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The Effect of High Pressure and Subzero Temperature on Gelation of Washed Cod and Salmon Meat

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Summary

The objective of the present work is to examine the influence of pressure up to 193 MPa at subzero temperature (without freezing of water) on myofibrillar proteins of salmon and cod meat and on the properties of gels obtained from washed mince of these fish. The solubility of proteins from myofibrils of cod and salmon meat suspended in 100 mM KCl solution increased after treating the samples with pressure above 60 MPa. The results of SDS-PAGE analysis showed that under these conditions two myosin light chains, tropomyosin and troponin T were released from myofibrils. The solubility of proteins in 0.9 M NaCl solution of washed fish meat after pressure treatment at 60 MPa and –5 °C decreased to about 80–90 % and at 193 MPa and –20 °C to 60 %. Pressurization of cod meat decreased only slightly the solubility of proteins in SDS and urea solution and the solubility of salmon meat was similar to that in the unpressurized sample. There were no differences in the electrophoretic pattern of proteins from untreated and pressurized cod and salmon meat in the range of 60 to 193 MPa and –5 to –20 °C. The pressure treatment of washed salmon and cod meat at a temperature below 0 °C induced gelation; on the other hand, hardness of gels was lower by 28 and 26 %, respectively, than that of gels formed by heating. The salmon and cod gels pressurized at 193 MPa and –20 °C and then heated were much harder than only pressurized or heated gels.

Key words: high pressure processing, subzero temperature, washed cod and salmon meat, gelation, properties of myofibrillar proteins

Introduction

Numerous studies have been carried out on high-pressure effect on the properties of muscle proteins. High pressure treatment exerts conformational changes in myofibrillar proteins that can lead to their depolymerisation and solubilisation, denaturation and aggregation depending on the pH, concentration and type of salt present, as well as parameters of the process (*1–10*).

From research and practical point of view, close attention is drawn to the pressure-induced gelation of fish myofibrillar proteins (*3,7,11–16*). This process was proposed as an alternative to traditional heat-induced gelation in preparation of different gel-based products from surimi and fish paste. The mechanism of pressure-induced denaturation/gelation is considered to be a complex process in which rearrangement and/or destruction of non-covalent bonds of the tertiary structure of proteins take place (*10,15*). The contribution of hydrogen bonds, hydrophobic interactions and electrostatic bonds in pressure-induced gels was evidenced (*13,17*). Furthermore, using differential scanning calorimetry (DSC), Angsupanich and Ledward (*18*) observed that after pressurization of cod meat at 100–800 MPa a new, quite stable structure stabilized by hydrogen bonds was formed. It was also found that covalent disulphide bonds at pressure higher than 300 MPa could stabilize the gel network (*17,19*).

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Gels formed by pressure treatment have improved some rheological properties when compared to heat-induced gels (*13,20*). Furthermore, the thermal gelation ability of muscle proteins may be enhanced by pressurization prior to heating (*3,4,21*). The properties of fish gels obtained by using high pressure depend on fish species, state of proteins, activity of endogenic proteases and transglutaminase (*11,14,21,22*). An important parameter affecting the properties of the formed gel is the temperature at which pressure treatment is applied (*13,16*). Fish protein gels with good properties may be formed by pressure treatment at refrigeration temperatures (*13,20*). Lowering of pressurization temperature below 0 °C increases the denaturation stage of myofibrilar proteins (*23*) and may affect their functional properties. Therefore, it is very interesting to study if gelation of fish proteins induced by pressure is also possible at such low temperature as –20 °C (in unfrozen system). Currently there is no literature data available on experiments conducted under these conditions. Understanding the impact that such high-pressure/low-temperature conditions exert on washed fish meat will create new opportunities in designing new products with improved functional properties and new direction in the development of fish surimi technology.

The objective of the present work is to examine the influence of pressure up to 193 MPa at subzero temperature (without freezing of water) on the myofibrillar proteins of salmon and cod meat and on the properties of gels from washed mince of these fish. The properties of gels formed by pressure treatment followed by heating were also studied. Gels induced by conventional heating were used as controls.

