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Changes in myofibrillar proteins during processing of salted cod (*Gadus morhua)* as determined by electrophoresis and differential scanning calorimetry

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Abstract

The effects of salt-curing, drying and rehydration on muscle proteins in cod (*Gadus morhua*) were studied during the processing of heavily salted cod or ‘‘bacalhau’’. The aim was to observe conformational stability and possible degradation or denaturation, with sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and differential scanning calorimetry (DSC). The salting process significantly decreased the heat stabilities of both myosin and actin. The decrease in water content during dry-salting did shift the transition temperatures slightly back to higher temperatures. The results, from the SDS-PAGE, showed that the myosin heavy chain (MHC) was cleaved into smaller sub fragments in the salting process with the two heavy meromyosin fractions (HMM S1 and S2) and the light meromyosin (LLM) fraction being the most abundant. Actin was less affected than myosin. © 2002 Elsevier Science Ltd. All rights reserved.

Keywords: Salted cod; Bacalhau; *Gadus morhua*; Protein denaturation; Differential scanning calorimetry (DSC); Electrophoresis (SDS-PAGE)

1. Introduction

Salting of fish is an old and traditional method to improve shelf life. Highly salted food products develop different organoleptic properties from the fresh product, which are often preferred by consumers. Salted cod, frequently referred to by the Spanish term for cod ‘‘bacalhau’’, has been produced in Iceland for centuries and has been one of the nations primary export products (Thorarinsdottir, Arason, Bogason, & Kristbergsson, 2001). The traditional markets for salted fish have been in Spain, Portugal and Latin America.

Salted cod is a heavily salted product, where the salt concentration reaches approximately 20%. The salt concentration affects the stability and denaturation of proteins and thereby such physicochemical factors as water-holding capacity (WHC). The addition of sodium chloride above the isoelectric point of proteins (pI), to meat systems, causes swelling and an increase of WHC (Honikel, 1989). The salt ions are believed to cause weakening of the interaction between oppositely charged side chains, which results in swelling. A measurable increase in WHC has been observed by the addition of more than 1% salt (0.17 M NaCl). Maximum swelling has been estimated at approximately 5% but, at higher concentrations, the myofibrilar proteins rapidly loose water through the salting-out process. Similar effects have been suggested for fish muscle (Akse, Gundersen, Lauritzen, Ofstad, & Solberg, 1993). At higher salt concentration in the muscle, or above 9–10%, the proteins may denature, resulting in stronger protein–protein bonds, shrinkage of the muscle and dehydration (Borgstrom, 1968; Hamm, 1985; Morrissey, Mulvihill & O’Neill, 1987; Offer & Trinick, 1983; Wilding, Hedges & Lillford, 1986).

Polyacrylamide gel electrophoresis (PAGE) is a technique in which proteins are placed in an acrylamide gel where the anode and cathode are located at opposite ends of the gel.

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The proteins may then be separated by charge and size. Electrophoresis is normally operated at high pH when the proteins take on a net negative charge. When the surface active agent, sodium dodecyl sulfonate (SDS), is added to the sample, it acts as a denaturant and a solubilizing agent, unfolding the peptide chains and converting them to a rod-like shape. The SDS binds to the proteins and cancels out the intrinsic charge of the proteins giving them the charge of SDS (Pomeranz & Meloan, 1987). The separation by electrophoresis in the SDS-PAGE system will then only be based on molecular weight, but not charge. The technique has proven to be an excellent tool for the determination of molecular weight and for analysing the number of subunits in the protein molecule. SDS-PAGE can be used, both as a qualitative, and quantitative, method in the identification of different proteins. SDS-PAGE has been used for identification of different muscle proteins and their subunits in fresh muscle and also to estimate the effects of storage and processing on the stability of proteins (Bechtel & Parrish, 1983; Garcia, Diez, & Zumalacarregui, 1997).

Differential scanning calorimeter (DSC) is a powerful technique for studying the thermodynamics of protein stability and it can provide basic understanding of protein denaturation. Proteins may be analysed in situ without solubilisation of the muscle tissue. DSC has been used to study the thermal properties of fish muscle proteins and to measure the extent of denaturation under various processing conditions. Hastings, Rodger, Park, Matthews, and Anderson (1985) studied the stability of muscle proteins, using DSC, and found the myosin to be unstable and easily and irreversibly denatured during both handling and processing. Actin, on the other hand, was more stable and its "denaturation" was largely reversible except when denaturation was conducted with heat. Fish muscle proteins are less stable than mammalian proteins against physical processes such as freezing and frozen storage.

