Physicochemical characteristics of surimi-like material made from the muscle tissues of freshwater mussels (*Sinanodonta woodiana* Lea, 1834)

Jerzy Stangierski¹ · Ryszard Rezler² · Bożena Grześ³ · Wojciech Andrzejewski⁴ · Piotr Konieczny¹

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Abstract

The aim of the study was to determine selected characteristics of surimi-like material (SLM) made from the muscle tissues of *Sinanodonta woodiana* (Lea, 1834) freshwater mussels. The research material consisted of unwashed mussel muscle homogenate as the control sample—C, mussel muscle tissue twice washed with water (SLM-W) and sample washed with NaCl at a concentration of 0.169 mol/L as well as water (SLM-S). A raw control sample and surimi like-materials were analysed using the SDS-PAGE technique. They were also tested using the DMTA method (dynamic mechanical thermal analysis) while heating the samples up to 80 °C and cooling to 20 °C. The thermal drip and texture of gels after heating (75 °C, 30 min) were also determined. The washing procedure had a significant impact on the protein composition of the SLMs. A significantly ($p < 0.05$) higher percentage of proteins with molecular weights of 270–273 kDa and 105–110 kDa (corresponding to specific filamin isoforms), as well as 42–43 kDa (corresponding to actin), were found in the SLMs compared to sample C. Correlation analysis confirmed a strong positive relationship between the percentage share of the above proteins and the values of the elasticity modulus ($r \geq 0.84$) and firmness ($r \geq 0.88$) of SLM gels. The SLM-S sample was characterised by the lowest significant ($p < 0.05$) thermal drip values. During heating, the rheological traits of all samples changed at two stages: from 20 to 50 °C and over 50 °C. The highest dynamics of variation in the elasticity modulus (G') value were noticed at temperatures exceeding 50 °C in all samples. The G' values in the SLM samples were significantly greater than the values in sample C. The analysis revealed a strong correlation ($r \geq 0.81$) between the determinants of the texture of the SLM samples and their rheological parameters. Sample SLM-W was the one with the highest firmness and elasticity values. The analysis showed that the textural properties of the SLM samples mainly resulted from the reaction of spatial matrices to mechanical interactions.

Keyword Mussel proteins · SDS-PAGE · Rheology · Gels · Thermal drip · Texture

✉ Jerzy Stangierski
jerzy.stangierski@up.poznan.pl

¹ Department of Food Quality and Safety Management, Faculty of Food Science and Nutrition, Poznań University of Life Sciences, Wojska Polskiego 31/33, 60-624 Poznań, Poland

² Department of Physics and Biophysics, Faculty of Food Science and Nutrition, Poznań University of Life Sciences, Wojska Polskiego 31/33, 60-624 Poznań, Poland

³ Department of Food Biochemistry and Analysis, Faculty of Food Science and Nutrition, Poznań University of Life Sciences, Mazowiecka 48, 60-623 Poznań, Poland

⁴ Division of Inland Fisheries and Aquaculture, Faculty of Veterinary Medicine and Animal Science, Poznań University of Life Sciences, Wojska Polskiego 71C, 60-625 Poznań, Poland

Introduction

More and more European consumers appreciate the nutritional, dietary and sensory values not only of fish but also bivalve molluscs such as oysters (*Ostreidae*), mussels (*Mytilidae*), and clams (*Bivalvia*). These bivalves are good sources of protein and minerals. They are easily digestible and compare favourably with other animal foods. Extracts from their soft tissues could be used as antibacterial, antifungal, or even anti-tumour agents [1].

The Chinese pond mussel (*Sinanodonta woodiana*, Lea 1834) is a species of freshwater mussel that is indigenous to Asia. It was brought to Europe together with farmed fish [2] and has spread all over Europe, the United States, Indonesia, the Philippines, Costa Rica and Haiti [3]. It is worth noting

that for many years *S. woodiana* has been acquired from the environment in Asia, where it is valued as a source of protein for humans and animals. However, extensive and multidirectional research is necessary to assess the possibility of using these mussels as food and feed. The research must include the functional properties of proteins, i.e. the physicochemical properties that influence the behaviour of proteins during processing, storage and food/feed consumption [4]. This fact inspired us to assess the functionality of surimi-like materials extracted from the muscle tissue of freshwater mussels. Numerous studies clearly proved and justified that the technology for making surimi from fish enables the recovery of different meat ingredients from the myofibrillar protein fraction, which is of the highest nutritional and technological value [5, 6]. The functional properties of proteins reflect their chain conformation, e.g. the sequence of amino acid residues in the polypeptide chain, the degree of their modification, the structure of proteins and the interaction between polypeptide chains. Regardless of the raw material from which a surimi-like extract was made, its functionality is chiefly assessed according to its capacity to generate high-quality gel with good consistence parameters [7]. The gelation of myofibrillar proteins, especially myosin, is important in making thermally processed meat products. The process affects the retention of water and fat, the consistency of the final products and general organoleptic assessment. At the same time, it is believed that other myofibrillar proteins such as actin, regulatory and cytoskeletal proteins (including filamin, among others) do not form gels. Nevertheless, they affect the viscoelastic properties of myosin gels [8]. The surimi myofibrillar protein extract made from fish, which was later heated, had better textural parameters than gels made from the fish muscle tissue which was thermally processed under the same conditions [9, 10].

