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CHARACTERIZING PROTEIN CHANGES CAUSED BY APPLICATION OF HIGH HYDROSTATIC PRESSURE ON MUSCLE FOOD BY MEANS OF DSC

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Present research concentrated on high pressure assisted thawing of fish fillets from commercially important fish species such as ocean perch, saithe and herring. DSC (Perkin-Elmer DSC 7) has been used to follow the pressure-induced changes of proteins. Results of pressure assisted thawing are compared to those of conventional thawing in water at 10°C and ambient pressure. The results obtained clearly demonstrate that changes in DSC curves become pronounced with advancing high pressure and are dependent on the species of fish.

Keywords: *DSC, fish, high pressure, quality, thawing*

Introduction

Thermal processing of foods has mainly been a two-dimensional process, time and temperature being the two process variables [1]. With the commercialisation of high pressure processes, pressure may now be used as a 'third dimension' in order to improve food quality and safety. Thawing generally occurs more slowly than freezing, potentially causing further damages to the texture of food that had been frozen. Rapid thawing at low temperatures can help to prevent the loss of food quality during thawing [2]. The thawing rate during conventional thawing processes is controlled by two main parameters outside of the product: the surface heat transfer coefficient and the temperature of the medium surrounding the sample. The heat transfer coefficient remains the only parameter affecting the thawing rate at atmospheric pressure. Hence, the small temperature difference between the initial freezing point and room temperature does not allow high thawing rates [3]. DSC proved to be a useful technique to study freeze-thaw processes [4, 5]. Pressure markedly influences the ice-water transitions, and the use of high pressure technology has a distinct potential to improve the kinetics of thawing and ice crystal characteristics [6]. Two different thawing processes can be defined: the pressure assisted thawing process where the phase transition is obtained by heating at constant pressure, and, the pressure induced thawing process where the phase change is induced by pressurisation [7, 8]. Research on high pressure assisted thawing of frozen fish and meat has shown the possibilities to significantly reduce the thawing time [9–12] as well also to minimise the drip volume after thawing [10] and subsequent cooking [13–16]. Reducing the drip loss and lowering the processing time can be seen as major advantages of high pressure assisted thawing. However, high pressure treatment is also connected with colour and texture changes [17–20]. These are obviously dependent on the level of pressure applied as well as on the pressurisation time. The main effect of high pressure is to provoke changes in hydrophobic and electrostatic interactions, with important consequences for the secondary, tertiary and quaternary structures in proteins. Covalent bonds are weakly affected by pressure. Pressure treatment of proteins can lead to significant conformational changes which influence functionality [21].

DSC has emerged as a technique of choice for the study of thermal transitions of food. The conversion of a protein from a native to a denatured state by heat is a co-operative phenomenon and is accompanied by a significant uptake of heat, seen as an endothermic peak in the DSC curve. For proteins, the thermally induced process detectable by DSC is the structural melting or unfolding of the molecule, the thermal denaturation of proteins being attributed to the rupture of intermolecular hydrogen bonds, the temperatures at which the bonds rupture being a measure of the thermal stability of proteins. Their determination under controlled conditions can provide direct comparison of the thermal stability of the different proteins. The enthalpy value which is correlated with the net content of the ordered secondary structure of a protein, is actually a net value obtained through the combination of endothermic reactions and exothermic processes, including protein aggregation and the break-up of hydrophobic interactions. A successful approach to the study of the native conformation of proteins is the subjection of the protein to physical

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and chemical stresses, followed by a determination of the effect of these stresses on its thermal denaturation. Differential scanning calorimetry is frequently applied according to recently published papers, which deal with fish quality and processing [22–29].

Recently, own research dealt with the impact of high pressure assisted thawing at 200 MPa on the quality of fillets from various fish species [30]. In this study apart from DSC measurements several other quality parameters, such as texture, colour, water holding capacity, drip loss were measured and it was found that some protein denaturation in the fish muscle was caused by pressure treatment at 200 MPa leading to quality differences between conventionally thawed and high pressure assisted thawed fish fillets. As a result, further research concentrated on the pressure range from 100 to 200 MPa, using the same methods as used earlier [30] and concentrating on fish species of commercial importance. The aim of the present paper is to show how quality parameters of fish species are influenced when pressure in the abovementioned range was used for thawing in comparison to fish fillets thawed conventionally using a water bath. Special emphasis is placed on DSC curves taken from the differently thawed samples, whereby the pressure range was widened to 0.1 and 300 MPa. Prior to the experimental results, the literature of differential scanning calorimetric investigations with special emphasis on high pressure treated fish and meat will be reviewed.