Changes in protein structure during DSC analysis have often been referred to as "transition" changes, with the basic DSC profile of muscle proteins being made up from a pattern representing myosin, actin, sarcoplasmic proteins and collagen peaks. One or more transitions were displayed for myosin, which is a multi-domain protein, depending on external factors, such as pH and ionic strength (Hastings et al., 1985; Wright, Leach, & Wilding, 1977). The myosin molecular domains differ considerably in the structure of the globular "head" and the helical "tail" (rod). Enzymes, such as trypsin may be used to cleave the myosin in the middle region, producing two fractions of the protein: heavy meromyosin (HMM) and light meromyosin (LMM). The heavy meromyosin has been shown to be composed of two regions, sub-fragment 1 (S1) and sub-fragment 2 (S2; Wright et al., 1977). According to Lawrie (1998) the HMM fraction contains all the ATP-ase and actin-combining properties found in myosin and these properties depend upon the number of free SH-groups in the molecule.

DSC patterns of myosin depend on the macrostructure of the myosin molecule. Formation of filaments, similar to the thick filaments in muscle, lends extra stability to a particular region of the myosin molecule and results in higher transition temperatures (Wright et al., 1977) but purification may lead to decreased heat stability. By comparing the thermograms of myosin with those of isolated myosin sub-fragments, Wright and Wilding (1984) were able to demonstrate that the three major transitions associated with the thermal denaturation of rabbit myosin could be assigned to different regions of the myosin molecule: the helical tail, the hinge-region and the globular heads.

Addition of salt affects the stability of proteins and can therefore be used to help with the observation of how the protein transitions are displayed in the DSC analysis. The effects of salt treatment on herring muscle showed that the transition temperatures were decreased by 5–10 K and the peak areas were also decreased by the salt addition (Hastings et al., 1985). Dialysis resulted in only partial recovery of the peak areas and restoration of the original transition temperatures. This was further supported by the studies of Schubring (1999), who showed that, after heavy salting of herring muscle, the thermograms were reduced to a single major transition peak and both thermal stability and transition temperature decreased. Wu, Akahane, Lanier, and Hamann (1985) studied the effects of salt addition (0–3%) on croaker surimi, by DSC. Three endothermic peaks were observed with T_{max} at 43, 54 and 71 °C, respectively. Since most water-soluble proteins were removed by washing during processing of surimi, it is unlikely that any of the three peaks were due to denaturation of sarcoplasmic proteins.

Chen (1995) observed no differences in DSC thermograms between shark muscle and its respective surimi (without added salt) and concluded that the mechanical energy input did not affect heat stability of proteins. However, this may only apply to the transition temperatures, since Kiowski and Mast (1988) observed a decrease in enthalpy represented by the size of the peak. Adding NaCl resulted in lower T_{max} for both the actin and myosin peaks. It was postulated that the increase in ionic strength solubilised myofibrilar proteins and induced free myosin associated with actin to form actomyosin. The DSC thermograms of cod muscle vary with the type of salt used. By adding dry salts to a final calculated strength of 255 mM, Weinberg, Regenstein and Lillfjord, (1984) demonstrated that NaI decreased transition energies of both actin and myosin when compared with either a no salt control, NaCl- or Na_2SO_4- treated samples. In contrast, $CaCl_2$ and $MgCl_2$ increased both height and broadness of the peaks.

In summary, it can be concluded that various salts have different effects on muscle proteins, and the concentration of the salts is also important in the protein denaturation process. Other factors, such as decreased water content of the product, may affect the thermal stability. The effect of relatively high salt concentrations, as used in the processing of salted fish, has not been reported. The aim of this research was to study the conformational stability and degradation of muscle proteins in the processing of high salt content fish, by DSC and SDS-PAGE.

2. Materials and methods

2.1. Materials

Cod (*Gadus morhua*) was caught by line to the south west of Iceland by commercial fishing boat. The cod was placed in bins with ample ice, immediately after gutting. Industrial grade salt, imported from Almeria, Spain was used in the experiment. All materials used for chemical analysis were of analytical grade.

2.2. Salting process

The salt processing was performed according to traditional methods used for the production of salted fish. After rigor mortis the cod was filleted by hand using standard industrial procedures. The fillets were submerged in brine with initial concentration of 16°Baume [analysed as 17.5% (w/w) NaCl in water] for 42 h at 2 ± 1 °C; the fish to water ratio was 1:1.6. After removal from the brine, the fillets were placed in plastic tubs and piled with alternating thin layers of salt into stacks, and kept for 14 days for dry-salting at 7 ± 1 °C. After dry-salting the fillets were packed into waxed cardboard boxes and kept at 3 ± 1 °C for approximately 3 weeks, after which time the salted fish was desalted and rehydrated at 3 ± 1 °C. The fillets were cut into three pieces, which were rehydrated by submerging the fish in a 1:5, fish to water ratio, for 30 h. Then the water was replaced with fresh water and the fish allowed to rehydrate for an additional 80 h submerged in a 1:4 ratio water bath.

