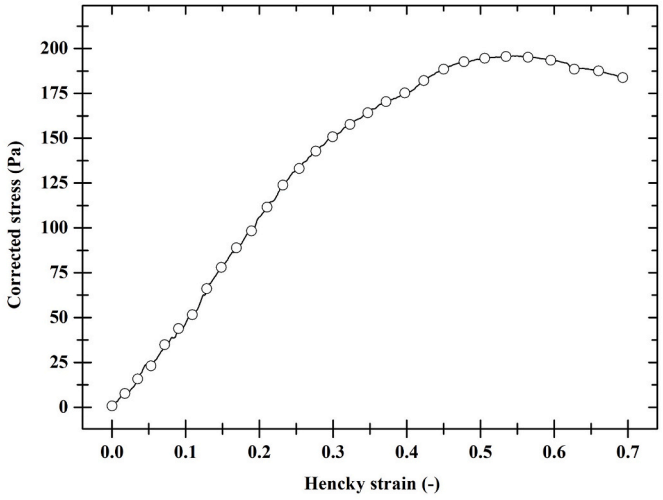

Fig. 3. The dependence of corrected stress (Pa) on Hencky strain (dimensionless) of the sterilised pork liver pâté (◯) after 7 days of storage (5 °C) measured at 25 °C (n = 9).

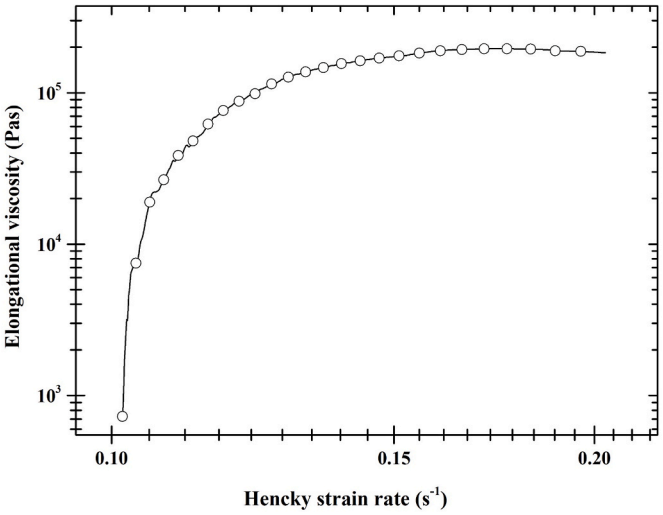

Fig. 4. The dependence of elongational viscosity (Pa·s) on Hencky strain rate (s^{-1}) of the sterilised pork liver pâté (◯) after 7 days of storage (5 °C) measured at 25 °C (n = 9).

2012). The above explanations are supported by the values of the phase angle δ and also by results of the application of Winter's critical gel theory (Table 2). The increase in the gel strength (A_F; Pa•$s^{1/q}$) was accompanied by a relatively high value in the interaction factor (q), representing the number of interacting particles in the three-dimensional structure. As a result of heat treatment, the product undergoes conformational changes in the secondary and tertiary structure of proteins (denaturation) leading to their aggregation, the formation of protein-protein bonds, and gel formation. Disulfide bridges, hydrophobic interactions, and hydrogen bridges are mainly involved in the formation of the three-dimensional matrix. Fat crystallization and changes in its polymorphic structure (especially the gradual transition from a metastable β' to a stable β crystalline modification) can also be expected to contribute to this process (Amako & Xiong, 2001; Rezler et al., 2021; Savadkoohi et al., 2013; Tornberg, 2005; Westphalen et al., 2006). Another factor that may contribute to the increasing intensity of the interactions between the proteins present is CMR. As was mentioned above, an example is the formation of an isopeptide bond between proteins via the ε-amino group of lysine (Buňka, Hrabě, & Kráčmar, 2004; Friedman, 1996; Lazárková et al., 2010; Li et al., 2021).

Furthermore, testing the samples in the area of large deformations led to the conclusion that the applied sterilisation heating resulted in a relatively high value of hardness and a low value in cohesiveness and adhesiveness (Table 2) of the studied sterilised pork liver pâté. Advanced evaluation of the texture analysis results (Figs. 3 and 4) led to the conclusion that the sterilisation mode used a sharp increase in corrected stress (σ_C; Pa) and elongational viscosity (η_E; Pa•s) in the first part of the curves, corresponding to the transient flow regimes. This is followed by a practically linear part of the curves corresponding to the squeezing flow regime, which is independent of the increasing values of the Hencky strain or Hencky strain rate, respectively. Heat treatment resulted in a shift in the transition point from the transient flow regimes to the squeezing flow regime. The application of this approach to evaluate the effect of heat treatment on pork liver pâté in the large deformation area was not found in available literature.

The DSC study showed that the sterilisation heating resulted in a value of the water freezable index (W_{fs}; rel. %) of 43.53 ± 0.86 %, which represent the content of freezable water, i.e. water that is not tightly bound in the three-dimensional structure. The freezing of water in food and the subsequent melting of ice can provide more information about the water organisation in food systems (Grabielle-Madelmont & Perron, 1983). The value of the W_{fs} supports the findings of textural and rheological analyses in small and large deformations, which indicate aggregation of the protein structure as a consequence of heat treatment. The latter phenomenon then has clear implications for changes in the binding of the water present (Parniakov et al., 2018; Savanović, Grujić, Rakita, Torbica, & Božičković, 2017; Yang & Mather). The DSC study of sterilised pâté was not found in available literature.