DSC investigations to follow high pressure effects on meat and fish

In one of the first applications of DSC to show the influence of high pressure on beef muscle proteins, it was reported that after treatment of 150 MPa for 3 h, the actin peak was eliminated while the myosin peak was reduced to an inflection in the curve. On the other hand the peak of sarcoplasmic protein did not change markedly [31]. Investigating by DSC different fish species after being treated with 200 MPa for 13 h, it was found that the two myosin peaks became small and were no longer distinguishable from each other, while the actin peak became indistinct [32]. Because the endothermic peak also became small or disappeared almost when unpressurised fish meat was treated with heat it is assumed that part of the mechanism of pressurised denaturation is similar to that of thermal denaturation. In all samples, the enthalpy values of all pressurised meat were markedly smaller than those of untreated meat. This decrease in ΔH was time dependent [33]. Since the enthalpy change may be reflected in changes in the structure of muscle in the meat, it may be assumed that the difference in the decrease of ΔH should depend on the species of fish.

In pressure-induced gel of Alaska pollock surimi with 300 MPa applied for 0.5 h, only one peak at 48°C was found which had a ΔH of 0.015 cal g^{-1}, indicating a partial denaturation of myosin. No peak was evident at higher temperature, indicating a complete denaturation of actin due to the pressure treatment [34]. Pressure-induced gelation of myosin in solution is initiated by head to head association and succeeded by aggregation of the helical tail region of myosin [35]. When fresh cod (*Gadus morhua*) fillet was treated in the pressure range from 100 to 380 MPa for 20 min, the DSC curves showed a shift of the myosin transition toward lower temperature as pressure increased. The intensity of this transition decreased drastically when a pressure greater than 100 MPa was applied. The actin peak was practically unaltered at pressures below 300 MPa, but could no longer be observed in samples treated with a pressure equal to or greater than 300 MPa. It is concluded that protein denaturation is very weak in cod treated for 20 min at 100 MPa, while it is important when pressure is equal or greater than 200 MPa [36]. In contrast to myofibrillar proteins, no significant differences in the DSC curves and denaturation of the intramuscular collagen were found in the pressure range from 100 to 300 MPa [37]. When carp (*Cyprinus carpio*) tissue was pressurised from 98 to 490 MPa for 13 h at 1°C, the endothermic peaks of DSC curves of pressurised meat were small compared with the untreated meat and a new clearly defined peak appears at around 38°C. This peak shifted to low temperature with increasing pressure. It appears that the phenomenon was the result of decreased myosin thermal stability. The decrease in ΔH for all pressurised samples indicated that the denaturation of protein in pressurised tissue occurred as a phenomenon similar to that of heat denaturation. Myosin was found to be more readily denatured than actin by pressurising [38]. When frozen carp muscles were thawed by high pressure at 100, 200, 300 MPa and in running water, it became obvious that the DSC curves from samples thawed at 100 MPa and in running water were approximately comparable to those of fresh carp. The endothermic peaks shifted to higher temperature regions with increases of pressure at 200 and 300 MPa. Protein denaturation of carp muscle caused by pressurisation was also recognised by a lowering of ΔH [39]. When minced pork has been pressure treated in the range from 100 to 800 MPa, the following was recognised: at 200 MPa the two myosin peaks observed in fresh meat merged to become a broad peak, whereas the actin peak temperature remained unchanged. However, above 400 MPa the distinct myosin and actin peaks were lost. Increasing the pressure to 200 MPa caused an insignificant decrease in the enthalpy for myosin and actin transitions. However, increasing the pressure from 200 to 400 MPa