As consequence of the conformational changes of molecular structures of protein during thermal treatment, significant changes of viscoelastic properties of mussels occur. The molecular mechanism of these transformations is complicated. In fact, the surimi-like material made from the muscle tissue of Chinese pond mussels contains not only the actomyosin complex and other myofibrillar proteins but also some sarcoplasmic proteins, as indicated by electrophoretic separation of proteins by SDS-PAGE [4, 11]. It significantly affects the rheological properties of the samples under analysis. Rheological properties refer to the physical interactions between the molecular elements of the structure in the system being studied [12, 13]. Although rheometric techniques are commonly used, so far there have been no studies on surimi-like extracts made from *S. woodiana* mussel meat. The authors of most studies described proteins isolated from the muscle of marine fish [10] or from other sources, e.g. crayfish [14]. In order to ensure advances in the knowledge of the mechanical properties of protein preparations physical

methods and techniques should be applied, which are sensitive to changes in intermolecular interactions occurring in the systems analysed. Oscillation rheometry expanded to the technique of dynamic-mechanical thermal analysis (DMTA) is one of the most universal and objective methods which can be applied in investigations of these types of systems, since it affords possibilities of determining the values of elasticity coefficients, coefficients of internal friction (viscosity) and other mechanical-rheological parameters at any frequency of non-destructive interactions of the developed system structure. In contrast to texture strength tests, DMTA allows the influence of changes in the molecular structure of surimi-like preparations made from the muscle tissue of freshwater mussels to be determined during the entire heat treatment cycle (heating and cooling) on their rheological properties.

Therefore, this study is devoted to determining the abundance of major proteins in the muscle tissue and surimi-like material samples being examined, and an analysis of the effect of heating surimi-like freshwater mussel products on physicochemical traits, which significantly affect the functional and technological quality of proteins. The temperature ranges tested were typical of the conditions found in commercial-scale meat processing.

Materials and methods

Sample preparation

Sinanodonta woodiana (Lea, 1834) freshwater mussels caught in Lake Gołławskie in Poland were used in the research. The research material consisted of live *S. woodiana* collected all at once in autumn 2019. They were kept in a pool with a constant flow of water for one month to clean their alimentary tracts. The material collected was divided into two parts so that the experiment could be conducted twice, on samples originating from the same source. After that, the shells of mussels were opened to separate the muscle tissue, i.e. the foot muscle and adductor muscle. The meat was initially shredded into a homogenous mass by means of a Braun Multiquick 5 MR 550 FPHC blender (Braun GmbH, Germany).

Production of surimi-like material (SLM)

As previous research has shown, washing freshwater mussel's *S. woodiana* soft tissue proves to be more effective when it comes to protein extraction when 0.5% NaCl solution and water is used rather than only water. Salt solution improves the efficiency of eliminating non-protein nitrogen substances from muscle tissue [11]. The increase in the myofibrillar proteins concentration and therefore an

improvement in the gelation capacity of surimi and surimi-like material after thermal processing was observed [15].

After initial shredding, the material was rinsed twice. The homogenates made from the muscle tissue of the mussels were rinsed with two variants of agents:

(1) The homogenate was washed twice with tap water in a ratio of 1:2 (water:homogenate; v:w)—the first variant (W);

(2) The homogenate was washed first with 0.169 mol/L aqueous solution of NaCl and second with tap water in a ratio of 1:2 (water:homogenate; v:w)—the second variant (S).

The same technological parameters were used in both variants. The homogenate was washed by means of a mechanical agitator (Velp Scientifica FC6S, Italy) at 150 rpm for 20 min. The suspension was centrifuged with an MPW 380R centrifuge (MERAZET, Poland) at 7000 rpm for 20 min, at a temperature of 6–8 °C. The precipitate obtained was washed with tap water in both variants once again. After washing, the connective tissue was separated on sieves with openings of 0.8 mm × 0.8 mm (Paxton Ltd. Glasgow). Next, the sinew fraction protein suspensions were centrifuged again with the MPW 380R centrifuge (MERAZET, Poland) at 7000 rpm for 20 min, at a temperature of 6–8 °C. The homogenate of the muscle tissue was used as the control sample (C). The control sample (C) and surimi-like materials (SLM) were refrigerated at 4–6 °C until measurements were taken, i.e. for about 15 h.

Basic chemical composition and pH measurements

The following methods were used in order to establish the basic chemical composition of surimi-like materials (SLM): dry matter, using the oven method [16], total protein content, in accordance with the Kjeldahl procedure, taking 6.25 as the conversion factor value [17], total fat content, evaluated using the Soxhlet method [18], and ash content, using PN-ISO 936:2000 [19]. The total carbohydrate level was calculated from the difference in the basic chemical composition.

The pH value was measured according to PN-ISO 2917:2001/Ap1:2002 [20] in a filtrate resulting from the samples being homogenised in cold distilled water. The pH value was measured with a CG 840 Schott Geräte pH-meter (Mainz, Germany) equipped with a glass electrode.

Sample preparation for SDS-PAGE

Each protein sample (10 mg) for SDS-PAGE (sodium dodecyl sulfate–polyacrylamide gel electrophoresis) was mixed with the buffer (150 µL) containing 8 M urea, 2 M thiourea, 0.05 M Tris–HCl (pH 6.8), 75 mM DTT, 3% (w/v) SDS, and 0.05% (w/v) bromophenol blue. Then all samples were heated in a dry block bath at 100 °C for 3 min. The protein concentration in 10 µL samples was determined using a

2-D Quant Kit (GE Healthcare BioSciences, Little Chalfont, UK).