The microscopic analysis (Fig. 5) clearly showed that sterilisation led to produce a stable matrix which were likely caused by denaturation of sarcoplasmic and collagen proteins which occurs between 65 and 75 °C and by actin denaturation which occurs between 78 and 83 °C (Tornberg, 2005). The fat was relatively equally distributed. This result could support the explanation of viscoelastic changes (see above and also Fig. 1). The coalescence of fat granules was not confirmed in LP and fat granules were homogenously distributed (Fig. 5; the fat particle was indicated "fg").

4. Conclusion

In the *in-situ* mode, the evolution of pork liver pâté stiffness during sterilisation heating with a target temperature of 122 °C held for 10 min

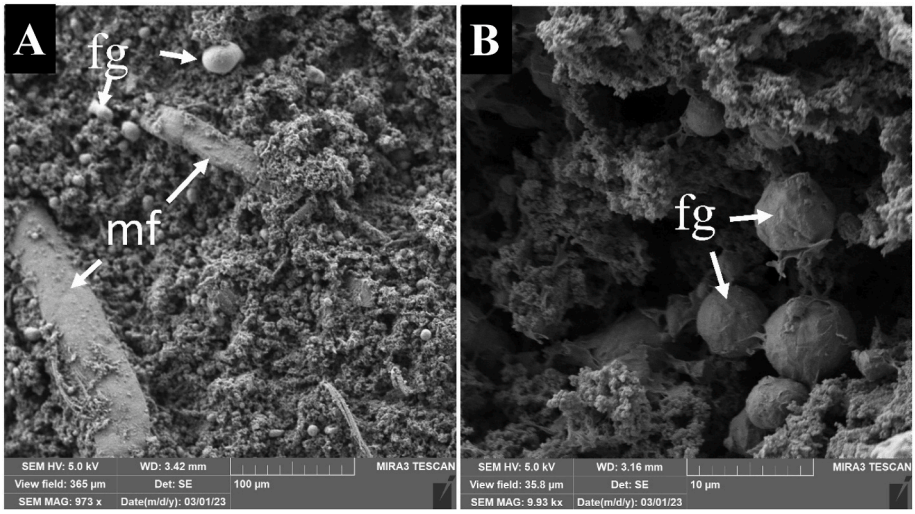

Fig. 5. Microstructure of the sterilised pork liver pâté (parts A and B). In part A, the bar represents 100 µm, and in part B, the bar represents 10 µm. Non-protein fat granules are presented as "fg" and muscular fibre as "mf".

was monitored using the storage and loss moduli and phase angle. After heat treatment and cooling, the final product was found to exhibit higher stiffness than the original meat batter. This conclusion was confirmed in the area of small and also large deformations after 7 days of storage at 5 °C and supported by microscopic analysis. The rheometer equipped with a pressure cell proved to be a valuable tool for *in situ* monitoring of processes during sterilisation heating. Furthermore, it was found that the heat treatment used ensures the sterility of the pork liver pâté, allowing these products to be used not only for retail for long-term storage at ambient temperature, but also for combat rations. The latter is particularly important when these products are included in the feeding of units operating in crisis situations, especially those units that are part of foreign humanitarian or military missions.

Detailed knowledge of the course of the viscoelastic properties development during sterilisation and about the quality and properties of sterilised pork liver pâté is crucial for control of these processes during production. The practical effect could be found e.g. during additives (such as hydrocolloids) dosage and when the effect of the latter mentioned substances on the mass is estimated and/or evaluated. This study could serve as an effective tool and methodical approach for studying and observing the changes during sterilisation and/or cooling. Additionally, the data presented in this study could be also useful when the appropriate time (moment) for pipeline transportof the mass is searched.

CRediT authorship contribution statement

Marketa Pětová: Conceptualization. **Zdenek Polášek:** Investigation, Methodology, Writing – review & editing. **Barbora Lapčíková:** Data curation, Investigation, Methodology, Writing – review & editing. **Lubomir Lapčík:** Investigation, Methodology, Supervision, Visualization, Writing – review & editing. **Leona Buňková:** Data curation, Investigation, Methodology, Supervision, Writing – review & editing. **Matej Pospiech:** Investigation, Methodology, Writing – review & editing. **Pavel Foltin:** Investigation, Methodology, Writing – review & editing. **Jaroslav Talár:** Data curation, Investigation, Methodology, Writing – review & editing. **Richardos Nikolaos Salek:** Investigation, Methodology, Visualization, Writing – review & editing. **Vendula Kůrová:** Investigation, Methodology, Writing – review & editing. **Katerina Křištofová:** Investigation, Methodology, Writing – review & editing. **Frantisek Buňka:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

Declaration of competing interest

we would like to submit the enclosed manuscript entitled "*Evaluation of the viscoelastic properties of pork liver pâté during sterilisation observed in situ*", which we wish to be considered for publication in "*LWT*". Moreover, no conflicts of interest exist in connection with the submission of this manuscript, which has been approved for publication by all the authors. I would like to declare on behalf of my co-authors that the work described is original research that has not been published previously, and is not currently being considered for publication elsewhere, in whole or in part. All the authors listed have approved the manuscript that is enclosed.

Data availability

Data will be made available on request.

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