caused a sharp decrease in the total enthalpy [40]. Cod fillet pressure treated at 100 to 800 MPa at room temperature for 20 min were subjected to DSC. The 100 MPa samples showed a significant loss of the myosin peak and at 200 MPa almost all of the myosin peak disappeared. At 300 MPa it was apparent that many of the sarcoplasmic proteins as well as the actin denature. At 400 MPa both actin and most of the sarcoplasmic proteins denatured while the myosin was already fully denatured at 200 MPa. Myosin in cod appears thus to be more effected by pressure than the same substance from red meat. Interestingly, after pressure treatment, a new low melting transition was seen around 32°C. This new structure appeared to form at pressures of 100 MPa or more and appears to be little effected by the degree of pressure (to 800 MPa) to which the fish is subjected. At pressures of 300 MPa and higher, further new structures are formed which appeared to melt in the range 40–60°C [39]. When isolated myofibrillar protein and myosin from cod or turkey were subjected to pressures of up to 800 MPa for 20 min, DSC measurements indicated that high pressure induced denaturation of myosin led to the formation of structures that contained hydrogen bonds and were additionally stabilised by disulfide bonds. These bonds were also important in heat-induced myosin gels [42]. Through DSC it was demonstrated for the first time that, depending on both pressure and temperature, comminuted meat pressurisation may efficiently preserve protein from subsequent thermal denaturation in combined pressure/heat processes of meat batters as well as fish mince [43–46]. However, protein denaturation by hydrostatic pressure at 10°C was directly related to pressure level and holding time, and intensified with increasing salt molarity. Pressure assisted freezing as well as pressure shift freezing of meat samples at 200 MPa and –21°C resulted in a strong protein denaturation in which actin practically disappeared and myosin and sarcoplasmic proteins were greatly reduced as shown in the corresponding DSC curves. However, connective proteins remained practically unaltered by pressurisation and/or freezing [47, 48]. Also for fish muscle tissue (turbot (*Scophthalmus maximus*) fillet) it was shown that conventional freezing followed by frozen storage for two days has little effect on the characteristic thermal transitions. However after pressure shift freezing (140 MPa, –14°C) and two days frozen storage, the DSC curve showed an important reduction of the myosin peak and the appearance of a new important peak at lower temperature connected with a significant decrease in total enthalpy. On the other hand, the actin peak was almost uninfected [49]. When DSC curves of high pressure thawed (200 MPa) fish fillets were compared with those of conventionally thawed samples great differences became obvious. Whereas the conventionally thawed samples showed patterns comparable to largely native proteins of fresh fish muscle, the patterns of high pressure thawed muscles confirmed a far-reaching denaturation of the muscle proteins as the result of applying high pressure [30]. When turbot fillet was subjected to high pressure treatment at 100, 140, 180 and 200 MPa for 15 and 30 min at 4°C, DSC showed a full denaturation of myosin at 200 MPa and the appearance of a new structure from a treatment at 100 MPa for 30 min. In addition the peak of actin appeared to be particularly reduced after the treatments at 180 and 200 MPa. The decrease in total enthalpy was pronounced with increasing pressure [50]. Modification of bovine myofibrillar proteins induced by high pressure processing has been investigated at pressures ranging from 50 to 600 MPa for 10 min at 20°C by DSC and showed that the proteins lost part of their total enthalpy during pressure treatment. ΔH started to decrease after treatment at 150 MPa, continued to decrease up to 500 MPa and then remained steady. While the myosin peak started to decrease at 200 MPa, the actin peak was not sensitive to pressure [51].

An interesting application of high pressure treatment on fish muscle is reported by Goodband [52]. By applying high pressure it may be possible to selectively denature the myosin molecule. By selectively denaturing the myosin head regions, so rendering them 'inert' prior to freezing, it ought to be possible to prevent/reduce thick filament associations during frozen storage. Reducing the thick filament association in the frozen state will allow the myofibrillar lattice to recover on thawing, and water losses from the fillet will be reduced. If pressure treatment can be tailored in such a way that neither the helical regions of myosin nor the actin in the thin filaments are denatured, then the myofibril structure will be largely preserved. The effect of increasing high pressure on the thermal properties of cod fillet has been monitored by DSC. It could be seen that the application of a pressure of 100 MPa to the cod prior to freezing, caused denaturation of parts of the myosin molecule. On increasing the pressure treatment to 200 MPa, denaturation of actin was observed accompanied by an increase in the amount of moisture lost on cooking. This process for preventing protein aggregation during frozen storage by high pressure treatment of fish muscle prior to freezing has been patented [53].

