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Effects of high-pressure processing on proteolytic enzymes and proteins in cold-smoked salmon during refrigerated storage

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Abstract

The present paper describes the effects of high-pressure processing on the activity of proteolytic enzymes in cold-smoked salmon and enzyme extracts for pressures up to 300 MPa. The activities of the three enzymes, cathepsin B-like, cathepsin B + L-like and calpains were reduced at all pressure levels of up to 300 MPa (at ca. 9 °C for 20 min) in crude enzyme extracts prepared from cold-smoked salmon. Calpain almost completely inactivated at 300 MPa. High-pressure did not influence general proteolytic activity but activated the enzymes in muscles at higher pressure levels studied until 18 days of storage. An increase in the activity of cathepsin B + L-like and calpain was seen after 12 days of refrigerated storage. Myosin heavy chain was less affected at higher pressure levels (300 MPa) only as shown by Sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE) of proteins and further refrigerated storage had no obvious effects on proteins.

Keywords: Cold-smoked salmon; High-pressure processing; Proteolytic enzymes; Spoilage; Cathepsin B-like; Calpains

1. Introduction

Cold-smoked salmon (CSS) is produced by salting and smoking of fresh salmon. CSS is commercially distributed vacuum-packed under refrigerated conditions. The temperature range (28–32 °C) used in the cold-smoking process is unlikely to inactivate the enzymes inherent to salmon tissue (Hultmann, Rørå, Steinsland, Skåra, & Rustad, 2004). A reduced shelf life, due to enzymic actions, was reported as outlined in a recent review (Lakshmanan, Piggott, & Paterson, 2003). Among inherent enzymes, proteolytic enzymes, such as cathepsins (lysosomal cysteine endopeptidases) and calpains (calcium-activated neutral proteinases) are the important ones. They have the capability to rapidly hydrolyze the muscle proteins at *post-mortem* pH of the muscles (Ho, Chen, & Jiang, 2000).

Among various catheptic enzymes, cathepsin L and cathepsin B have been found to be responsible for the extensive softening of muscle in mature salmon and spawned chum salmon (*Onchorynchus keta*), respectively (Yamashita & Konagaya, 1990a, 1990b, 1991). Konagaya (1982) also recorded an increased catheptic activity during spawning migration of chum salmon. These enzymes are known to digest both myofibrillar proteins (actomyosin) and connective tissues (Yamashita & Konagaya, 1990a). Cathepsin L has also been associated with the production of jelly-like softening in some seafoods (Aoki & Ueno, 1997; Lund & Neilsen, 2001; Toyohara et al., 1993) and degradation of myosin (actomyosin) into low molecular (LM) fragments at pH 5.5–7.0 as observed in mackerel. Calpains could hydrolyse the Z-line of pig, beef and fish myofibrils at pH 6.5–7.5 (Ho et al., 2000).

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Though, autolytic enzymes did not seem to produce off-flavours/off-odours, their impact on textural quality brought a limit to the shelflife and hence an early downgrading of the product. Increasing salt concentrations reduced catheptic activity, but enzymes were still active at salt levels relevant to smoked salmon (Reddi, Constantanides, & Dymaza, 1972). Recently, Hultmann et al. (2004) have studied the effects of smoking temperature on proteolytic activity in fresh and cold-smoked salmon. Smoking temperatures of up to 29.9 °C did not influence the activity of cathepsin B-like enzymes or general proteolytic activity (GPA), with high smoking temperatures causing reduced myofibrillar extractability and decrease in myosin heavy chain (MHC) levels. This emphasizes the need to develop an effective method to control enzyme activity in CSS.

High-pressure processing (HPP) of foods can either deactivate or enhance enzymic activity in food systems, as shown by various studies on real and model food systems. A clear reduction in autolytic activity was observed at pressures above 200 MPa at elevated temperatures, and it was less affected by step-pulsed than by continuous pressurization (Hurtado, Montero, Borderias, & Solas, 2001a). This varies from product to product and with the process variables adopted. A significant reduction in autolytic activity to a level of 68% was observed in two cases of chill stored octopus, which were treated at 7 °C (400 MPa for 15 min or 400 MPa for 35 min pulses) and 40 °C (400 MPa for 15 min or 400 MPa for 35 min pulses) (Hurtado, Montero, & Borderias, 2001b). There was no difference in the autolytic activity between control and pressurized samples during subsequent storage. High-pressure at 100–300 MPa for various durations (up to 30 min) on enzyme extracts from bluefish and sheephead, affected the enzyme activity, especially cathepsin C, collagenase, chymotrypsin and trypsin-like enzymes (Ashie & Simpson, 1996). Enzyme extracts from fish were more susceptible than their mammalian counterparts. High pressures at 100–200 MPa can cause the release of endogenous proteases which otherwise participate in meat conditioning (Ohmori, Shigeshisa, Taji, & Hayashi, 1991). High pressures at 101.3–202.7 MPa induced the destruction of lysosome membranes and caused leakage of lysosomal enzymes into sarcoplasmic fluid (Ohmori, Shigehisa, Taji, & Hayashi, 1992). A similar result was observed in pressure treated cod, where increased proteolytic activity at 200 MPa at pH 3.3 and 9.0, and decreased activity at pressures above 800 MPa were found (Angsupanich & Ledward, 1998).