2.3. Sampling

Samples were taken from the fillets, fresh and after each processing step. For chemical analysis, two pieces (approximately 6 cm each) were cut from the fillets, from the tail part and near to the head. The samples were then frozen immediately and stored at −24 °C, until analysed. Prior to chemical analysis, the fish was skinned by hand and minced in a Braun mixer (type 4262; Braun, Kronberg, Germany). Samples for DSC and SDS-PAGE were taken dorsally from the fillets next to the head and analysed immediately after sampling.

2.4. Determination of pH, water, salt and protein content

Moisture content (g/100 g) was calculated as the loss in weight, during drying at 105 °C for 4 h (ISO, 1983). Salt content was determined by the method of Volhard (JAOAC, 1937, 1940). The total protein contents of the fish muscle and brine were estimated by the Kjeldahl method (ISO, 1979) with the aid of a Digestion System 40, 1026 digester (Tecator AB, Hoganas, Sweden) and calculated using total nitrogen (N) × 6.25. The pH of the muscle was measured by inserting a combined glass electrode (Red rod C2401-7, Radiometer, Copenhagen, Denmark) directly into the cod mince. This method was a modification of the procedure by Kramer and Peters (1981), who measured pH in fillets by inserting the electrode directly into the approximate middle of the fillets.

2.5. SDS-PAGE

Electrophoresis of the proteins in the supernate was carried out on a Pharmacia LKB PhastSystem (Pharmacia LKB, Uppsala, Sweden). Using a PhastGel 4–15 polyacrylamide gradient gel and PhastGel SDS buffer strips, made from 3% Agarose IEF which contained 0.20 M tricine, 0.2 M Tris and 0.55% sodium dodecyl sulfate (SDS), pH 8.1. The polyacrylamide gradient gel had a 13 mm stacking gel zone (4.5% T, 3% C) and a 32 mm continuous 5–15% gradient cross linker. The thickness of the gel was approximately 0.45 mm. The sample buffer used for electrophoresis was composed of 0.02 M Tris, 2 mM EDTA, 5% SDS and 1% DDT (dithiothreitol), pH 8.0.

A 10-g sample of minced/whole muscle was dispersed by homogenisation in an Ultra Turrax mixer (T25, IKA-Labortechnik, Staufen, Germany) for 60 s in 290 ml of cold deionised and distilled water. The dispersed samples were then immediately mixed with the sample buffer in a 1:1 proportion. The samples were boiled in water for 2 min, cooled on ice and centrifuged at 2–5 °C for 15 min at 10,000 rpm. The samples were separated on the Pharmacia PhastSystem (Ingolfsdottir, 1996). This dilution of the sample gave a suitable protein concentration for the electrophoresis and staining condition used.

Gels were removed from the electrophoresis unit immediately after the run and placed in the development unit for staining. The staining solution was composed of 0.1% PhastGel Blue R in 30% methanol and 10% acetic acid in distilled water. The destaining solution was composed of 30% methanol and 10% acetic acid in distilled water. The preservation solution was

composed of 5% glycerol and 10% acetic acid in distilled water (Pharmacia LKB Biotechnology). After staining, the gels were allowed to dry in a Petri dish for 5–10 h. The gels were scanned, analysed and compared to standards with the aid of two software packages: Multi-Analyst version 1.1 (Bio-Rad Laboratories, Inc., Herts, UK) and GelCompar II, 1.01, (Applied Maths BVBA, Kortrijk, Belgium).

2.6. Differential scanning calorimetry (DSC)

Differential scanning calorimetry was performed on a Perkin-Elmer DSC-7 (Perkin-Elmer, Norwalk, USA). The instrument was temperature-calibrated, using water and indium. Enthalpy was calibrated with indium. Empty pans were used as reference. Slices, free of connective tissue, were dissected from whole muscle. Samples, weighing 20–25 mg (accuracy of ±0.01 mg) were sealed in Perkin-Elmer volatile sample pans and scanned at a heating rate of 10 °C/min over the range 15–110 °C.