Experimental

Materials and methods

Fish samples

The samples were processed as previously reported [30]. The fish species include ocean perch (*Sebastes*

marinus) (length 17–25 cm) caught during the 244th research trip of the FRV 'Walther Herwig III' west of Greenland, saithe (*Pollachius virens*) (length 48–57 cm), bought at the Hamburg fish market and herring (*Clupea harengus*) (length 25–31 cm), commercially caught in the Baltic Sea. Only herring was used as skin-on fillet the other samples were skinless. All samples were stored frozen at –24°C for between 2 to 4 months prior to the thawing experiments.

Thawing process

In order to evaluate high pressure assisted thawing of foodstuffs, the same transportable high pressure unit was used as described earlier [30]. Pressure was built up using a high pressure reciprocate pump (DSXHW, Haskel Ltd., California, USA) which automatically compensates for pressure dependent density changes (volume changes) of the treated fish sample. The pressure transmitting medium (silicone oil) was chosen because of its suitable chemical and thermophysical properties within the relevant pressure and temperature range. A water bath (Haake 6P, Karlsruhe, Germany), equipped with a thermostat (Haake D1), was used to thaw the fish samples. The temperature of the circulating water was set to 10±0.5°C. Compared to ambient conditions and similar medium temperature, the phase transition time within the pressure range investigated can be reduced by 40 to 60%.

Differential scanning calorimetry

The measurements were performed using a Perkin-Elmer DSC 7 device equipped with a Perkin-Elmer Intra cooler II and Pyris software. The fish samples consisting of small (15±3 mg) pieces of ordinary muscle after thawing were weighed (±0.1 mg) into 60 μL stainless steel pans (LVC 0319-0218) and sealed. At least three samples were heated from 10 to 95°C at a scanning rate of 10 K min^{-1} with a sealed empty pan as reference. The instrument was calibrated for temperature and enthalpy using indium and naphthalene as standards. The transition temperature (T_{max}) was recorded and the transition enthalpy (ΔH) was calculated from the peak area using the Pyrus software and expressed in J g^{-1} sample material. Results are displayed in the figures as average curves.

Methods to assess quality

Several other physical methods such as texture and colour measurement, determination of water binding capacity and drip loss beside the sensory evaluation of selected quality parameters of thawed and thawed then cooked fillet were used to determine the quality as influenced by thawing. These methods are described in detail earlier [30]. Results of this investigation will be reported elsewhere and will be discussed here only when necessary to explain the effects of different pressure levels of protein denaturation derived from the DSC curves.

Results and discussion

Thermal behaviour

The thermal behaviour of fish fillets measured by DSC as influenced by different levels of high hydrostatic pressure used for thawing is shown in Figs 1–3. The calculated respective enthalpy values are shown in Tables 1–3. As muscle tissue is a complex system comprising three classes of proteins: sarcoplasmic, myofibrillar and connective tissue, the DSC curves for whole muscle are also very complex. The first peak at low temperature is connected with myosin and related proteins, while the last one at higher temperature is connected with actin, the other important myofibrillar protein. The peaks between the above two are connected with sarcoplasmic proteins and/or collagen [54–57]. Thawing of saithe fillet at ambient pressure does not markedly influence the DSC curve (Fig. 1, curve a), so that the properties of fresh fish are almost preserved. The influences of freezing and frozen storage on the transition temperature and enthalpy, if there were any, will be mirrored by this curve. Appropriate results have been published recently [58]. Thawing at elevated pressure has a marked influence on the DSC curves (Fig. 1, curves b–g, Table 1). Already at 50 MPa a new peak at lower temperature in the range of 32–35°C appears, while the myosin peak is reduced and diminished with progressive elevation of the pressure and until it possibly disappears above 250 MPa. At this pressure additionally a small transition at higher temperature becomes obvious. The actin peak is seen to be almost stable up to 100 MPa