There is a need to control the activity of spoilage enzymes in CSS to improve its quality. Considering the applications of HPP in other foods, we are particularly interested to study the effects of high-pressure processing on the activity of proteolytic enzymes and changes in proteins in CSS during refrigerated storage.

2. Materials and methods

2.1. Raw material, storage conditions and experimental set up

Cold-smoked salmon (CSS) was purchased from a local supermarket in vacuum packs and stored at −20 °C until used. After thawing, packs were mixed well, and ca. 15–20 g aliquots of fish were vacuum packed (90 micron, 140 × 170 mm, Cryovac, UK) and kept at 0 °C using ice prior to HPP. All sample packs were placed in ice on sampling days to minimise the temperature effect. Unless otherwise mentioned, double-distilled water was used for all procedures, and enzyme storage tubes and assay tubes were sterile to avoid bacterial contamination. The experiment consisted of two sets of samples; CSS samples and crude enzyme extracts from CSS. Thus the present study allowed differentiation of high-pressure effects on enzymes inherent to fish muscles, and in solution. After pressurization, the samples were stored at 4–5 °C and enzyme activities were analysed until 18 days, at intervals.

2.2. Product characteristics

Approximately 50 g samples were drawn from three randomly-selected retail packs and analysed for their product characteristics. pH was determined by diluting the homogenised sample with distilled water (1:1) and using a pH meter, and moisture content (AOAC, 1995a), and salt (AOAC, 1995b) were by standard procedures. Water phase salt (WPS) content was calculated from the contents of salt and moisture.

2.3. Preparation of crude enzyme extract

10 g of sample were homogenised with 50 ml ice cold double-distilled water for 2 min and the homogenate allowed to stand in ice for 30 min with occasional stirring. After 30 min, the homogenate was centrifuged at 14,600*g* at 4 °C for 20 min. The supernatant was filtered through a 0.45 μm sterile filter unit and stored at −80 °C prior to analysis.

2.4. High-pressure processing

The vacuum-packed CSS samples were high-pressure treated at the Department of Food Science, Queen's University Belfast, Belfast, using a Food Lab 900-High Pressure Food Processor (Stansted Fluid Power Ltd., Stansted, UK). The size of sample holder was 21 cm × 7 cm. The pressure levels used were 0.1 (control), 100, 150, 200 and 300 MPa at ca. 9 °C for 20 min. The

pressure come-up time was 200 MPa min^{-1} and pressure release time was 2 min. Approximately 2 ml of crude enzyme extracts from the same batch of CSS as used above were also pressurized at the indicated pressure levels. 10% vegetable oil and 90% water (v/v) was used as the pressure transmitting medium and cold-circulating liquid (50% monoethylene glycol and 50% clean tap water) was used to help maintain the temperature in the pressure vessel. The temperature increase due to adiabatic heating was 2 °C for each 100 MPa.

2.5. General proteolytic activity

The Barrett and Heath (1977) method was used to determine the general proteolytic activity of samples. Into a 1.5 ml sterile Eppendorf tube was added 0.4 ml of buffer solution containing 0.2 M phosphate, and 0.1 M citric acid (pH 6.0 or 6.5) (McIlvaine, 1921), 0.4 ml enzyme extract and 0.4 ml 1% haemoglobin solution. The tubes containing the reaction mixture were incubated at 25 °C for 2 h in a water bath and 0.200 ml 20% TCA was added after 2 h to end the reaction. The tubes were then incubated at 4 °C for 15 min to allow precipitation of unhydrolysed proteins and centrifuged at 6000*g* at 4 °C for 10 min (Eppendorf centrifuge 5415C, Fisher Scientific UK, Loughborough, UK). The protein content was determined from 100 μl of supernatant using BIO-RAD protein kit (Bio-Rad Laboratories, UK), measuring the absorbance at 750 nm (Beckman DU-7500 spectrophotometer), based on Lowry's method (Lowry, Rosebrough, Farr, & Randall, 1951). Bovine serum albumin was used as a standard. The total proteolytic activity was expressed as mg TCA-soluble peptides liberated/g wet weight/h. The results are based on three replicate measurements.