3. Results and discussion

3.1. Salt, moisture and protein content

The salt content of the cod muscle reached 7.24% after brine salting, and the water content decreased from 81.84 to 75.86% (Table 1). The muscle was further dehydrated to 56.63% after dry salting and storage, when salt content increased to 20.3%. The slight loss in total proteins from fresh material through rehydration (Table 1) was believed to be due to the loss of water and salt-soluble proteins, since the conformational stability and denaturation of the myofibrilar proteins, were greatly affected by the salt concentration in the muscle. This was in agreement with what has been reported for the effect of salt on muscle tissue when salting-in and swelling of the muscle is believed to occur at lower salt concentration ($\leqslant$ 5%). But salting-out and denaturation of proteins have been reported to occur at higher concentrations, above 9–10% (Akse et al., 1993; Duerr & Dyer, 1952).

Table 1
Chemical content of the muscle at various stages of the process (mean±S.D. of three fillets)

State of process	Moisture (%)	Protein (%)	Salt (%)	pH
Fresh cod	81.84±0.40	17.5±0.44	0.36±0.08	6.91±0.01
After brine salting	75.86±3.29	16.9±0.60	7.24±0.23	6.74±3.88
After dry salting	57.64±0.56	21.9±0.51	20.5±0.03	6.18±0.21
After storage	56.63±0.36	22.8±0.40	20.3±0.02	6.13±0.10
After rehydration	84.06±0.71	15.0±0.73	0.84±0.06	6.66±0.03

3.2. Electrophoresis

The protein band for myosin heavy chain (MHC) appeared at 190 kDa and the actin band at 45.6 kDa. The bands for the myosin light chains appeared in the range of 18–25 kDa in the fresh cod muscle (Fig. 1). This was confirmed by comparison with the results of Stefansson (1994) and Ingolfsdottir (1996). The intensity of the MHC band decreased more during the salting and dry storage than the actin band. Parallel to the decrease in the intensity of the MHC band, the intensities of two protein bands with molecular weights of 130–140 kDa and approximately 80 kDa increased. These protein bands were likely parts of the myosin molecule, based on denaturation properties of the protein. The 80 kDa band may represent the LMM part of the myosin since Wright and Wilding (1984) reported similar values for LMM of 70, 74, and 78 kDa, respectively. The band at 130–140 kDa was believed to represent the HMM and likely the HMM-S2 sub-fragment of myosin. After rehydration of the dried fish, the MHC (190 kDa) molecule all but disappeared but the LMM increased slightly (77 kDa) (Fig. 2) and the HMM band remains.

The results are in agreement with what has been reported earlier on the effect of salt concentration, above 9–10% on muscle proteins (Duerr & Dyer, 1952). The proteins are believed to denaturte by a complicated process, due to the extreme changes in water and salt content of the muscle. Research, based on the effects of storage (Bechtel & Parrish, 1983) and salt-treatment of muscle foods, has shown that myosin (or the MHC), is more sensitive to both handling and processing than actin. Garcia, Diez, and Zumalacarregui, (1997) studied the changes in proteins during the ripening of Spanish dried beef "cecina", which is an intermediate moisture food product. The muscle was salted, washed, smoked and dried, followed by a ripening period. During the processing, the extractability of sarcoplasmic and myofibrilar proteins decreased by approximately 80%. This was thought to be due to denaturation of proteins and/or proteolysis. Changes observed in the myofibrillar proteins included disappearance of MHC, troponin C and myosin light chain from the smoking phase and throughout the remainder of the drying and storage process. Three components, with molecular weights of 75, 70 and 65 kDa, were observed during ripening.

Increasing the concentration of salt above some limit is believed to result in the depolarisation of myofibrils, increased hydrophobicity and aggregation of the proteins (Morrissey, Mulvihill, & O'Neill, 1987). Increased hydrophobicity is related to increased exposure of hydrophobic groups in the proteins, which in turn facilitates protein denaturation. Denaturation was further shown to lead to crosslinking of SH-groups, which stabilizes proteins at higher salt concentrations (Akse et

al., 1993). According to Buttkus (1970), SH-groups participated in denaturation and aggregation of myosin by sulfhydryl-disulfide (SH-SS) exchange reactions. He found the extent of denaturation to be species-dependent, by showing that trout myosin, in the presence of traces of heavy metals, was more susceptible to oxidation than rabbit myosin. Heavy metal contamination affected enzyme activity and the tendency of myosin to aggregate. ATP and inorganic phosphate are believed to decrease the rate of aggregation but KCl to increase it with increasing concentration. Decrease in the total content of SH groups in pork muscle has also been observed during curing and ripening of pork muscle (Dazzi, Chizzolini, & Modensi, 1982). Tambo, Yamada, and Kitada (1992), studied the changes in myofibrilar protein of fish muscle caused by soaking in NaCl solution, in the range of 1–3 M (5.85–17.6% w/w). By increasing the concentration of NaCl, the rate of inactivation of myofibrilar Ca-ATPase in the meat was increased. When the NaCl concentration was low, cross-linking reaction of MHC in meat proceeded rapidly at the early stage of the soaking, but its rate was suppressed at the later stages. Regardless of the concentration, the cross-linking progress was dependent on the integrated value of salt concentration in the meat and the soaking time. Ito, Kitada, Yamada, Seki, and Arai (1990) reported similar results in their study on the cross-linking reaction of MHC in cured Walleye Pollack meat. Concentration and soaking period affected the degree of cross-linking by dehydration at 30 °C. In minced meat, containing 2.0–2.1 M NaCl (11.5–12%), no further changes were observed during the dehydration period, since the MHC had already been cross-linked during soaking in NaCl.