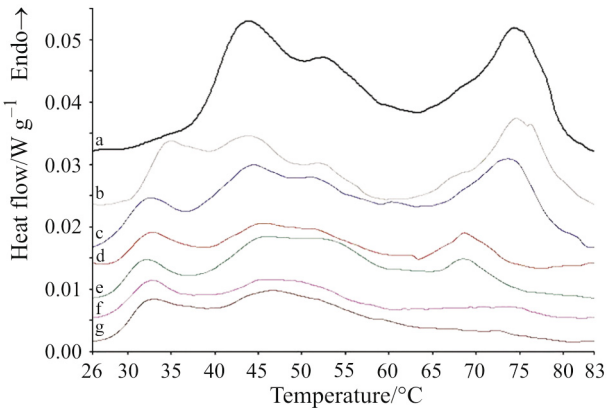

Fig. 1 DSC curves taken on thawed ordinary muscle of saithe as affected by high pressure treatment (a – conventionally thawed, b – 50 MPa, c – 100 MPa, d – 150 MPa, e – 200 MPa, f – 250 MPa, g – 300 MPa)

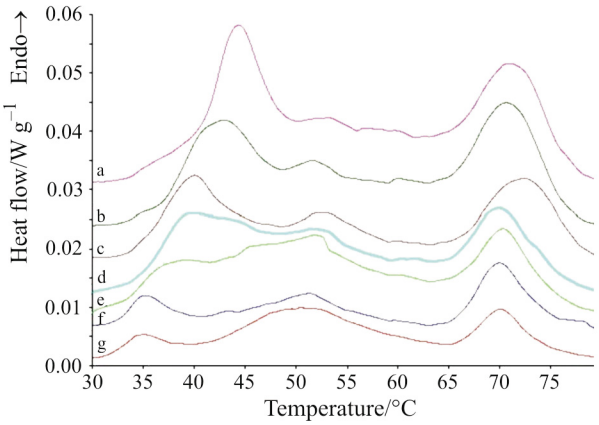

Fig. 2 DSC curves taken on thawed ordinary muscle of ocean perch as affected by high pressure treatment (a – conventionally thawed, b – 50 MPa, c – 100 MPa, d – 150 MPa, e – 200 MPa, f – 250 MPa, g – 300 MPa)

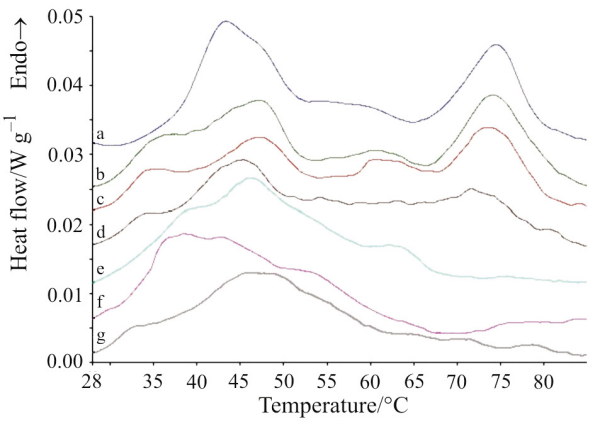

Fig. 3 DSC curves taken on thawed ordinary muscle of herring as affected by high pressure treatment (a – conventionally thawed, b – 50 MPa, c – 100 MPa, d – 150 MPa, e – 200 MPa, f – 250 MPa, g – 300 MPa)

with only slight reduction in enthalpy (Fig. 1, curves b, c). Thawing at 150 and 200 MPa is then connected with a lowering of both the transition temperature and enthalpy for actin until disappearance after thawing at 250 and 300 MPa. The existence of a new low temperature melting peak already noted at low levels of high pressure thawing as well as the occurrence of a new melting area at medium temperature around 50°C is in accordance with earlier reports on DSC investigation of cod fillet subjected to high pressure treatment in the range from 0.1 to 800 MPa [41]. This could be expected because both saithe and cod are fish in the same taxonomic family. There is also an agreement in the fact that myosin appears to be far more sensitive to pressure compared with actin. Furthermore, it can be noted that freeze/thaw in combination with high pressure treatment alone obviously exert a greater denaturation on muscle proteins, because actin completely disappeared at lower temperature as already reported in [41]. The DSC pattern of ocean perch is different from that of saithe (Fig. 2). Two well-defined transition peaks for myosin and actin can be seen. Between both, two small, broad transitions occur possibly indicating sarcoplasmic and connective proteins. Raising the pressure during thawing to 100 MPa, is connected with a shift of the myosin transition to a lower temperature (Table 2). This shift continuous over the entire high pressure range connected with a progressive reduction of its transition enthalpy. The second transition peak appears to be stable as far as temperature is concerned and even increasing in enthalpy with elevation of pressure. The third transition peak can be seen only in the curves a and b (Fig. 2) and appears therefore to be most sensitive to pressure. In contrast to saithe, the actin peak of ocean perch does not disappear over the entire range of pressure assisted thawing investigated. However, the transition enthalpy for actin shows a continuous decrease with increasing pressure. On the direct effect of pressure on G- and F-actin, the following results were reported [59]: (*i*) actin is irreversibly denatured >150 MPa without ATP, but >250 MPa with ATP. The amount of protein denatured by pressure is dependent on the initial protein concentration. (*ii*) ATP protects actin from pressure-induced denaturation. DSC curves resulting from thawed herring muscle as influenced by increasing high pressure (Fig. 3) show at a glance that in this case actin ap-