SDS-PAGE conditions

The electrophoretic separation (SDS-PAGE) of protein samples (control sample and surimi-like materials) in 15% polyacrylamide gel was carried out in the vertical system using the SE 250 Mighty Small II 10 cm × 8 cm unit (Amersham Biosciences, Little Chalfont, UK). The amount of protein in each sample applied on the gel was 12 µg. Electrophoresis was conducted at room temperature and a constant current 20 mA per gel. Proteins were visualised by staining in a solution 0.05% (w/v) Coomassie Brilliant Blue R-250; 50% (v/v) methanol; 10% (v/v) acetic acid for 1 h and destained by diffusion in 10% (v/v) methanol, 4.5% (v/v) acetic acid for several hours. All gel images were acquired using an Image Master VDS (Pharmacia Biotech, Vienna, Austria) imaging system and analysed using Image Master® 1D Elite v. 4.0 software. Quality identification of selected peaks was performed on the basis of molecular weight (MW) expressed in kDa using a PageRuler Plus Protein Ladder 10 to 250 kDa (Thermo Scientific, Waltham, MA, USA).

Mechanical and rheological properties

A Dynamic Mechanical Thermal Analyser DMWT (COBRABiD—Poznań, Poland), operating on the principle of free vibration technique, was used for measuring purposes [21]. A parallel plate with a 50-mm diameter and 1.0-mm gap measuring system was used. 25 g/kg of NaCl was added to the homogenate and surimi-like samples. The sample (sample layer 1 mm thick and 50 mm in diameter) perimeter was covered with a thin layer of high-temperature-resistant silicone grease (GE Electronics, Rockford, IL) to prevent dehydration of the sample edge and moisture evaporation from the sample. The frequency of the systems' own vibrations was 1.2 Hz. The rheological properties of the systems were tested during heating in the temperature range from 20 to 80 °C. After reaching the final temperature, the resulting gels were tested during cooling to 20 °C. The systems were heated/cooled at a rate of 2.0 °C/min. The following components of the complex modulus of elasticity were calculated: elasticity modulus (G'), loss tangent ($\tan \delta$) and dynamic viscosity (η). The elasticity modulus is associated with the part of potential deformation energy which is maintained in the course of periodical deformations. The loss tangent ($\tan \delta$) is a measure of internal friction. It describes the relative quantity of energy dissipated in the material in the course of one deformation cycle.

Additionally, rheological measurements of the viscoelastic properties of the protein gel systems were performed using the DMA method (dynamic mechanical analysis). For

this purpose, samples were prepared in a similar way to the texture tests. Samples of the surimi-like preparation placed in the aluminum vessel (diameter 50 mm and height 1 mm) in the measuring chamber of the rheological analyser were heated at 75 °C for 30 min. Next, the samples were cooled to 20 °C at a rate of 2.0 °C/min. After cooling in the measuring chamber of the rheological analyser, the sample was placed in a refrigerator at 4–6 °C for about 15 h. The measurements were taken at 20 °C. The temperature of the chamber and measurement plate was measured with an accuracy of ± 0.2 °C.

Gels preparation

The homogenate as control sample and all samples of surimi-like materials were mixed with 25 g/kg of sodium chloride. The contents were then placed in plastic test tubes 20 mm in diameter (approx. 27 g of sample per tube). The test tubes were sealed and placed in a water bath GFL 1083 (BIONOVO, Poland). The samples were being heated in water at 75 °C for 30 min. Next, the samples were cooled in cold water and placed in a refrigerator at 4–6 °C for about 15 h.

Thermal drip

The gels obtained by cooling were taken out from the tubes and weighed. Thermal drip was expressed as the ratio ($\times 100\%$) of the difference in weight between the heated and the raw sample relative to the weight of the raw sample.

Texture analysis

Texture was analysed with a Texture Analyser TA-XT2i (Surrey, UK), which used the stress relaxation test to measure firmness (N) and elasticity (%). A sample with a height and diameter of 20 mm was compressed to 50% of its height and remained in that position under a constant load for 1 min. Force–time deformation curves were obtained with a 25-kg load cell applied at a cross-head speed of 2.0 mm/s. The other operating conditions of the apparatus were as follows: pre-test speed and post-test speed—1.5 mm/s, data acquisition rate—200 PPS, trigger force—10 g.

Statistical analysis

The experiments were conducted twice (two production batches). Analyses were carried out in at least three replications and the average data were expressed as mean $\pm$ SD. The electrophoretic analysis (SDS-PAGE), rheological and textural measurements were analysed statistically with SPSS software ver. 13.0 (SPSS Inc., USA). The significance (Tukey's test) of the results at $p < 0.05$ was tested by means of

one-way ANOVA. The one-factorial (ANOVA) analysis of correlation between textural and rheological parameters, and the proteins group were employed.