Materials and Methods

Preparation of washed meat and gels

Fresh filets of cod (*Gadus morhua*) and salmon (*Salmo salar*) were purchased on a local market in Gdynia, Poland. They were skinned and minced in a meat grinder (ϕ(mesh)=3 mm, model 986.86 Zelmer; Zelmer S.A., Rzeszów, Poland). The mince was washed twice by mixing it with water (1:3, by mass per volume) for 15 min at 0 °C and centrifuged at 5000×*g* (MPW 350R centrifuge; MPW Med. Instruments, Warsaw, Poland) for 30 min at 4 °C. Next, 0.15 M NaCl solution (Polish Chemical Reagents SA, Gliwice, Poland) was added to the washed meat (1:3, by mass per volume). The resulting slurry was mixed for 15 min at 0 °C and centrifuged at 5000×*g* for 30 min at 4 °C. Sodium chloride (2.5 %, by mass; Polish Chemical Reagents SA) and crumbled ice were added to prepare a batter with the required final gel moisture of 78 %. The mixture was blended for 15 min. The temperature of the mixture did not exceed 10 °C. The prepared batters were filled into flexible plastic casings (Podanfol S.A., Chodzież, Poland) of 35 mm in diameter. The filled casings (100 mm length) were subjected to pressure of 193 MPa at –20 °C and in some cases heated in water bath to achieve 90 °C in the core of the sample and kept under these conditions for 15 min (total heating time was about 50 min). Gels induced only by heating (conventional gelling procedure) were made by a setting step at 25 °C for 60 min, followed by heating (90 °C for 15 min). After cooking, all samples were cooled in 10–15 °C running water and stored overnight at 4 °C before analysis.

Isolation of myofibrils

Minced meat was homogenized for 5 s at 7000 rpm followed by 20 s at 12 000 rpm using Silent Crusher M D-91126 (Heidolph Instruments GmbH & Co, Schwabach, Germany) with six volumes of buffer (20 mM Tris-HCl, 100 mM KCl, 5 mM EDTA, pH=7.6; Merck Millipore, Darmstadt, Germany) and centrifuged at 4000×*g* (MPW 350R centrifuge; MPW Med. Instruments) for 15 min at 4 °C. The pellets were resuspended in six volumes of the buffer, blended for 2 min and subsequently centrifuged at 4000×*g* for 15 min at 4 °C. These operations were repeated five times. The final pellets were resuspended in six volumes of buffer and homogenized for 15 s at 7000 rpm and centrifuged at 4000×*g* for 15 min at 4 °C. The same operations were repeated twice. The homogenate was filtered through a mesh nylon net (20 μm pore size; Merck Millipore) and centrifuged at 1000×*g* for 10 min at 4 °C and washed with the same buffer in order to remove the connective tissue. The pellets were resuspended in 100 mM KCl and homogenized at 7000 rpm for 15 s. The biuret method was used to determine protein concentration (*24*). The final concentration of protein was adjusted to 5 mg/mL. The samples were transferred to glass tubes, closed with stoppers without leaving any air bubbles inside, and kept for 10–15 min at 0 °C before pressurization.

Pressure treatment

Pressurization was carried out at 60, 110 and 193 MPa at –5, –10 and –20 °C, respectively, as described by Malinowska-Pańczyk *et al.* (*23*). The samples and a metal spring were placed in a cylindrical metal vessel filled with water and closed without leaving any air bubbles inside. The pressure vessel was gradually immersed with the closed side down in a cooling bath at the appropriate temperature. Pressure was generated as a result of increasing the volume of the forming ice I in a sealed vessel filled with water and kept at temperatures below 0 °C. The increase of pressure up to 207 MPa causes lowering of the freezing point of water to –22 °C (*25*). After pressurization the vessel was warmed in the upper part to 15 °C and placed for a few minutes in a water bath at 20 °C. The whole process, including pressurization and decompression steps, lasted 50 min (40+10 min).