3.3. Differential scanning calorimetry (DSC)

In the fresh cod muscle, T_{max} for myosin was determined as 43.5 °C, for actin as 73.6 °C and for sarcoplasmic proteins as 59.3 °C respectively. The T_{max} for sarcoplasmic protein was high, though not significantly different from reported values (Table 2). Myosin transitions were determined as the peaks labelled 1, 2 and 4 with overlapping transitions (Fig. 3) and the actin transition peak was labelled as No 7. Peaks labelled as 3, 5 and 6 were determined to be from the transitions of the sarcoplasmic proteins. The different domains in myosin gave rise to more than one transition peak. The results for the cod muscle, prior to processing, were in general agreement with earlier findings for fresh cod (Hastings et al., 1985; Poulter, Ledward, Godber, Hall, & Rowlands, 1985) and for other species as well (Wright et al., 1977).

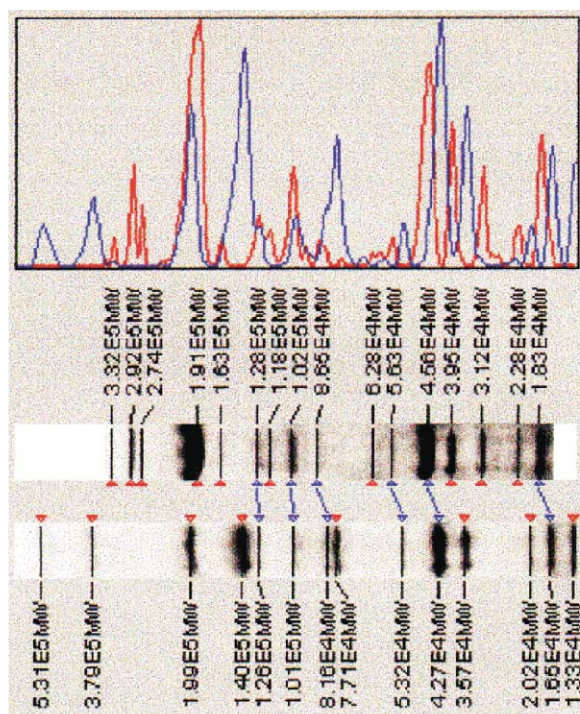

Fig. 1. SDS-PAGE analysis. Upper lane: fresh cod; myosin at 1.91 E5 MW (191 kDa), actin at 4.56 E4 MW (45.6 kDa) and MLC at 2.28 and 1.83 E4 MW (22.8 and 18.3 kDa, respectively). Lower lane: 16°Baume after storage; myosin at 1.99 E5 MW (199 kDa), HMM-S2 at 1.40 E5 MW (140 kDa), LMM at 7.71 E4 MW (77.1 kDa), actin at 4.27 E4 MW (42.7 kDa).

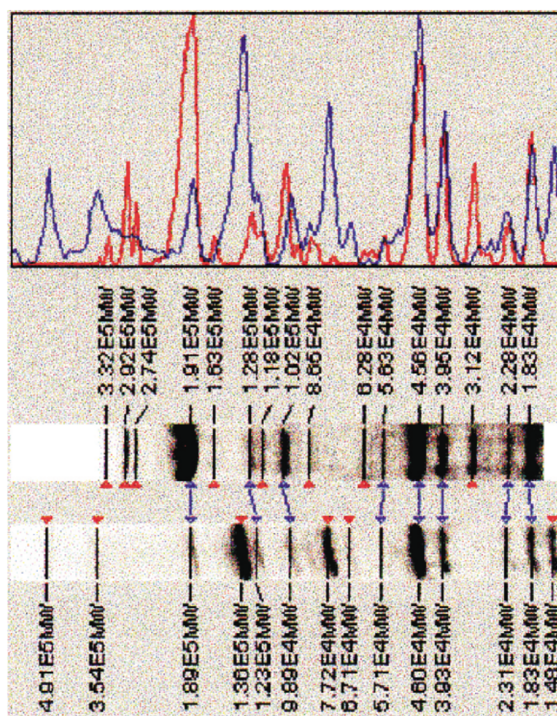

Fig. 2. SDS-PAGE analysis. Upper lane: fresh cod; myosin at 1.91 E5 MW (191 kDa) and actin at 4.56 E4 MW (45.6 kDa). Lower lane: 16°Baume after rehydration; myosin at 1.89 E5 MW (189 kDa), HMM-S2 at 1.36 E5 MW (136 kDa), LMM at 7.72 E4 MW (77.2 kDa), actin at 4.60 E4 MW (46.0 kDa).