Results and discussion

Proximate composition and pH value

The size and quality of protein recovery yields depend on the composition and quality of the raw material [22]. The mean basic composition of minced mussels was as follows: water 82.15% (± 0.23), protein 10.93% (± 0.18), fat 0.82% (± 0.02), ash 1.02% (± 0.01), carbohydrates 5.09% (± 0.23) and pH 6.89 (± 0.08). The composition of this product was slightly different to that published in the literature. According to Saiful and Lumenta [23], the mean chemical composition of *S. woodiana* muscle tissue obtained in Taiwan was as follows: water 80.66%, protein 11.59%, fat 0.26%, ash 3.06% and carbohydrates 4.20%. In turn, Surasani et al.'s [22] study on mussels established the following basic composition: water 79.88%, protein 8.40%, fat 1.04% and ash 2.63%.

In these studies, the time and place where the mussels were caught may have affected the basic chemical composition. As was established in a study on the chemical composition of *Parreysia* spp. freshwater mussels, protein and fat content varies depending on the season [24]. The composition of mussel bodies may depend on external factors, for example, habitat conditions (the temperature, quality and quantity of nourishment available). It may also result from the physiological state and metabolism of individual specimens [25, 26]. Therefore, the recommendation for future studies would be to determine the optimal period of acquiring mussels for production in order to obtain the optimal percentage content of valuable components.

Table 1 presents the mean basic composition per dry matter of surimi-like materials obtained from the homogenate of *S. woodiana* meat mussels. The production of the surimi-like extract from mussels led to a decrease in the amount of dry matter in the samples. This fact is in agreement with the observations from surimi production using mechanically recovered chicken meat [5]. In a comparison of surimi from Alaskan Pollock, chicken breast and pork leg, which had been washed twice, the protein content was a significantly lower in the preparations made from the soft tissues of mussels, while the water content was similar [27].

Washing with water only and with a 0.5% NaCl solution caused the pH of the surimi-like material to increase slightly to a mean value of 6.96, as compared with the homogenate samples (Table 1). The functional properties' characteristics of raw materials of animal origin, such as gelation capacity and thermal drip, depend significantly on the active acidity of meat. The most important functionality criteria are three

Table 1 Proximate composition and pH value of control sample (C) and surimi-like materials (SLM) (% dry matter)

Type of sample	Dry matter (%)	Protein (%)	Fat (%)	Ash (%)	Carbohydrate (%)	pH
Control sample (C)	17.85 ^c ± 0.15	61.23 ^a ± 0.59	4.59 ^b ± 0.03	5.66 ^a ± 0.31	28.52 ^c ± 0.23	6.89 ^a ± 0.04
SLM-W (2xH ₂ O)	10.64 ^a ± 0.09	62.50 ^b ± 0.56	2.82 ^a ± 0.02	10.34 ^b ± 0.62	24.34 ^b ± 0.19	6.93 ^{ab} ± 0.05
SLM-S (1xNaCl and 1xH ₂ O)	11.48 ^b ± 0.10	63.24 ^b ± 0.58	2.79 ^a ± 0.02	11.15 ^b ± 0.48	22.82 ^a ± 0.20	6.98 ^b ± 0.05

a, b, c—means values in columns denoted by different letters differ statistically significant ($p < 0.05$; $\pm$ standard deviation; $n = 6$)

Fig. 1 Electrophoretic separation of proteins in 15% polyacrylamide gel (SDS-PAGE). Sample explanations: 1–2 porcine lumbar muscle, 3–4 homogenate of the mussels (C); 5–6 sample washed with a solution of NaCl and water (SLM-S); 7–8 sample washed twice with water (SLM-W)

types of interaction between the preparation proteins and other components. The first type is the protein–water interaction, which determines the protein–fat and protein–protein interactions to a considerable degree. These functional properties of proteins become modified when the environment changes. In view of this fact, we can suppose that the different pH values of the preparations will also determine their functionality [11].

Protein profile analysis by SDS-PAGE

The type of rinsing solution affected the degree of protein extraction in the muscle tissue of mussels and the final content of proteins in the surimi-like materials. The protein bands with molecular weights (MW) of 270–273 kDa, 224–228 kDa and 42–43 kDa were characterised by the

Fig. 2 Densitograms of homogenate (C) and surimi-like materials (SLM-S and SLM-W) from pond mussel separated by SDS-PAGE. Explanations: 1—peptide with MW of 270–273 kDa; 2—peptide with MW of 224–228 kDa; 3—peptide with MW of 105–110 kDa; 4—peptide with MW of 42–43 kDa

highest intensity (Figs. 1 and 2). In our previous study [9], we indicated that the first two of the aforementioned protein bands (270–273 kDa and 224–228 kDa) in this position on gel may correspond to specific filamin isoforms. The presence of other proteins in these bands, particularly myosin, cannot be excluded. Myosin heavy chain (MHC) isoforms are usually detected in this position on polyacrylamide gel. The characteristic band pattern corresponding to this protein may also indicate this (Fig. 1). In turn, the third protein band (42–43 kDa) may correspond to actin.

Two different isoforms of filamin with MW of 270 and 230 kDa were identified earlier in molluscan smooth muscle (*Mytilus galloprovincialis*) using MALDI-TOF/TOF MS. They were named FLN-270 and FLN-230, respectively. Both of these isoforms contain the C-terminal dimerization and the N-terminal actin-binding domain typical of filamins [28]. Different amounts of myosin with a molecular weight of 225–228 kDa and actin with a molecular weight of 46 kDa were found in three samples (AM—adductor muscle, IO—internal organs and FO—foot muscle) of *Anodonta woodiana* [4]. In this case, the highest percentage of myosin was found in the adductor muscle (14.27%), whereas the highest percentage of actin was found in the internal organs (19.92%).