Solubility test of myofibrillar proteins in salt solutions

To test the solubility of myofibrilar proteins in salt solution, pressurized and unpressurized samples of myofibrils in 100 mM KCl were centrifuged at 4000×*g* (MPW 350R centrifuge; MPW Med. Instruments) for 15 min at 4 °C. Biuret method was used to determine protein concentration in the supernatant (*24*).

The solubility of washed meat proteins in 0.9 M NaCl solution was assayed according to Malinowska-Pańczyk *et al.* (*23*). Samples were homogenized for 5 s at 7000 rpm followed by 30 s at 12 000 rpm using Silent Crusher M D-91126 (Heidolph Instruments GmbH & Co). The homogenate was centrifuged at 4500×*g* for 30 min at 4 °C and the protein concentration in the supernatant was determined using biuret method (*24*).

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Solubility of proteins in a solution containing SDS and urea with or without β-mercaptoethanol

Untreated and meat samples treated at 193 MPa were mixed with 20 mM Tris-HCl buffer (1:9 by mass per volume, pH=8) and homogenized for 3 s at 700 rpm (Silent Crusher M D-91126; Heidolph Instruments GmbH & Co) and for 30 s at 12 000 rpm. The homogenates were divided into two parts. Solution I (2 % SDS, 8 M urea, 20 mM buffer Tris-HCl, pH=8; Merck Millipore) was added to the first part, and solution II (2 % SDS, 8 M urea, 2 % β-mercaptoethanol, 20 mM Tris-HCl buffer, pH=8; Merck Millipore) was added to the second part (1:1 by volume). The mixtures were transferred to Nalgene™ (Thermo Scientific, Waltham, MA, USA) plastic tubes, purged with nitrogen, capped, heated at 100 °C for 2 min, and agitated constantly overnight at ambient temperature. The samples were subsequently centrifuged at 2000×*g* (MPW 350R centrifuge; MPW Med. Instruments) for 5 min. Protein in the supernatant was determined using RC DC™ protein assay kit (Bio-Rad, Hercules, CA, USA). Protein in meat was determined using Kjeldahl method (*26*).

SDS-PAGE electrophoresis

Electrophoresis was performed in accordance with Laemmli (*27*). Samples of washed meat and soluble fractions of myofibrils were dissolved in 0.125 M Tris-HCl, 4 % SDS, 20 % (by volume) glycerol, 0.2 M dithiothreitol and 0.02 % Bromophenol Blue (all from Merck Millipore) (pH=6.8) and heated at 100 °C for 1 min. A volume of 30 μL of samples was placed on the 4 % stacking gel. Separation was performed on 12 % gel. Molecular mass standard from 250 to 10 kDa (HyperPage II Prestained Protein Marker, Bioline Reagents Ltd, London, UK) was used as a reference. Samples were separated on the Hoefer SE660 (Hoefer Inc, Holliston, MA, USA) vertical electrophoresis system.

Texture profile analysis and penetration test

Texture profile analysis (TPA) of the samples prepared as described in paragraph Preparation of washed meat and gels was performed in a universal testing machine (model 5543; Instron Engineering Corp., Canton, MA, USA) as described by Bourne (*28*). The gels were removed from their castings and cut to 3.0-cm height. Before analysis, the samples were tempered for 1 h at room temperature. Compression was applied using a flat plunger (a cylindrically shaped piston; 50 mm diameter) at the deformation rate of 2 mm/s. The samples were compressed in two consecutive cycles to 50 % of their initial height. The TPA parameters (hardness, springiness, cohesiveness and chewiness) were determined according to Mochizuki (*29*).

In the puncture test, the gels were penetrated to the breaking point using the equipment as above with a round-ended stainless steel plunger (*d*=5mm, model 5543; Instron Engineering Corp., Canton, MA, USA). Cross-head speed was 10 mm/min and a 10-load cell was used. The gel strength was calculated by multiplying breaking force (in N) with breaking deformation (in mm) determined from the force-deformation curves.