3.4. DSC analysis of the salting process

The salt-curing led to some shifts in transition temperatures and decrease in peak area (Fig. 4). The transition peaks became lower and broader and less separable, compared to the thermogram of the fresh cod. After brine-salting, significant changes in the transition peaks were observed (Fig. 4b). The peaks shifted to lower temperatures (T_{max}) and it was not possible to distinguish between the respective transitions of sarcoplasmic proteins and myosin. This was likely due to an expected decrease in sarcoplasmic proteins and partial break-down of the myosin during the brining step, indicating disassociation of the MMC, which was supported by the SDS-PAGE analysis. The peak observed at 67 °C was believed to be from the transition of actin, which has been shown to lower its transition temperature and to be more resistant to denaturation than myosin (Hastings et al., 1985). However, in the thermogram for salted herring, with 14% salt and 7% acid, Hastings et al., (1985), did not observe a transition peak for actin, which may have been due to the effect of the acid or species, since the present study indicated some retention of actin. As previously discussed, the salt content had increased to 7.2% and the water content decreased to 75.9% during the brine-salting step. The increased salt content of the muscle after the brining step resulted in decreased stability of the proteins, as measured by DSC.

After the dry-salting step, the two main peaks observed were at approximately 50 and 70 °C (Fig. 4c) and a small transition was at 60 °C. The transition peak at 70 °C was from the actin molecule and the other two peaks were likely from the HMM myosin transitions. Similar transitions have been reported for croaker surimi, when the sarcoplasmic proteins had been washed out (Wu et al., 1985). Although the leaching of sarcoplasmic proteins is far greater in surimi processing than in the heavy salting of cod, the transitions were believed to be from myosin. During dry-salting, the average water content of the fillets decreased from 82.8 to 57.6% and the salt content increased to 20.5%.

After storing of the dry-salted cod, a slight decrease in moisture content was observed. The peaks from myosin and actin were very low and broad, overlapping each other, and were displayed as one major transition peak (Fig. 4d). The actin peak could, however, be observed at approximately 72 °C and transitions at approximately 60 °C were believed to display denaturation of myosin. The results were in agreement with the DSC analysis of muscle proteins in herring after 1 month of heavy salting (Schubring, 1999). The drying of cod muscle has been shown to increase transition temperatures (Hastings et al., 1985) and changes were thought to be due to

Table 2
Peak maximum temperatures (T_{max}) for the main muscle proteins, analyzed with DSC

Analysed in:	Myosin (°C)	Sarcoplasmic proteins (°C)	Actin (°C)	References
Cod muscle	43.5±0.2	59.3±0.9	73.6±0.7	
Cod muscle	44±2	55±5	74±2	Geirsdottir (1998)[a]
Cod muscle	~44	~58	~73	Hastings et al. (1985)
Rabbit muscle	~60	~67	~80	Wright et al. (1977)
Cod muscle	~45	~54	~74	Poulter et al. (1985)
Rabbit muscle	~60	~67	~80	Wright et al. (1977)

[a] Literature review—average of reference values found in other reports.