Washing the mussel preparations with a sodium chloride solution and with water (S), as well as washing them with tap water two times (W), significantly increased ($p < 0.5$) the proportion of proteins with MW of 270–273 kDa, 105–110 kDa and 42–43 kDa, as compared to the control sample (Table 2). The band of the protein with MW of 105–110 kDa is usually associated with paramyosin [30]. Konieczny et al. [4] showed a significantly ($p \leq 0.05$) higher proportion of paramyosin in the adductor muscle (24.01%) compared to samples from internal organs (17.51%) and foot muscle (16.97%) of *S. woodiana*. Méndez-López et al.

[28] identified protein with MW of 105 kDa as proteolytic fragment FLN-270 (filamin isoform with MW of 270 kDa). The analysis of the proteins' percentage below 42 kDa in the samples washed with a salt solution (S) and water (W) showed that they were significantly ($p < 0.05$) lower than in the control sample (Table 2). When the muscle structures of pond mussels *S. woodiana* were washed with a sodium chloride solution and then once with water (S), the preparation released a significantly larger ($p < 0.05$) amount of proteins with 224–228 kDa MW and also 42–43 kDa MW than when the muscle structures were washed twice with running water (W). The analysis of the percentages of 30 and 15 kDa proteins revealed the opposite effect. The relative contents of these proteins in the preparation washed with pure water (W) were significantly higher than in the sample washed with a sodium chloride solution and then with water (S).

Dynamic mechanical analysis of samples during heating

The homogenate and surimi-like from *S. woodiana* mussels were a mixture of various proteins. This fact was confirmed by the electrophoretic separations (SDS-PAGE) of proteins acquired from the pond mussels. Thus, this mixture can be treated as a two-phase dispersion system (continuous hydrocolloid phase and dispersed phase). The continuous phase is an aqueous, colloidal solution of proteins and small-molecule, water-soluble compounds. In contrast, the disperse phase, which is insoluble, concentrated components of myofibrillar proteins and connective tissue.

Figure 3 shows changes in the elasticity modulus (G') during thermal treatment of the three types of samples under analysis (C—control; SLM-W—water; SLM-S—salt). The heating process enabled two main areas of changes in the elasticity modulus value to be identified. The first area

Table 2 The percentage share of proteins in *Sinanodonta woodiana* control sample (C) and surimi-like materials (SLM-W and SLM-S) separated by SDS-PAGE

Molecular weights of proteins (kDa)	The percentage share of proteins		
	Control sample (C)	SLM-W (2xH ₂ O)	SLM-S (1xNaCl and 1xH ₂ O)
270–273	18.60 ^a ± 0.20	21.27 ^b ± 0.74	20.72 ^b ± 0.65
224–228	13.94 ^{ab} ± 0.16	13.62 ^a ± 0.19	14.80 ^b ± 0.66
105–110	4.52 ^a ± 0.20	6.59 ^b ± 0.11	6.07 ^b ± 0.36
42–43	17.32 ^a ± 0.64	22.98 ^b ± 0.40	24.78 ^c ± 0.46
30	1.91 ^b ± 0.15	1.87 ^b ± 0.16	1.21 ^a ± 0.10
20	4.52 ^a ± 0.54	4.74 ^a ± 0.18	4.29 ^a ± 0.17
15	6.75 ^c ± 0.46	5.01 ^b ± 0.13	3.54 ^a ± 0.12
< 224–228 ÷ 42–43 >	18.59 ^b ± 1.34	14.12 ^a ± 0.70	14.96 ^a ± 0.34
< 42–43	27.79 ^b ± 1.75	21.66 ^a ± 0.72	20.81 ^a ± 0.54

a, b, c—mean values in rows denoted by different letters differ statistically significant ($p < 0.05$; ± standard deviations; n = 6)

Fig. 3 The temperature dependencies of the elasticity modulus (G') in the mussel protein systems (C, SLM—W, SLM—S) during heating and cooling

spanned temperatures ranging from approximately 20 °C to 50 °C. The other area included temperatures exceeding 50 °C. The final temperature interval, i.e. over 50 °C, was characterized by the highest dynamics of variation in the elasticity modulus value (G'). This may have been caused by different intensities of intermolecular processes, which affected the rheological properties of the protein systems during heating.

During the thermal treatment within the applied temperature interval, the elasticity modulus values (G') in the system of protein surimi-like materials (SLM—W and SLM—S) were significantly greater than the values in the control system (C). G' values different from zero (samples: C—200 Pa, W—270 Pa, S—320 Pa), which were noted in the rinsed homogenates and in the control system at 20 °C, show that the aggregation of protein molecules occurred during pre-treatment [30].

The non-dissolved molecules of myosin, paramyosin and actin (no NaCl), bind to each other through sulfhydryl groups (—SH) to form filamentous structures. The cross-linking in the low temperature range may have been caused by the interaction between the heads of part of the free myosin molecules, as well as myosin and actin or paramyosin. As a result of heating, there are changes in the physicochemical properties of the proteins contained in homogenates [31]. During thermal denaturation at 30–35 °C, native tropomyosin dissociates from F-actin.