Folding test

The folding test was performed according to Lee (*30*). A slice (*d*=3.5 cm, *h*=3 mm) was folded over twice and the extent of its folding or crack formation were measured. The score was: AA=does not crack when folded twice, A=does not crack when folded once but cracks after two foldings, B=gradually cracks after being folded once, C=cracks immediately after first folding, and D=breakable by finger pressure without folding.

Statistical analysis

The results of solubility tests are presented as average values of at least six replications with standard deviation. Texture profile analysis parameters, gel strength and gel forming ability show the average values and standard deviation of at least eight replicates. Analysis of variance (one-way procedure) was performed to evaluate the differences between treatments using the Statistica v. 8.0 (*31*).

Results and Discussion

Protein solubility in salt solutions

The solubility of proteins from isolated fish myofibrils suspended in 100 mM KCl solution increased after treating the samples with pressure of 60, 110 and 193 MPa at –5, –10 and –20 °C, respectively. The content of dissolved proteins from salmon and cod myofibrils treated with pressure of 193 MPa was higher by about 2 and 1.5 times respectively than of those in the unpressurized samples (Table 1). This phenomenon has been observed in pressure-treated suspension of myofibrils isolated from sheep muscle (*1*), rabbit (*32*) and bovine muscle (*8*). Suzuki *et al.* (*32*) suggested that pressurization of isolated myofibrils lead to depolimerization of actomyosin, myosin polymer and F-actin, while McArthur and Wilding (*33*) supposed that hydrophobic interactions, which can probably participate in stabilization of myofibrils, are disrupted by pressure and consequently could lead to their increased solubility in low-ionic-strenght solutions.

Solubilisation of myofibrillar proteins in 100 mM KCl solution induced by pressure was confirmed by SDS-PAGE. Two myosin light chains, tropomyosin, troponin T and actin were released from myofibrils pressurized at 60 and 110 MPa (Fig. 1). Suzuki *et al.* (*32*) observed that also troponin I and C, and M protein were present in the supernatant obtained from the suspension of rabbit skeletal

Table 1. The solubility of proteins isolated from myofibrils of cod and salmon meat pressurized in 100 mM KCl solution

p/MPa	γ(protein)/(mg/mL)	
	Cod	Salmon
0.1	$(0.19\pm0.02)^a$	$(0.29\pm0.07)^a$
60	$(0.34\pm0.08)^b$	$(0.27\pm0.09)^a$
111	$(0.38\pm0.084)^b$	$(0.40\pm0.02)^b$
193	$(0.43\pm0.07)^b$	$(0.40\pm0.01)^b$

The results are expressed as mean value±standard deviation (S.D.), *N*=6; values in a particular column followed by different letters differ significantly ($p<0.05$)

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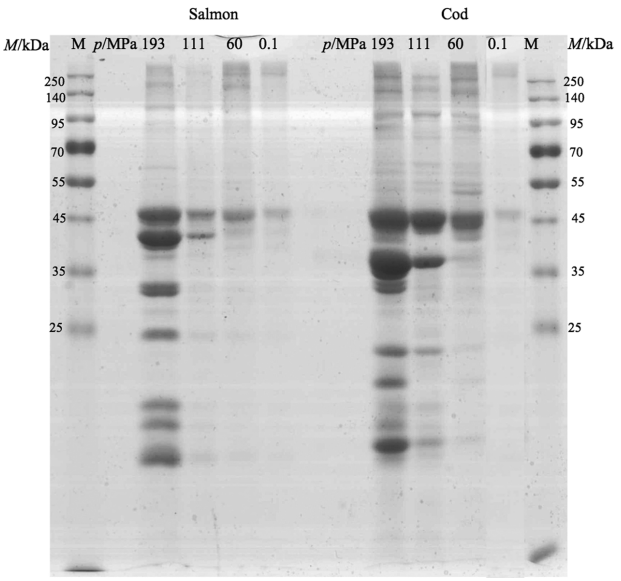

Fig. 1. SDS-PAGE profiles of soluble proteins from myofibrils pressurized in 100 mM KCl solution. M=markers

muscle myofibrils pressurized at 150 MPa. Increase of pressure up to 200 MPa caused slight solubility of myosin heavy chain, while solubility of troponin I decreased. Furthermore, the same authors found that the content of myosin heavy chain increased significantly when pressure of 300 MPa was used. However, Jung *et al.* (*8*) reported mainly the increase in the solubility of two myosin light chains of bovine myofibrils treated with pressure above 100 MPa. However, both papers showed that the pressure-induced changes in the ultrastructure of myofibrils could be responsible for releasing particular proteins.