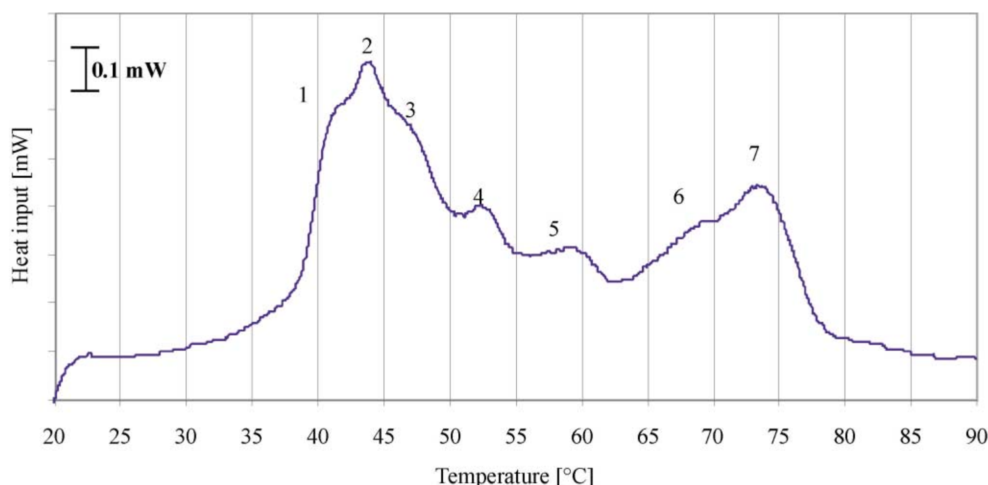

Fig. 3. DSC thermogram of muscle proteins in whole muscle of fresh cod (used as control).

inter- and intramolecular electrostatic interactions and hydrogen bonds, which became established in sites that were previously occupied by water. Difficulties in determining changes in the myosin peaks due to processing have been discussed by Wright and Wilding (1984). They stated that the various sub-fragments of myosin showed different behaviour due to changes in the environment and that myosin may have one or more transitions. Ionic strength and pH each played an important role in the conformational stability of the fragments. At low ionic strength the myosin head section (HMM) had the lowest transition temperatures but at higher ionic strength, one of the transitions for the myosin rod (LMM) displayed the lowest transition temperature. In the present study changes in T_{max} were observed and there may have been some rotation of the peak sequence with respect to $T_{max.}$

Earlier studies used far lower salt concentrations than are needed for the traditional salted-cod product (bacalhau) and are therefore not fully comparable. The DSC thermogram of croaker surimi demonstrated that the addition of 3% salt, shifted peak temperatures to lower transition temperatures, i.e. from 43, 54 and 71 to 37, 51 and 66 °C, respectively (Wu et al., 1985). The croaker surimi product is reported to be depleted of sarcoplasmic proteins due to the processing, involving mincing and washing with water. The mincing of fish meat with salt, has shown to lead to solubilisation and dissociation of myosin into individual molecules and a partial unfolding of the alfa-helix region of the myosin heavy chain (Chen, 1995). During heating in the DSC analysis, changes in conformation of myosin molecules in shark surimi, including solubilisation and denaturation of the myosin tail, where thought to occur at lower temperatures (30–43 °C). At higher temperatures (47–57 °C) the transition of head portions of the myosin molecule could be observed. Barbieri and Ghillani (1997) studied the curing of pork *longissimus dorsi* muscle by injection of brine (25%) and cooking. The denaturation of myofibrilar proteins started with a denaturation of myosin heavy chain (MHC) that resulted in soluble agglomerates or separation of parts of the MHC, depending on the rate of cooking. These results indicated that the MHC was the most heat sensitive of the salt soluble proteins, which is in agreement with the results presented here.

3.5. DSC thermograms after rehydration

Some restoration of the transition peaks could be seen after rehydration of the fish. However, all peaks were lower and broader (Fig. 5), which led to the conclusion that the conformational stability of the proteins had decreased compared to fresh cod and some changes in protein structure occurred, particularly in the myosin molecule. The large MHC peaks had all but disappeared. The transitions peaks at 34, 44, 49.4 and 57 °C (labelled 1b, 2b, 3b and 4b), were believed to show the transition of the different domains of myosin and the peak at 69.1 °C (5b) to be due to the transition of actin. Peak (1b) was believed to be from transitions from the helical tail of myosin (LMM), based on results from Wright and Wilding (1984). Peak (2b) may have been from the myosin heavy chain MHC, as was peak 2 in the fresh cod, which was, however, shown to break down in the SDS-PAGE analysis and was therefore more likely to be from the S1 fraction of HMM. A significant decrease in the peak size was observed, indicating breakdown or denaturation of the molecule. The maximum transition temperature (T_{max}) of myosin was determined to be 43.5 °C for the fresh cod, but 49.7 °C (± 1.7) for the rehydrated muscle as peak (3b). This could have been the same transition as peak No. 4 for myosin in the fresh muscle tissue, but was more likely from the HMM-S2 fraction of myosin, corresponding to the 140 kDa band from the SDS-PAGE analysis.