2.6. Assay of cathepsin B and B + L enzymes

2.6.1. General

The activities of both cathepsin B-like enzymes and cathepsin B + L-like enzymes were assayed by the Barrett and Kirschke (1981) method.

2.6.2. Cathepsin B-like enzymes

0.1 ml enzyme extract and 0.1 ml of substrate solution (0.0625 mM of *Z*-Arg-Arg-7-amido-4-methylcoumarin HCl in 100 mM Bis–Tris, 20 mM EDTA, 4 mM dithiothreitol, pH 6.5 at 4 °C) were incubated at 4 °C for 10 min. The reaction was stopped with the addition of 3 ml 1% sodium dodecyl sulphate in 50 mM Bis–Tris (pH 7.0). When enzymes split the substrate, 7-amino-4-methylcoumarin (AMC) is liberated; its fluorescence was measured at an emission wavelength of 460 nm after excitation at 360 nm (RF-5301 PC Spectrofluorophotometer, Shimadzu, Kyoto, Japan).

2.6.3. Cathepsin B + L-like enzymes

0.1 ml of enzyme extract was incubated with 0.1 ml of substrate solution, 20 μM of Z–Phe–Arg-4-methoxy-β-naphthylamide HCl (buffer solution of 0.1 M sodium citrate and 0.2 M sodium phosphate, pH 6.5) at 4 °C for 10 min. The reaction was stopped after 10 min by adding 3 ml 100 mM monochloroacetate, 30 mM sodium acetate and 70 mM acetic acid, pH 4.3 at 4 °C. The amount of fluorescence of AMC was measured at an emission wavelength of 460 nm after excitation at 360 nm.

2.7. Assay for calpains

Activity of calpains was measured by the method of Sasaki, Kikuchi, Yumoto, Yoshimura, and Murachi (1984). 0.1 ml of enzyme extract and 0.1 ml of substrate solution (0.125 mM L-Methionine-7-amido-4-methylcoumarin triflouroacetic salt in 100 mM Bis–Tris, 5 mM $CaCl_2$, pH 6.5 at 4 °C) were incubated for 10 min at 4 °C. By adding 3 ml 30 mM monochloroacetic acid, 21 mM acetic acid and 9 mM sodium acetate (pH 4.3 at 4 °C), the reaction was arrested after 10 min. Fluorescence of AMC was measured at an emission wavelength of 460 nm after excitation at 360 nm.

2.8. Sodium dodecyl sulphate–polyacrylamide gel electrophoresis of proteins

Laemmli's (1970) method of Sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE) was used to identify changes in proteins in experimental samples during refrigerated storage; 0.5 g of homogenised sample was mixed with 10 ml 0.125 M Tris buffer (pH 7.5) that contained 8 M urea, 2% SDS, and 2% 2-mercaptoethanol, homogenised with sterile hand homogenisers and stirred overnight at 18 °C. The tubes containing the sample were then centrifuged at 12,000*g* for 20 min at 16–18 °C and the supernatant was removed. An aliquot of sample (0.6 ml) was mixed with an equal amount of denaturing buffer (0.125 M Tris buffer, pH 6.7 containing 10% SDS, 0.002% 2-mercaptoethanol and 0.002% bromophenol blue). The sample mixture were boiled at 100 °C for 5 min, placed immediately in ice and 10 μl of this solution was used for SDS–PAGE analysis of proteins using 8% polyacrylamide gels at 130 V and 30–40 mA. The gels were stained with Coomassie brilliant blue and the unknown bands in the sample were identified by comparison with those of broad range protein molecular weight markers (10–225 kDa) (Promega, UK).

2.9. Statistical analyses

Analysis of variance (ANOVA) was carried out with Minitab 13.1 (Minitab Inc., State College, PA, USA).

3. Results and discussion

3.1. Product characteristics

The CSS used in the present experiment had a moisture level (mean ± SD) of 65.3 ± 2.1%, salt, 4.2 ± 0.3% and pH, 6.21 ± 0.01. The WPS was 6.0 ± 0.5%. Table 1 shows pH and moisture content of the samples on the 0 and 18th days of storage. Storage time had a significant effect on pH of the fish muscle ($p < 0.05$), whereas pressure did not influence either moisture or pH levels in the pressurized samples ($p = 0.087$).