Table 1 Transition temperatures T_{max} (°C) and transition enthalpies ΔH (J g^{-1}) measured by DSC on ordinary saithe muscle after conventionally and pressure assisted thawing

Pressure/MPa	Peak 1		Peak 2		Peak 3		Peak 4	
	T_{max}	ΔH	T_{max}	ΔH	T_{max}	ΔH	T_{max}	ΔH
0.1	–	–	43.2	0.47	53.3	0.07	74.5	0.87
50	34.5	0.15	44.1	0.11	52.8	0.02	74.6	0.84
100	32.3	0.13	44.3	0.35	51.5	0.03	74.1	0.56
150	32.7	0.12	45.8	0.30	–	–	68.6	0.16
200	31.9	0.16	45.8	0.60	–	–	68.9	0.10
250	32.6	0.11	–	–	49.6	0.18	–	–
300	32.4	0.14	–	–	47.3	0.21	–	–

Table 2 Transition temperatures T_{max} (°C) and transition enthalpies ΔH (J g^{-1}) measured by DSC on ordinary muscle from ocean perch after conventionally and pressure assisted thawing

Pressure/MPa	Peak 1		Peak 2		Peak 3		Peak 4	
	T_{max}	ΔH	T_{max}	ΔH	T_{max}	ΔH	T_{max}	ΔH
0.1	44.3	0.66	53.3	0.03	59.8	0.02	71.2	0.69
50	42.7	0.47	51.7	0.04	60.1	0.01	70.9	0.72
100	40.9	0.44	53.1	0.10	–	–	72.5	0.58
150	39.2	0.32	52.8	0.04	–	–	70.1	0.43
200	37.1	0.01	52.3	0.07	–	–	70.4	0.40
250	35.1	0.11	51.3	0.16	–	–	69.9	0.32
300	34.5	0.07	51.2	0.49	–	–	70.0	0.27

Table 3 Transition temperatures T_{max} (°C) and transition enthalpies ΔH (J g^{-1}) measured by DSC on ordinary herring muscle after conventionally and pressure assisted thawing

Pressure/MPa	Peak 1		Peak 2		Peak 3		Peak 4	
	T_{max}	ΔH	T_{max}	ΔH	T_{max}	ΔH	T_{max}	ΔH
0.1	–	–	43.3	0.77	58.8	0.04	74.5	0.50
50	34.7	0.06	47.6	0.28	61.1	0.07	74.1	0.57
100	36.9	0.03	47.6	0.12	60.3	0.06	74.6	0.31
150	33.7	0.02	45.3	0.27	54.3	0.01	71.6	0.13
200	38.6	0.02	46.5	0.30	63.9	0.03	–	–
250	36.5	0.14	45.6	0.05	–	–	–	–
300	–	–	45.9	0.63	–	–	–	–