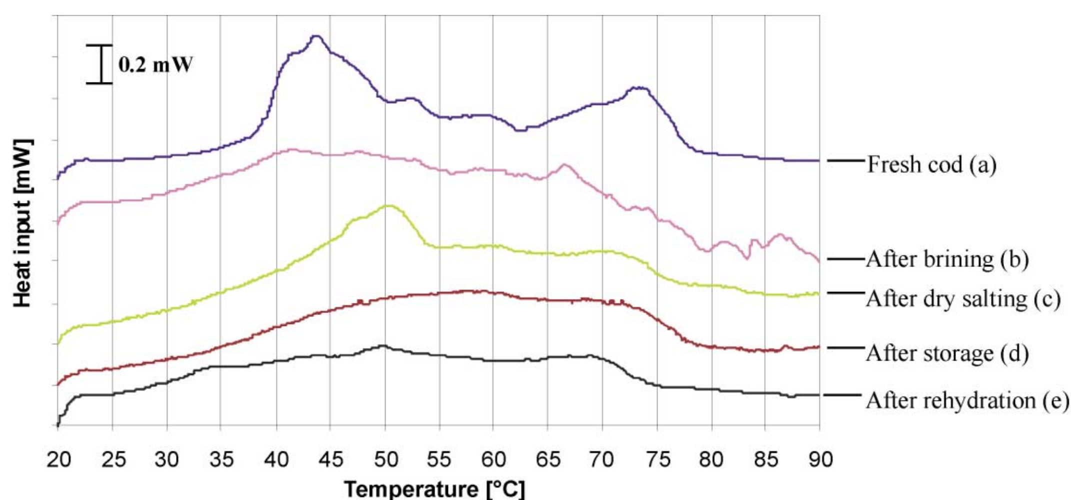

Fig. 4. DSC thermograms of fresh and the salted cod fillets after each processing step.

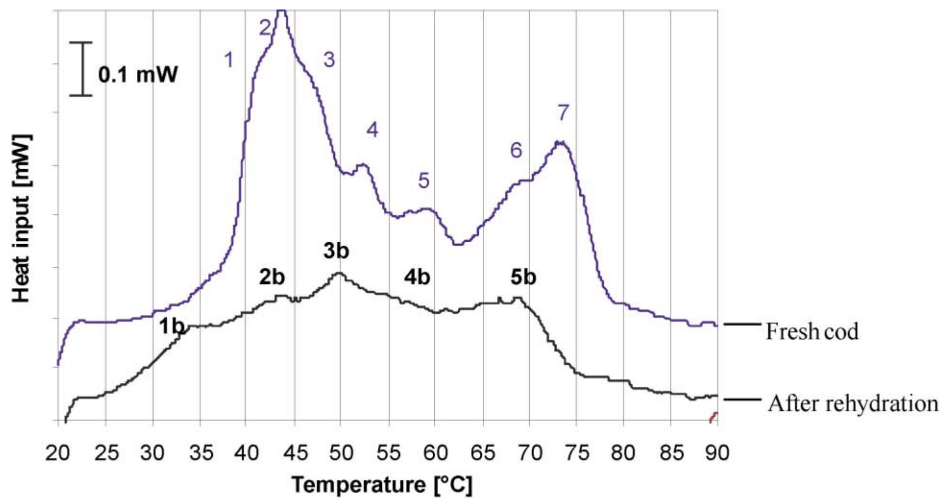

Fig. 5. DSC thermograms of fresh (the same thermogram as in Fig. 3) and salted cod fillets after rehydration.

The determination of T_{max} proved to be more straightforward and more reliable for actin than for myosin, since only one peak (5b) was displayed for actin at 69.1 °C (± 0.2) in the rehydrated cod which was near the 73.6 °C found for the fresh cod. Considering the results from the SDS-PAGE analysis, actin was affected to a lesser extent by the process than myosin.

Hastings et al., (1985) observed that the salt treatment of cod muscle shifted the transition temperatures to lower values and they only found a partial recovery of peaks and restoration of original transition temperatures after dialysis treatment of the samples. They stated that salt addition resulted in decreased thermal stability of muscle proteins, which indicated that the protein structure had been destabilized. However, the salt treatment was not as extreme as in our study in which the salt concentration changed from about 0.3 in the fresh material to about 20% and the water content decreased from 82 to 57%.

4. Conclusions

The salting process significantly decreased the heat stability of both myosin and actin. The protein denatured at lower temperatures and with less energy input. However, the decrease in water content, during dry salting and storage, seemed to shift the transition temperatures slightly back to higher temperatures. The enthalpy, represented by peak areas, was significantly smaller after the salting and rehydration steps. The conformational stability of myosin and actin was less than in the fresh material. The myosin heavy chain (MHC) was the most vulnerable to denaturation by heavy salting, and the heavy chain fractions (HMM S1 and S2), along with the light meromyosin (LMM), were the most abundant after salting and rehydration along with actin. The actin molecule appeared to be more resistant according to the SDS-PAGE analysis, although the molecule clearly underwent some conformational changes.

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