3.2. General proteolytic activity

Figs. 1 and 2 depict the changes in GPA of crude enzyme extracts and pressure treated CSS, respectively. The GPA at pH 6.0 was greatly reduced by treatment up to 150 MPa in enzyme extracts compared with treatment at 200 MPa and above ($p < 0.05$), whereas this was not the case at pH 6.5 where treatment pressure had no effect ($p = 0.235$) (Fig. 1).

The GPAs of control and pressurized CSS during storage are given in Fig. 2. As expected there was great variation within each sample of CSS, as shown by the standard deviations. Pressure treatment had no effect on GPA at either pH, but storage time affected GPA at pH 6.5 ($p < 0.01$). It is interesting to note that the effect of high-pressure on general proteolytic activity was less in the muscle of CSS than in crude enzyme extracts. This suggests that the enzymes are more resistant to pressure when they are intact in muscle and susceptible if they are in solution. Overall, a higher GPA was recorded at pH 6.0 (0.10 ± 0.03 mg peptides/g wet weight/h) than at pH 6.5 (0.08 ± 0.05 mg peptides/g wet weight/h) ($p < 0.05$). Hultmann et al. (2004) found similar results in cold-smoked salmon, which was smoked at different temperatures of up to 29.9 °C and Stokes and Rustad (1995) also obtained similar results. Salmon protease needs a higher temperature and pH for maximum activity (Stokes & Rustad, 1995). The effect of HP treatment on the autolytic activity of octopus (*Octopus vulgaris*) muscle varied with pressurization temperature. Pressurization temperature of 40 °C was found to be more effective than 7 °C and

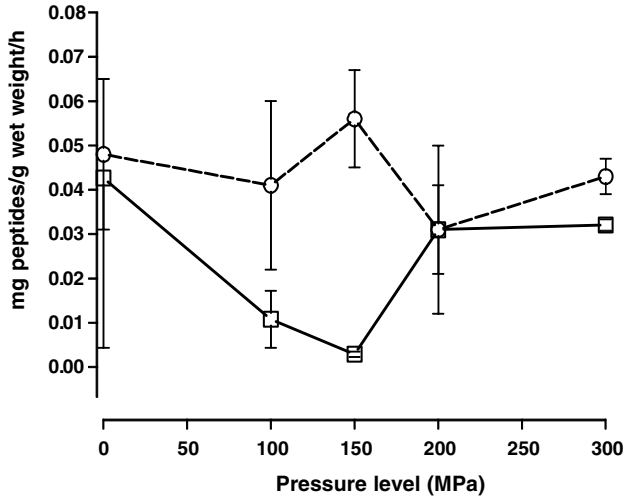

Fig. 1. Proteolytic activity of enzyme extracts from cold-smoked salmon, expressed as TCA-soluble peptides/g wet weight/h. pH 6.0, (□); pH 6.5, (○).

reduced proteolysis was observed from myofibrillar proteins (from MHC as analysed by SDS–PAGE method) (Hurtado, Montero, Borderias, & An, 2002). Autolysis was reduced when pressures were above 450 MPa in octopus muscle (Hurtado, 2000).

3.3. High-pressure effects on the activity of enzymes

3.3.1. Enzyme extracts

As shown in Fig. 3, all three enzymes tested were highly susceptible to high pressures, with greater inactivation at 300 MPa. Calpains seemed to be affected to a greater extent by lower pressure levels ($P < 0.05$). Up to 200 MPa, the activity of cathepsin B-like enzymes was almost close to that of the control and a variation was observed at 300 MPa ($P < 0.005$). Cathepsin B&L like enzyme was largely inactivated at 300 MPa, and the effect of HP was clear from the lower pressure levels used in this study (100 MPa) ($P < 0.001$).

3.3.2. Activity of cathepsin B and B + L enzymes

Figs. 4–6 show changes in the levels of cathepsin B-like, cathepsin B&L-like and calpain in HP-treated CSS during refrigerated storage for up to 18 days.

Table 1
Changes in moisture content and pH of control and pressure-treated samples during storage (Mean ± SD)

Pressure levels[a] (MPa)	Moisture content		pH	
	0 day	18th day	0 day[b]	18th day[b]
0.1	72.26 ± 3.92	67.11 ± 1.96	6.27 ± 0.01	6.12 ± 0.06
100	68.09 ± 0.01	64.87 ± 3.09	6.30 ± 0.03	6.01 ± 0.12
150	63.48 ± 2.07	65.78 ± 0.01	6.30 ± 0.02	6.14 ± 0.15
200	67.10 ± 0.49	65.92 ± 0.19	6.32 ± 0.02	6.22 ± 0.01
300	63.92 ± 0.79	64.81 ± 0.06	6.29 ± 0.02	6.39 ± 0.02

[a] Pressure level was non-significant on both pH and moisture contents.
[b] Storage time was significant on pH ($p < 0.05$).