It has been shown that changes in myofibrillar protein solubility differ depending on whether the isolated myofibrils or whole meat is pressurized (*32*,*33*). Such differences have also been observed in this work. The solubility of proteins in 0.9 M NaCl solution of washed salmon and cod meat pressurized at 60 MPa decreased to about 80–90 % and at 193 MPa and –20 °C down to 60 %, indicating that under these conditions denaturation of proteins takes place (Fig. 2). Similar results were obtained previously for unwashed cod and salmon meat (*23*). The loss of protein solubility in salt solutions was observed also after pressurization of Norway lobster (*Nephrops norvegicus*) meat at 200 MPa and 5 °C and at –20 °C (in the pressure-shift freezing process) (*2*), as well as in paste from arrowtooth flounder (*Atheresthes stomias*) pressurized at 400 and 600 MPa (*3*,*4*). Mammalian proteins and those of fish living in tropical waters are more resistant to denaturation/aggregation induced by pressure (*7*,*9*,*23*,*34*,*35*). A similar relationship is observed in the thermal process. It has been found that the thermal stability translates into pressure stability. The greater pressure and thermal stability of proteins from warm-adapted species than that from those adapted to cold habitats results from differences in their molecular structures (*36*).

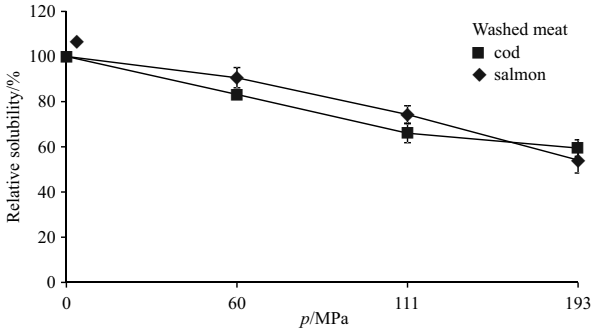

Fig. 2. The solubility of washed cod and salmon meat proteins in 0.9 M NaCl solutions after treatment at 60–193 MPa

Solubility of proteins in SDS solutions and SDS-PAGE analysis

Pressure treatment of washed cod meat only slightly decreased the solubility of proteins in SDS and urea solution, and the solubility of washed salmon meat was similar to the control (Table 2). These results indicate that a pressure of 193 MPa at subzero temperature does not cause covalent bond formation, including disulphide linkages, therefore 100 % of protein solubility in SDS and urea solution containing β-mercaptoethanol was achieved. Montero *et al.* (*22*) also reported that disulphide bonds do not participate significantly in the pressure-induced (300 MPa) aggregation of Atlantic horse mackerel (*Trachurus trachurus*) proteins. Similar results were obtained with pressurized gels from tilapia meat pastes (*36*). On the other hand, Gilleland *et al.* (*17*) showed that pressure treatment at 300 MPa and 5 °C of surimi paste from Alaska pollock (*Theragra chalcogramma*) decreased protein solubility in SDS and urea solution from 90 % (the untreated sample) to about 40 %. After including β-mercaptoethanol into the solubilizing medium, the sample was thoroughly solubilized, indicating that all these covalent bonds formed in pressurized paste corresponded to disulphide bonds. Only when incubation of the sample at 25 °C preceded the pressure treatment, the solubility in the presence of β-mercaptoethanol was lower. According to the authors, forming of ε-(γ-glutamyl)lysine bonds by transglutaminase was responsible for the decrease of protein solubility under these conditions.