Within the temperature range from 40 to 45 °C, the actomyosin complex becomes dissociated [32]. As a result of partial unfolding of myosin, the structuring processes become intensified and they are particularly noticeable in rinsed protein systems [9]. This process resulted in the maximum elasticity modulus values (G') at a temperature of approximately 40 °C (Fig. 3). The thermally-induced partial

Fig. 4 The dependency between the loss tangent ($\text{tg } \delta$) and temperature in the mussel protein systems (C, SLM—W, SLM—S) during heating

unfolding of the muscle protein structure in the systems being studied resulted in the formation of a spatial protein matrix with elastic properties. The density of its segments depended on the protein type and concentration [33].

In comparison to the control sample, the increased elasticity (G') of the rinsed systems (water—W and salt—S) was manifested by their reduced capacity to disperse mechanical energy (Fig. 3). The analysis of temperature-dependent variations in the loss tangent ($\text{tg } \delta$) values confirms this fact (Fig. 4). Within the range of temperatures applied for thermal treatment, the $\text{tg } \delta$ values of the protein surimi-like materials were lower than those noted for the control sample. The elasticity modulus values (G') in the control sample were lower than in the homogenates rinsed twice with water (W) or those rinsed with the NaCl solution and water (S). This may have been the effect of the activity of muscle proteases contained in the sarcoplasmic protein fraction [34], which caused the degradation of myosin. The level of these proteins in the rinsed samples may have been lower than in the control sample. Therefore, the effect of proteases was less noticeable. The differentiation of the elasticity modulus values (G') above the maximum temperature (40 °C) in the protein systems was caused by the dissociation of bonds between actin and myosin [35]. A further increase in the temperature over 50 °C caused conformation changes within both free myosin molecules and actomyosin chains (transformation of the α -helix chain into a ball). The transformation of myosin chains from a spiral into a ball damages the spatial network structure. It favours rapid aggregation caused by hydrophobic interactions and the formation of further disulfide bonds between polypeptide chains [36].

The relaxation transitions, which were manifested by maximum values on the curves of dependencies between the loss tangent (Fig. 4) and temperatures of about 55 °C

(in the homogenate samples rinsed with NaCl and water), 57 °C (in the sample rinsed only with water) and about 58 °C (in the control sample), were caused by the progressing denaturation of myosin and the beginning of the formation of the spatial network of the gel [37].

The diversification of denaturation temperatures and the beginning of the gelation process may have been caused by different levels of sarcoplasmic proteins in the samples of protein fractions under analysis. This resulted from the fact that during the sol–gel process, sarcoplasmic proteins coagulated and adhered to microfibrillar fibres, which caused gel formation problems [38].

The dynamic increase in the elasticity modulus value (G') in the systems above a temperature of 55 °C was caused by further conformation in myosin molecules. The rapid increase in the elasticity modulus over 60 °C was also caused by paramyosin cross-linking and structuralisation in the hydrocolloid continuous phase resulting from the thermal transformation of sarcoplasmic proteins [39].

At temperatures exceeding 70 °C, significant transformations occur in the G-actin monomer [40]. Although the polymerisation of native actin particles does not occur during thermal gelation, the resulting bonds between the tails of free myosin and actin molecules (there was interaction between the heads at low temperatures) contributed to the elastic reaction observed in the system [39]. Sulfhydryl groups (–SH), which are oxidised at temperatures exceeding 70 °C, cause the formation of very strong –S–S– covalent bonds in the protein. Gelation results not only from the oxidation of sulfhydryl groups into disulfide bonds but also from intra- and inter-molecular sulfhydryl/disulfide interchanges, producing a variety of cross-links without a net change in the concentration of sulfhydryl or disulfides [42]. As a consequence, there are more bonds between protein aggregates, and denatured proteins are included in the existing network [37, 43]. Simultaneously, heating intensifies proteolytic effects [44]. It was particularly noticeable in the control sample (C). The hydrolysis of myosin and actomyosin limited the efficiency of polymerisation of these proteins. This was manifested by dispersion of the elasticity modulus (G') (Fig. 3) and a decrease in the dynamic viscosity (η) (Fig. 5) at temperatures higher than 60 °C. Consequently, the structuralisation processes occurred later than in the rinsed homogenate systems.

As mentioned above, the mussel homogenates form a two-phase dispersion system. The physical properties of the continuous and dispersed phases, as well as the interactions between them, determine its mechanical-rheological properties. The approximate relation between the stiffness modulus of high-elasticity polymer spatial networks and the concentration of segments in these networks is described as follows [45]:

Fig. 5 The dependency between the dynamic viscosity (η) and temperature in the mussel protein systems (C, SLM–W, SLM–S) during heating and cooling

$$G' \cong n_s RT$$

where n_s —the concentration of segments, R —gas constant, T —temperature.

As can be seen, at a particular protein concentration, the increase in the system stiffness results from the extension of spatial network nodes by binding new segments of molecules formed as a result of interaction between the protein polypeptide chains. This results in the structuralisation and association of water, which can bind to hydrophilic groups of polypeptide chains, which could not be accessed before. This fact was confirmed in the study conducted by Lanier et al. [30]. The researchers proved that hydrogen bonds were formed in β -protein structures of myosin isolated from sea fish muscles heated above 55 °C and cooled to 25 °C. It stabilised and increased the elasticity of the protein gel network and it improved the gel elasticity. This mechanism of variation in the values characterising rheological and mechanical properties of the systems is reflected by the course of temperatures of the elasticity modulus G' (Fig. 3).