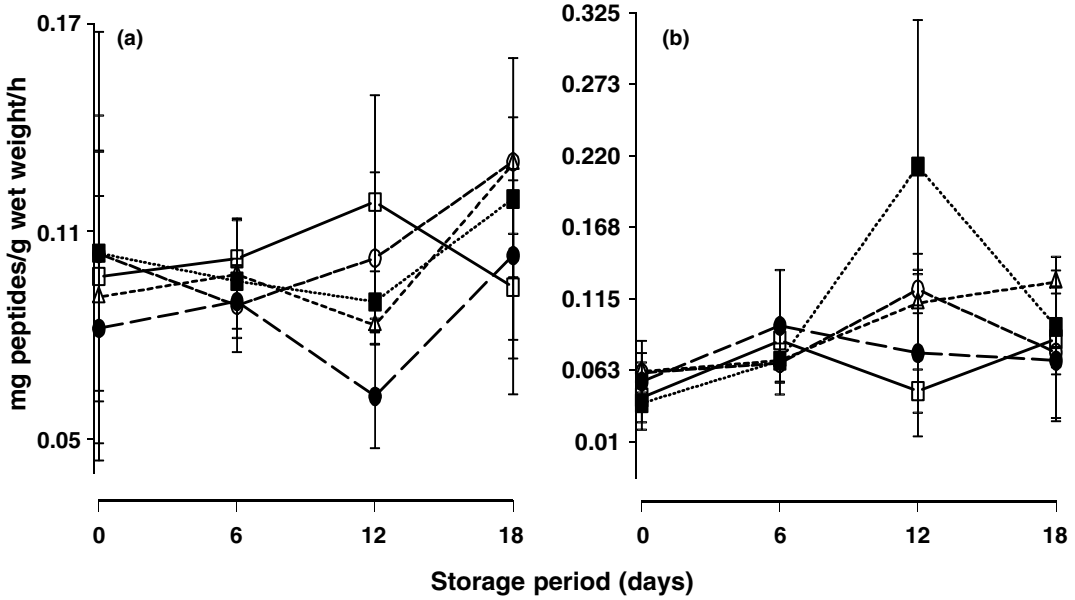

Fig. 2. General Proteolytic activity of control (□) and pressure-treated CSS: 100 MPa (○), 150 MPa (△), 200 MPa (▲) and 300 MPa (●) during refrigerated storage. GPA is expressed as TCA soluble peptides/g wet weight/h at different pH 6.0 (a) and pH 6.5 (b) levels.

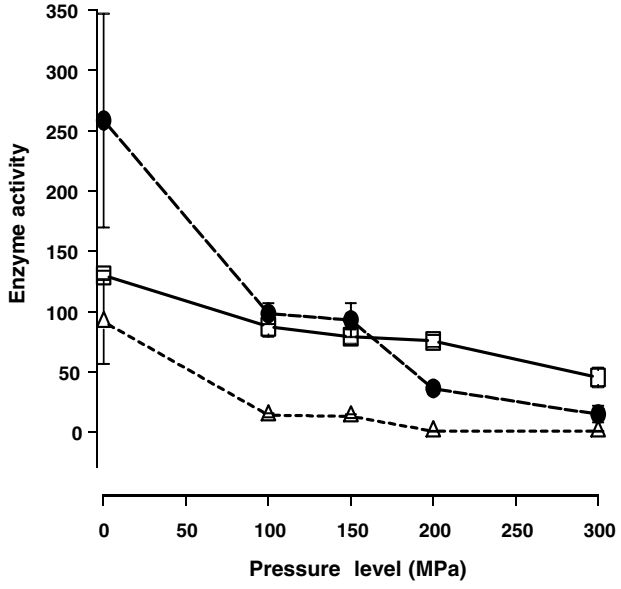

Fig. 3. Effects of high-pressure on proteolytic enzymes in crude enzyme extracts from cold-smoked salmon given as increase in fluorescence intensity/g wet weight/10 min and mentioned as enzyme activity in graph. Cathepsin B (□), Cathepsin B&L (●) and Calpain (△).