Table 2. The solubility of washed cod and salmon proteins in SDS/urea solution with or without β-mercaptoethanol unpressurized and pressurized at 193 MPa

Solution	Solubility/%			
	Cod		Salmon	
	p/MPa			
	0.1	193	0.1	193
SDS/urea	87.6[a]	82.2[b]	73.9[a]	74.0[a]
SDS/urea/β-mercaptoethanol	100[a]	100[a]	100[a]	100[a]

The results are expressed as mean values, *N*=6; values in a particular row followed by different letters differ significantly ($p<0.05$). SDS=sodium dodecyl sulphate

Figs. 3 and 4 show electrophoretic profiles of proteins from the untreated and pressurized washed meat samples. They differ slightly depending on the kind of tested meat. However, there were no differences in the electro-

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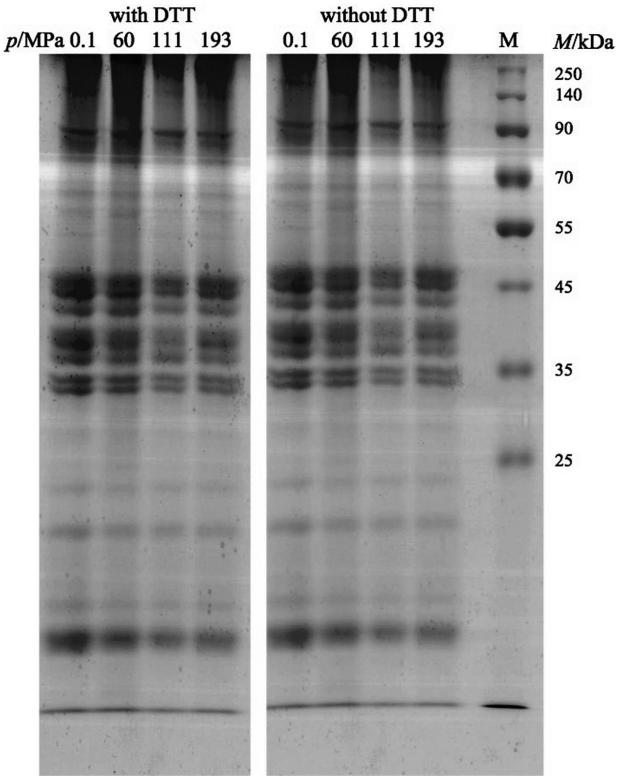

Fig. 3. SDS-PAGE profile of pressurized washed cod meat proteins; M=markers, DTT=dithiothreitol

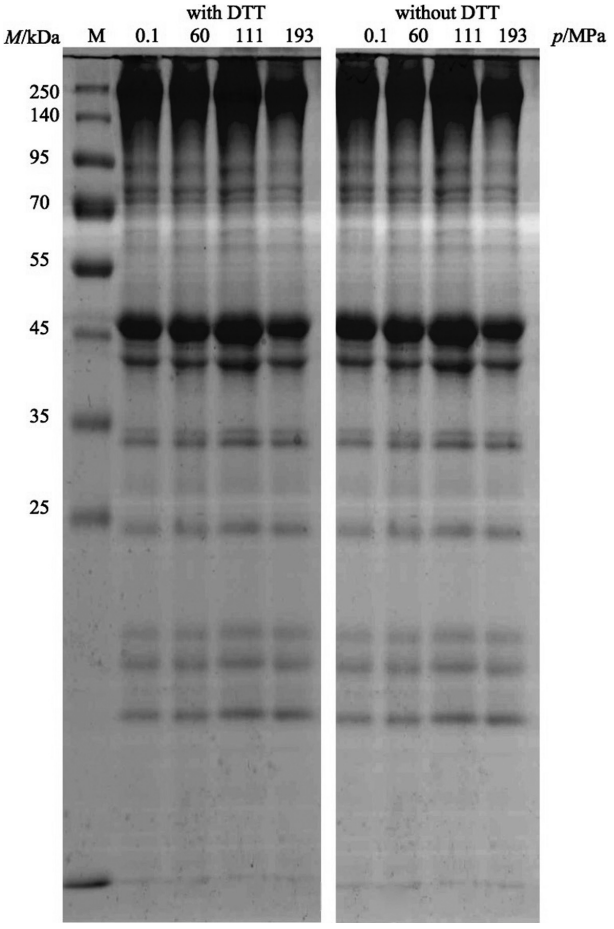

Fig. 4. SDS-PAGE profile of pressurized washed salmon meat proteins; M=markers, DTT=dithiothreitol