Dynamic mechanical analysis of samples during cooling

During cooling from 80 to 20 °C, the elasticity modulus (G') in all the systems increased monotonically. Apart from that, within the whole range of temperatures, the rinsed homogenate systems (SLM–W; SLM–S) were characterised by greater values of the elasticity modulus and increment than the control sample (C). Variation in the loss tangent values ($\tan \delta$) was analysed according to the temperature-dependent variation in the elasticity modulus (Fig. 6).

Within the whole range of temperatures, the $\tan \delta$ values tended to decrease. It indicated reduced relative capacity of

Fig. 6 The dependency between the loss tangent ($\text{tg } \delta$) and temperature in the mussel protein systems (C, SLM—W, SLM—S) during cooling

the systems to disperse mechanical energy. These changes were less intense in the rinsed homogenates. The increase in the elasticity modulus (G') and the decrease in the energy dissipation capacity of the protein systems, which were observed during cooling, may have been caused by the higher density of hydrogen bonds between polypeptide chains and water molecules per gel volume unit [30]. This points to the increase in the concentration of segments of the three-space protein matrix, which exhibited elastic properties.

The spatial structures formed by elastic myosin and actomyosin associates were characterised by a greater capacity to exhibit the elastic reaction induced by mechanical effect than the hydrogel formed by thermal transformation of soluble proteins, especially sarcoplasmic ones [40]. The hydrogel did not affect the elastic reaction of the whole system but it filled the viscose spatial network. This fact was reflected by the values of the dynamic viscosity (η) (Fig. 5) and loss tangent ($\text{tg } \delta$) (Fig. 6).

Thermal drip assessment

Thermal drip is one of the most important technological parameters, because it directly affects the chemical composition of final products, and in consequence, it also affects the rheological and textural properties [31]. The amount of thermal drip mainly depends on the ratio of water to protein, which affects the ability of muscle tissue proteins to bind water. It also depends on the fat content [34]. The systems analysed in the research had low fat content. Therefore, we can assume that it did not influence this determinant considerably. At the same time, there was a statistically significant difference in the content of water and protein between

the control sample (C) and the surimi-like materials (SLM) (Table 1).

The analysis of the samples showed that the protein systems obtained by washing (SLM-W and SLM-S) were characterised by significantly lower thermal drip values than the control sample (C). The following leakage values were noted in the samples: homogenate (C)— $20.63\% \pm 1.87$; the sample washed twice with water (W)— $10.53\% \pm 0.89$; the sample washed with saline and water (S)— $6.10\% \pm 0.66$ ($p < 0.05$). It is possible that this effect was caused by the different content of protein fractions in the preparations, as was evidenced by the results of electrophoretic analysis. When the muscle tissue were washed, sarcoplasmic proteins are leached. During the sol–gel transformation, sarcoplasmic proteins coagulate and adhere to myofibrillar fibres. This hinders the formation of gels [38]. Thus, they affect the spatial matrix, which maintains free water and fat. The content of sarcoplasmic proteins in the washed homogenates was lower than in the control sample. Therefore, the thermal drip was much smaller in them.

Rheological and textural properties of gels

The final mechanical, rheological and textural properties of cross-linked systems are not only the effect of changes in their molecular structure induced by thermal treatment, but also their reorganisation during storage [45]. This fact was confirmed by measurements of the rheological and textural parameters of the gels after about 15 h of cold storage (Table 3). The cold storage of the gels made from the samples caused an increase in their elasticity G' , as compared with the G' values measured when the systems were cooled to 20 °C (Fig. 3). This increase was caused by the degree of cross-linking of their spatial structures. It was the consequence of the formation of cross-links between part of the polypeptide chains which developed during the cold storage of the preparations [30].

Our analysis showed that the values characterising the mechanical and rheological properties of the mussel homogenate systems reflected the conformation changes occurring in the spatial structure of proteins as a result of thermal treatment. Therefore, we can suppose that their mechanical properties are interrelated. Two mechanical determinants of the texture were identified: firmness (N) and elasticity (%) (Table 3). The firmness value might be determined by the density of segments constituting the external matrix of proteins, which was directly related to the elasticity modulus (G') calculated during measurements of mechanical and rheological properties. The rinsed homogenate systems were characterised by greater firmness and elasticity than the control sample ($p < 0.05$).

There are many experiments confirming the beneficial effect of the washing process on the texture properties of

Table 3 The parameters of the texture and selected rheological traits of gels made from the protein systems (control sample and surimi-like materials SLM) after heating and cooling to 20 °C and after cold storage (marked in bold)