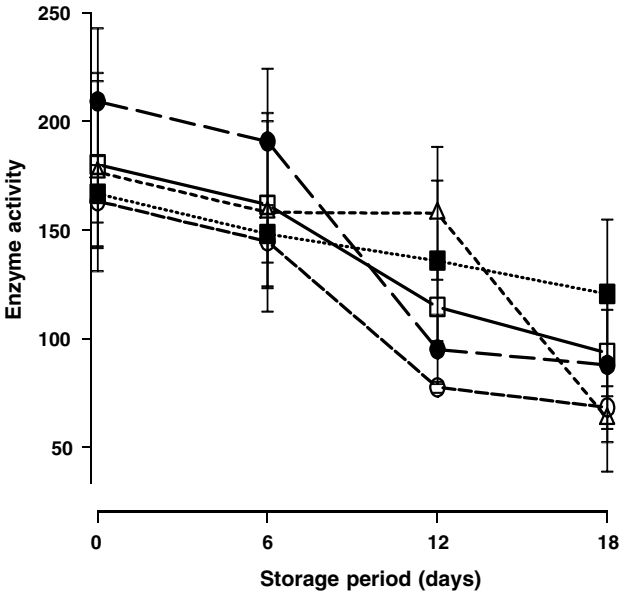

Fig. 4. Changes in levels of Cathepsin B levels in control (□) and pressure-treated CSS: 100 MPa (○), 150 MPa (△), 200 MPa (▲) and 300 MPa (●) during storage, given as increase in fluorescence intensity/g wet weight/10 min and mentioned as enzyme activity in graph.

A gradual decrease ($p = 0.000$) in the activity of cathepsin B-like enzymes was observed at all pressures until the last day of storage (Fig. 4). However, the remaining activity of the enzyme was still high enough to contribute to proteolysis and muscle softening during further storage. Hultmann et al. (2004) found the same results and concluded that smoking also causes increased activity of cathepsins and, therefore, no heat inactivation of enzymes. Moreover, a salt level relevant to the CSS investigated in this study was not enough to inactivate cathepsin B-like activity (Hultmann et al., 2004; Reddi et al., 1972).

In contrast, the overall activity of cathepsin B + L was substantially reduced by increasing pressure ($p < 0.01$), from 126 to 50 ± 49 as increase in fluorescence intensity/g wet weight/10 min for control and 300 MPa treated fish, respectively. Activity decreased slightly up to the 12th day of refrigerated storage, and thereafter there

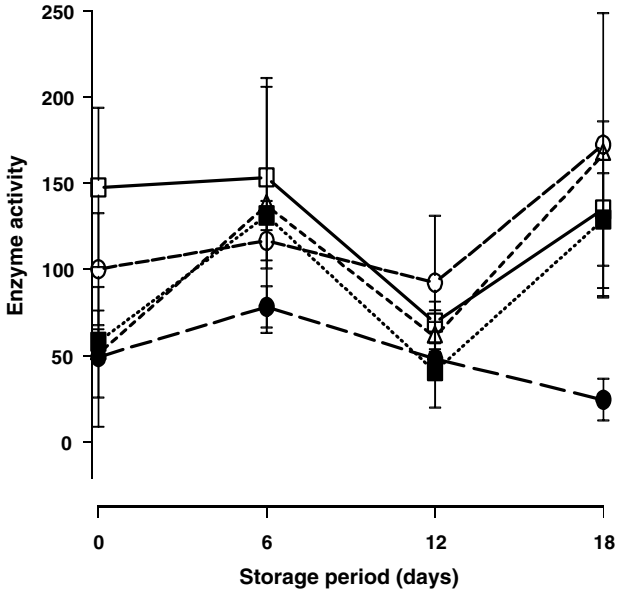

Fig. 5. Changes in levels of Cathepsin B&L levels in control (□) and pressure treated CSS, 100 MPa (○), 150 MPa (△), 200 MPa (▲) and 300 MPa (●) during storage, given as increase in fluorescence intensity/g wet weight/10 min and mentioned as enzyme activity in graph.