pears to be more sensitive to pressure compared to myosin. This high melting point protein decreases in enthalpy with increasing pressure and disappears after thawing at 200 MPa. In contrast, myosin alternates slightly in its transition temperature and does not denature even at highest pressure applied for thawing. A possible explanation is given by [52] which could show that for cod at a particular pressure level the very complex molecule will be partly stabilised by selectively denaturing the myosin head regions, thus rendering them 'inert' prior to freezing. Here it is hypothesised that in herring the same is the case also, which means pressure stabilises the special regions of the molecule and so preserves the whole molecule against denaturation. Shifts appearing in the transition temperature can be seen as expression for this phenomenon. The thermal denaturation of myosin was shown to occur in three (at least) partly independent cooperative endothermic processes [60]. As herring has the highest fat content among the species investigated (Table 4), it can also be assumed that myosin is possibly protected against denaturing [61], as also in cod treated at 50 MPa, which causes the appearance of a low temperature transition peak, which disappears only after thawing at 300 MPa. The transition assigned to sarcoplasmic/connective tissue proteins in the temperature range between 55 and 64°C is of very low enthalpy (Table 3) and disappears after thawing at 250 MPa. It can be summarised that the thermal behaviour of fish muscle proteins, measured by DSC, appears to be species specific and is influenced by applying high pressure in the range from 50 to 300 MPa compared to that of conventional thawing at ambient pressure. Some phenomenon, such as the existence of a low temperature melting peak as well as stabilised myosin molecule are obviously caused by high pressure treatment. Even the high pressure treatment at 300 MPa does not result in fully denatured fish muscle proteins. The reflection of the protein denaturation as observed by DSC in terms of chosen quality parameters mainly defined by physical methods as well as sensory evaluation will be consecutively given in brief.

Table 4 Proximate composition (%) of fish species used for thawing

Species	Protein	Fat	Moisture	Ash
Ocean perch	18.3	6.8	73.6	1.0
Saithe	19.6	1.0	79.0	1.1
Herring	16.2	16.3	66.3	1.1

Influence of different thawing conditions on the quality of the fish fillet

The chemical composition of the fish tissue used for thawing is shown in Table 4. According to their fat content, the species can be divided into lean (saithe),

Table 5 Sensory assessment of thawed and thawed then cooked fillet from saithe and ocean perch as influenced by high pressure thawing (scores are given in the range from 0=no deviation from freshly caught fish to 3=markedly changes in the respective quality attribute)

Thawed at MPa		Thawed				Thawed then cooked			
		texture	odour	colour	gaping	odour	colour	taste	texture
Saithe	0.1	1.6	0.3	1.2	0.1	1.7	1.2	1.2	1.9
	100	0.3	0	0.3	0	1.3	1	1	1.3
	150	0	1.0	0	0	1.0	0.9	1.7	1.3
	200	0	1.0	0	0	1.3	0.3	1.3	2.7
Ocean perch	0.1	0.7	0.1	1	0	0.1	0.7	0.3	1.6
	100	0.3	0.3	0	0	1	0	1	1
	150	0	1	0	0	0.7	0.7	1	0.7
	200	0.7	1.3	0	0	0.7	0	1.7	1.3

Table 6 Sensory assessment of thawed and thawed then cooked herring fillet as influenced by high pressure thawing (scores are given in the range from 0=no deviation from freshly caught fish to 3=markedly changes in the respective quality attribute)

Thawed at MPa	Raw				Cooked				
	skin appearance	flesh colour	texture	odour	odour	skin appearance	flesh colour	texture	taste
0.1	1.6	2.1	1.7	0.6	0.4	1.0	1.3	1.3	1.7
100	1.0	1.3	0.7	1.3	0.7	0	0.7	0	1.0
150	1.3	0.7	0.5	1.7	1.0	0.3	0.2	0.5	1.0
200	0.7	1.0	0	2.0	1.7	0.3	0.2	0.3	2.3

Table 7 Colour differences measured on fillet as influenced by high pressure assisted thawing (conventionally thawed samples were used as control)

Thawed at MPa		Thawed				Thawed then cooked			
		ΔL^*	Δa^*	Δb^*	ΔE^*	ΔL^*	Δa^*	Δb^*	ΔE^*
Ocean perch	100	10.6	0.8	2.2	10.8	3.1	0.4	0.3	3.1
	150	24.2	1.7	5.1	24.8	4.1	0.2	0.7	4.2
	200	23.1	1.2	3.7	23.4	3.1	0.2	1.6	5.6
Saithe	100	3.0	0.8	2.0	3.7	2.0	0.5	0.05	2.9
	150	18.8	2.4	1.6	19.0	2.9	0.3	0.03	2.9
	200	18.4	1.6	1.1	18.5	2.2	0.01	1.5	2.7
Herring	100	6.7	1.7	2.7	7.4	0.7	0.3	0.2	0.8
	150	14.1	1.7	4.0	14.8	4.1	0.1	0.6	4.1
	200	16.8	3.0	3.8	17.7	4.4	0.5	0.1	4.4