Type of sample	Firmness (N)	Elasticity (%)	G' (Pa)	G' (Pa)	η (Pas)	η (Pas)
Control	2.7^c ± 0.3	21.4^b ± 3.1	43,960.0 ^c ± 1613.3	48,600.7^c ± 1350.6	1260.0 ^c ± 96.6	1800.3^b ± 75.4
SLM (washed 2xH ₂ O)	5.4^a ± 0.5	35.6^a ± 2.6	51,428.4 ^b ± 1577.3	53,001.5^b ± 1890.8	1419.3 ^b ± 98.3	2201.2^a ± 89.9
SLM (washed 1xNaCl + 1xH ₂ O)	4.3^b ± 0.4	34.1^a ± 2.1	54,131.0 ^a ± 1643.4	55,710.4^a ± 1450.3	1703.2 ^a ± 100.1	2295.1^a ± 98.6

a, b, c—means values in columns denoted by different letters differ statistically significant ($p < 0.05$; $\pm$ standard deviation; textural test $n = 10$; rheological test $n = 6$)

products obtained from comminuted meat raw material [13, 46]. Removing some of the dissolvable proteins from raw material in the course of washing results in an increase in the concentration of proteins of a high molecular mass, i.e. 270–273 and 224–228 kDa (Table 2). It is manifested by a significantly higher capacity to form strong and elastic gel after thermal treatment [8]. Therefore, it can be assumed that the resulting spatial network of surimi-like material (SLM) systems is characterised by a greater segment density, which could have contributed to their significantly ($p < 0.05$) greater firmness (Table 3).

This is also confirmed by Ramirez et al.'s [47] studies regarding fish surimi. According to their results, the washing process significantly increased the firmness of steam-boiled samples by 30% ($p \leq 0.05$) in comparison with samples which were not subjected to washing. At the same time, the elasticity modulus G' determined for SLM showed a higher value in comparison with the control sample (Table 3). On the other hand, the greater firmness of the water washed sample (W) in comparison with the sample washed with salt solution (S) could have been caused by a significantly higher proportion of low-molecular proteins ($p < 0.05$), i.e. of 30 and 15 kDa MW taking part in the formation of the spatial network. In these systems, the protein network was formed by macromolecular proteins (myosin, actomyosin and paramyosin associates) and by low-molecular-weight protein fractions. Therefore, their spatial network was characterised by a higher density of segments, which resulted in the greater strength of the surimi-like extracts.

The other texture parameter, elasticity, specifies the ability of the system to recover its original shape. During the elastic deformation of real systems, some mechanical energy causing deformation is absorbed by internal friction (viscosity). Therefore, the degree of elasticity might be partly related to the dynamic viscosity (η) calculated during measurements of mechanical and rheological properties. The samples which were twice rinsed with water or with the solution of salt and water were characterised by a similar elasticity ($p < 0.05$) (Table 3).

While the firmness value development was influenced, primarily, by the structural organisation of the spatial matrix forming the continuous phase, the elasticity value was

additionally influenced by hydrogel mechanical-rheological traits affected by thermal transformations of soluble proteins, mainly sarcoplasmatic [40, 48]. In the system examined, sarcoplasmatic proteins form very poorly networked spatial matrices [49]. This leads to a weak response to mechanical deformations and the gel obtained is of a viscoelastic nature. Hence, the lower content of myofibrillar proteins and the higher concentration of sarcoplasmatic proteins in the control system explains its lower elasticity value in comparison with samples subjected to washing (Table 3).

The analysis revealed a strong correlation between the determinants of the texture of the mussel homogenates and their rheological parameters. The coefficient of correlation r between the firmness and elasticity modulus (G') for the control sample and samples subjected to the washing process using salt and water and twice with water amounted to 0.80, 0.83, and 0.81 respectively, whereas the coefficient of correlation between elasticity and dynamic viscosity (η) for the control sample (C) and protein preparations S and W amounted to 0.94, 0.96 and 0.95, respectively.

It is evident from the analysis of rheological properties of the protein systems examined here that their elastic properties are conditioned by increased protein presence of a specific molecular weight. It is corroborated by the correlation analysis of the elasticity modulus (G') with the composition of proteins determined using the SDS-PAGE technique. Correlation coefficients (r) between G' and proteins from the control sample (C), samples subjected to the process of washing with salt and water (S) and twice with water (W) were calculated. The r values for proteins of molecular weight MW were as follows: 270–273 kDa—C $r = 0.88$, S $r = 0.90$, W $r = 0.89$; 105–110 kDa—0.86, 0.88, 0.87; 42–43 kDa—0.84, 0.91, 0.88. Similarly, high values were found for the correlation coefficient $r \geq 0.88$ for the systems analysed between firmness and the content of the above-mentioned protein groups.

Conclusion

The washing procedure had a significant impact on the protein composition of the experimental surimi-like materials (SLM) obtained. Washing the pond mussel preparation

with a saline solution and with water (S), as well as washing the preparation twice with pure water (W) significantly ($p < 0.05$) increased the percentage of proteins with 270–273 kDa, 105–110 kDa and 42–43 kDa MW compared to the control sample.

In the systems of surimi-like materials, thermally induced conformation changes of proteins led to the development of the protein spatial matrix of segment density preconditioned by the procedure used for their preparation. The high protein concentration of high molecular mass and proteins of 42–43 kDa MW in SLM resulted in an increase in both their elastic properties and the value of texture determinants in comparison with the control sample. Firmness differences between samples washed twice with water and salt solution and water could have been caused by the greater proportion of low molecular protein of 30 and 15 kDa in the development of the spatial network. On the other hand, a greater proportion of sarcoplasmatic proteins in the development of the system spatial structure of the control system in comparison with samples subjected to washing led to a decrease in its elasticity. The research results showed that small deformations measured by means of dynamic rheometry were correlated with measurements of the texture parameters. This study gives insight into the biophysics of surimi-like material made from rinsed mussels and it takes research into a new scientific area.

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