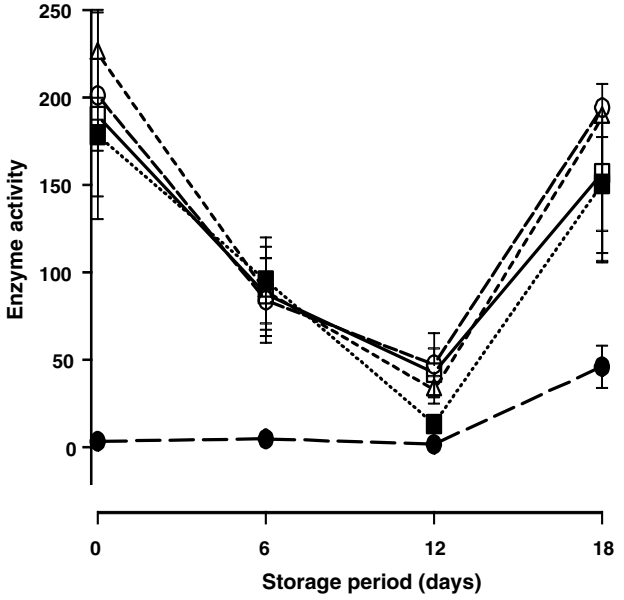

Fig. 6. Changes in levels of Calpain levels in control (□) and pressure-treated CSS: 100 MPa (○), 150 MPa (△), 200 MPa (▲) and 300 MPa (●) during storage, given as increase in fluorescence intensity/g wet weight/10 min and mentioned as enzyme activity in graph.

was a reactivation of the enzyme (Fig. 5) ($p < 0.01$). A study by Yamashita and Konagaya (1990a, 1990b) provided evidence of high activity of cathepsin L-like enzymes in chum salmon during spawning migration. This led to the conclusion that this enzyme was also participating in the softening of salmon muscles along with other cathepsins, such as cathepsin B-like enzymes.

High-pressure does not generally seem to have a substantial effect on either of these enzymes (Figs. 4 and 5). On the one hand, a gradual decline on storage of the activity of cathepsin B-like enzymes in pressurized samples was close to that of the controls. On the other hand, there was a reactivation of cathepsin B + L like enzymes in all samples except the one treated at 300 MPa on the 12th day of storage onwards. Two phenomena are shown on HP effects of enzymes: enzyme activation observed at comparatively low pressures (approx. 100 MPa) and enzyme inactivation at much higher pressures (>300 MPa). The studies of HP on enzyme activity from other seafoods or foods support the results obtained. Ashie and Simpson (1996) observed that proteolytic enzymes lose their original activity, to 80%, due to HP (300 MPa for 30 min), but a partial recovery in these enzymes was observed during subsequent refrigerated storage. Cathepsin L was not inactivated when surimi, prepared from Pacific Whiting (*Merluccius productus*), was pressurised up to 240 MPa (Chung, Gebrehiwot, Farkas, & Morrisey, 1994). Ohmori et al. (1991) have found that acid proteases (such as cathepsin L) were more resistant to high-pressure than other proteases studied in beef rounds, pressurized from 100 to 500 MPa for 10 min at 25 °C. Cathepsin B-like enzymes were found to be sensitive $\geqslant$ 200 MPa, but could keep only 50% of their activity at 500 MPa. Thus the authors have shown the resistance of cathepsin L-like enzymes to high pressure and this explains why pressurised samples behaved similarly to controls in the present study. The increase in the specific activity of enzymes in pressurized CSS was ascribed to their release from lysosomes (Homma, Ikechi, & Suzuki, 1994).

3.3.3. Activity of calpains

Activity of calpains decreased during the initial period of storage, until 12 days at 4–5 °C, and thereafter a return to the original activity was recorded in all samples (Fig. 6). At 300 MPa, the activity of calpain from salmon tissues was greatly reduced until the 12th day of storage ($p < 0.01$), but increased at the 18th day. Pressures <300 MPa had virtually no effect compared with controls. In contrast, the activity of crude enzyme extract was affected by all pressure levels used in this study with a marked effect $\geqslant$ 100 MPa (Fig. 3). The results from the present study are in reasonable agreement with those of Homma, Ikechi, and Suzuki (1995). The authors studied the effect of HP on calpains in meat and found reduced activity in enzyme extracts pressurized at $\geqslant$ 100 MPa. The activity of calpains in muscle was also found to decrease from 200 MPa, in contrast with the present study. Increased activity of calpains in pressurized muscles could be due to release of Ca^{2+} from sarcoplasmic reticulum (Suzuki, Okamoto, Ikeuchi, & Saito, 1993). From electron micrographs of the pytoanthimonate-fixed fibre bundles, which were prepared from pressurised muscles, the authors have shown that pressure induces the release of Ca^{2+}