phoretic pattern between the untreated and pressurized samples of particular meat. The results also show that disulphide bonds in meat proteins are not formed under these conditions. Angsupanich *et al.* (*19*), using SDS-PAGE, found that disulphide bonds participate in aggregation of myosin in myofibrils pressurized at above 600 MPa isolated from turkey and cod meat but not at 200–400 MPa. Furthermore, some literature data show that enzymatic degradation of proteins is not excluded during pressure treatment of fish and mammalian meat (*5,6,22*). However, such degradation did not appear in the samples of salmon and cod meat pressure-treated at below 0 °C (Figs. 3 and 4). If proteolytic enzymes are responsible for the degradation and if this reaction takes place during pressurization, our results can be easily explained: the temperature of –20 °C during pressure treatment is too low for enzymatic reaction.

Texture and gelling properties of washed fish meat

The results of the texture profile analysis of fish gels are presented in Table 3. Pressurization of washed salmon and cod meat at subzero temperature induced gelation; however, hardness of the gels was lower by 28 and 26 %, respectively, than the hardness of the gels formed by heating. Better properties of heat-induced gels than those obtained by pressure treatment at 193 MPa may be caused by setting step used only before heating. During this stage, crosslinking of the proteins by endogenous transglutaminase can take place, resulting in the formation of

Table 3. Texture profile analysis parameters of cod and salmon gels at p=193 MPa, t=–20 °C and/or cooked at t=90 °C for 15 min

Cod treatment	Hardness/N	Springiness	Cohesiveness	Chewiness
A	(33.3±2.1)^a	(0.90±0.03)^a	(0.80±0.07)^a	(27.4±5.3)^a
B	(46.1±1.5)^b	(0.90±0.04)^a	(0.70±0.09)^a	(31.9±8.8)^b
C	(64.9±3.9)^c	(0.90±0.01)^a	(0.70±0.05)^a	(45.7±23.1)^c
Salmon reatment				
A	(35.5±1.3)^a	(0.80±0.02)^a	(0.60±0.1)^a	(22.9±4.7)^a
B	(48.0±2.5)^b	(0.80±0.04)^a	(0.70±0.05)^a	(31.1±1.2)^b
C	(98.6±5.6)^c	(0.90±0.01)^a	(0.70±0.02)^a	(67.2±5.7)^c

The results are expressed as mean value±S.D., N=8; values in a particular column followed by different letters differ significantly ($p<0.05$). A=193 MPa/–20 °C, B=90 °C/15 min, C=193 MPa/–20 °C, followed by 90 °C/15 min

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harder gels than the gels obtained by pressurization (*37*). Some authors also observed that pressurization of washed fish meat at 3–20 °C caused gelation, but their textural properties were worse than of gels obtained in the heat process without the setting step (*12,14,20,38*). On the other hand, Uresti *et al.* (*3*) showed the opposite effect; the hardness of the gels formed by pressure treatment of unwashed arrowtooth flounder meat was higher than the hardness of the heated gels. Proteolytic enzymes from the muscles can be responsible for weaker mechanical properties of the heated samples. Uresti *et al.* (*4*) observed enzymatic degradation of proteins during incubation of paste from arrowtooth flounder meat at 60 °C. It is possible that in the samples of arrowtooth flounder meat cooked at 90 °C some proteolysis of myofibrillar protein appeared before reaching this final temperature. Chung *et al.* (*11*) showed that when a protease inhibitor was present in the samples, the pressure-induced surimi gels from Pacific whiting (*Merluccius productus*) were characterized by lower shear stress and shear strain than the heat-induced gels. The differences in the reported data can also result from the parameters of the process. In the pressure range of 100–400 MPa, the properties of gels can be better than those obtained by heating; however, they can worsen with further increase in pressure (*7,14*). The mechanical properties of gels also depend on a test used for their determination. The breaking force and deformation (puncture test) were higher in pressure-induced gels, while their hardness (compression test) was lower (*12,20,38*). In our work, we observed the same direction of changes in the hardness and gelling strength. There was also a dependence between the hardness and the folding test. The pressure-induced gels from salmon and cod meat were characterized by lower hardness (Table 3) and lower value of the folding test (Table 4) than the gels formed in the thermal process.