medium (ocean perch) and fatty (herring) fish. Sensory evaluation (Tables 5 and 6) indicates a slight rancid odour and taste only for herring thawed at 200 MPa. At lower pressure (100, 150 MPa), no signs of the promotion of rancidity in the fish flesh caused through pressure treatment are noticeable. One of the most obvious quality changes caused by high pressure thawing is that of colour. Discoloration, the consequence of pressure treatment was widely observed [30]. The differences between the conventionally and high pressure thawed samples indicate a marked influence of high pressure treatment >100 MPa (Table 7). Remarkable colour changes (indicated by very strong colour differences ΔE^*) are mainly caused by a pronounced increase in lightness, L^*, due to pressure treatment. Smaller changes are to be seen in both redness, a^*, and yellowness, b^*. The intensity of colour changes is nearly doubled when pressure is raised to 150 or 200 MPa. After heat treatment ΔE^*, the colour differences between conventionally and pressure assisted thawed samples become in agreement with [19] much smaller than in raw fillets. The water holding capacity is characterised by measuring the water loss during thawing (thaw drip) and the expressible moisture after compression to 70% of both thawed and thawed then cooked samples. The thaw drip decreases with increasing pressure (Fig. 4), the decrease for herring being linear. Compared with conventionally thawed samples, the decrease in thaw drip is impressive. This behaviour is in agreement with other reports [10, 13, 15, 16, 30].

Table 8 Expressible moisture (%) measured on thawed and thawed then cooked fish fillets as a function of high pressure treatment

Thawed at MPa	Thawed			Thawed then cooked		
	ocean perch	saithe	herring	ocean perch	saithe	herring
0.1	3.8±0.8*	4.3±1.2*	7.1±2.1*	4.8±0.7	5.4±0.9	4.9±0.1
100	11.6±2.1	10.9±2.8	10.4±2.9	4.7±0.8	5.2±1.1	6.7±1.2
150	8.6±1.5	10.4±1.7	14.0±3.6	5.1±0.5	6.6±0.9	6.1±1.8
200	10.3±1.4	9.1±1.4	13.5±3.1	4.5±0.6	6.2±1.3	7.1±1.0

*asterisk indicate significant differences (p<0.05) within a column

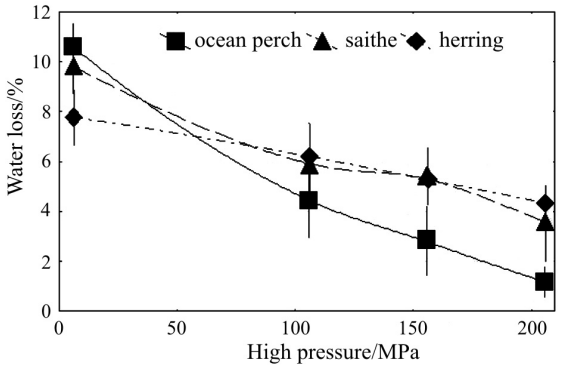

Fig. 4 Thaw drip of fish fillets as affected by pressure used for thawing

However, when the water holding capacity of thawed samples is assessed as expressible moisture (Table 8), pressure treatment caused a significant increase in water loss, while between different pressure levels no clear trends are to be seen. A strong increase in the water loss on cooking by applying high pressure has been reported [52]. Contrary, in thawed then cooked samples no influence of pressure can be observed.

Conclusions

Pressure assisted thawing of frozen fish fillets in the range from 0.1 to 300 MPa affected progressively the denaturation of muscle proteins with higher pressure. DSC curves show mainly that myosin is denatured with increasing pressure, while actin is the more resistant myofibrillar protein. This behaviour appears to be species specific as in herring the actin has been shown to be the most sensitive muscle protein. Also the appearance of new low temperature melting peaks caused by pressure treatment during thawing, has been observed for both saithe and herring indicating that responses of the different fishes on high pressure are species specific. DSC provides a valuable tool for investigating the protein denaturation caused by pressure assisted thawing. The changes in quality attributes observed for sensory assessment, colour and water holding capacity which are pronounced by elevated pressure can be attributed to this protein denaturation.

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