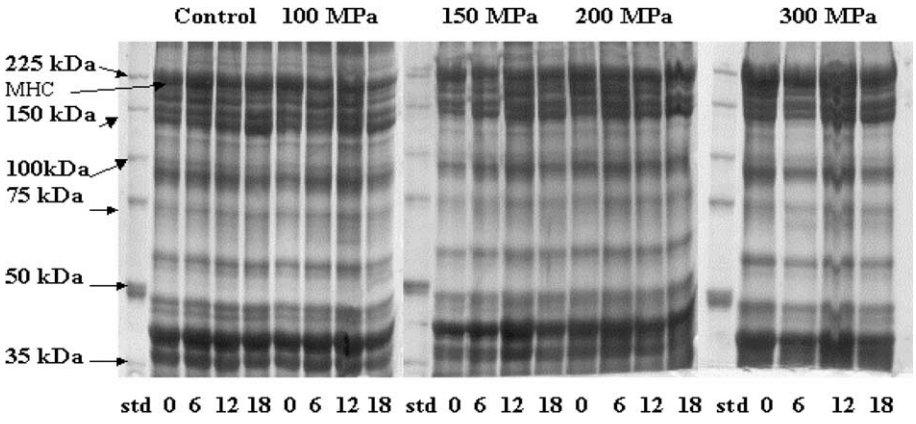

Fig. 7. SDS–PAGE of protein from control (0.1 MPa) and pressure-treated (100, 150, 200 and 300 MPa) cold-smoked salmon during refrigerated storage. kDa represents molecular weight of proteins; std, broad range protein standard, and subscripts, 0, 6, 12, 18 days of storage.

from sarcoplasmic reticulum. The increased levels of Ca^{2+} could have caused the increased activity of calpains, resulting in higher levels recorded in the present study. This could result in some beneficial effects, such as meat tenderisation.

3.4. SDS–PAGE

Changes in proteins in control and pressurized samples during refrigerated storage were investigated with SDS–PAGE (Fig. 7). There were only minor differences between storage days within each treatment. The myosin was not affected (as shown by the intensity of the bands) until the last day of refrigerated storage in control samples, indicating less proteolysis up to the 18-day storage period used in the present experiment. A similar but slight decrease in intensity of MHC was observed in the samples pressurized up to 200 MPa. This is concomitant with the reduced GPA noted in all samples (see previous section). At 300 MPa, disintegration of myosin bands, to a lesser extent, was seen at the start of storage, and further storage had no apparent effect on proteins. The relative intensity of protein bands between 150 and 225 kDa also decreased in 300 MPa-treated samples compared with control and other pressurized samples. Immediately after pressurization, no degradation of myosin was observed in chicken muscle pressurized to the range of 200–600 MPa for 5 min at 2 °C (Kamiyama et al., 2001). Similarly, no significant change was seen between control and pressure-treated (up to 800 MPa) cod with respect to protein changes (Angsupanich & Ledward, 1998) during further storage. The present results are consistent with these two studies. However, there was a slight decrease in the intensity of MHC in the pressurized samples (up to 200 MPa) on the last storage day, and this could be due to an increase in the specific activity of proteolytic enzymes in fish muscles (Homma et al., 1994). Cathepsins could have contributed to the decrease in MHC intensity, though there was an increase in the enzymic activity of calpain. This is because only a little proteolysis of myofibrillar protein was found in Chinook salmon (*Oncoryhnchus tshawytscha*) and reproducibility was obtained when calpain was incubated with myofibrils (Geesink, Morton, Kent, & Bickerstaffe, 2000). Using SDS–PAGE, Lund and Neilsen (2001) studied the changes in myofibrillar proteins from fresh and smoked salmon until 23 and 21 days, respectively, stored at 0 °C. They found similar results with few changes observed related to myosin, and a stable pH was one of the suggested reasons for the appearance of the fragments during storage without major changes to myosin. Though pH was affected by storage time ($p < 0.05$), the changes were minor up to 18 days of storage in the present study (Table 1). There were small falls in pH in all samples except those pressurized at 300 MPa, where there was an increase.

4. Conclusions

The present study has demonstrated high-pressure effects on the proteolytic enzymes from cold-smoked salmon in muscles and in solution. High-pressures at lower levels seem to induce proteolytic activity in fish muscles, and to decrease activity at higher pressure levels (>300 MPa). Activity was substantially reduced by HP treatment of enzyme extracts in solution. The increase in the relative activities of catheptic enzymes during further refrigerated storage was not due to an increase in specific activity, but due to an increase in their content in the extract. The increased catheptic activity was attributed to disruption of lysosomic membranes by pressure (Homma et al., 1994). Also, the temperature used to smoke the CSS used in this study was not known, but smoking itself increases the proteolytic activity in salmon muscle (Hultmann et al., 2004; Lund & Neilsen, 2001). Hence it is difficult to say that high-pressure alone has contributed to the increased activity, though HP has been shown to have this effect. Further studies are needed of the combined effects of all process variables on the enzymic activity in fresh and cold-smoked salmon. This would allow

development of a suitable intervention method for better quality control options.

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