Table 4. Cod and salmon gel strength and gel-forming ability

Cod treatment	Gel strength/(N·mm)	Gel-forming ability
A	(69.8±1.4)^{a}	B
B	(81.8±1.0)^{b}	A
C	(177.1±16.3)^{c}	AA
Salmon treatment	Gel strength/(N·mm)	Gel-forming ability
A	(34.1±0.7)^{a}	C
B	(68.1±3.9)^{b}	A
C	(189.1±12.3)^{c}	AA

The results are expressed as mean value±S.D., *N*=8; values followed by different letters differ significantly ($p<0.05$). A=193 MPa/–20 °C, B=90 °C/15 min, C=193 MPa/–20 °C, followed by 90 °C/15 min

The data on rheological properties of gels heated after pressure treatment are scarce. Gilleland *et al.* (*17*) reported that heat-induced surimi gels and gels from surimi pressurized before heating were characterized by similar tensile stress. However, this study showed that the hardness of gels from washed salmon and cod meat pressurized at 193 MPa and –20 °C and then heated was much higher than that of the gels from the only pressurized or only heated samples (Table 3). Uresti *et al.* (*3,4*) reported that hardness of heat-induced arrowtooth flounder gels increased when pressurization was used prior to heating. This effect was higher at a pressure of 400 than of 600 MPa. According to the authors, better molecular organization of gels is achieved at lower pressure and improvement of mechanical properties is possible after cooking of the samples.

The gels from pressurized and heated washed cod and salmon meat had the highest chewiness (Table 3). The springiness and cohesiveness of all samples were similar irrespective of the gel preparation conditions. The data presented in Table 4 also show that the pressurized and heated samples had the highest scores of the gel strength and folding test, while the gels induced only by pressure were characterized by the lowest values.

The mechanism leading to the formation of gels having improved rheological properties as a result of two processes: pressurization and heating, still remains unclear. Our research has shown that, similarly as at temperature above 0 °C, pressurization at subzero temperature causes denaturation and depolymerization of myofibrilar proteins (Fig. 1 and Table 1) (*3,4,17*). It has been hypothesized that these changes lead to opening up the structure of the protein and may result in a higher number of available locations on the substrate, which facilitated the active crosslinking of myosin (*17*). High pressure also increases the hydrophobicity and sulfhydryl content in actomyosin. This may be partially the reason for enhanced strength of the gels (*10*). Some authors suggested that pressure-induced denaturation and unfolding of meat proteins lead to exposition of reactive lysine and glutamine residues and facilitate formation of ε-(γ-glutamyl)lysine linkage by endogenous transglutaminase (*39*). Although many studies on the mechanism for inducing gelation of myofibrillar proteins by the pressure have already been carried out, many questions still remain unanswered. More research is needed to explain which changes in the proteins under high pressure are responsible for the enhancement of the thermal gelation of meat proteins.

Conclusion

The pressure treatment at subzero temperature of cod and salmon myofibrils suspended in diluted salt solution leads to the release of some myofibrillar proteins, but in the pressurized washed meat it leads to the loss of protein solubility in 0.9 M salt solution. The disulphide bonds do not participate in denaturation/aggregation of proteins developed by moderate pressure at subzero temperature. The pressure treatment under these conditions induces gelation of washed cod and salmon meat; however, the hardness of the gels is lower than that of the gels formed in the thermal process. On the other hand, pressurization at subzero temperature before cooking allows the achievement of high synergistic effect in the increase of hardness of the gels and gelling strength.

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