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The effect of brine composition and pH on the yield and nature of water-soluble proteins extractable from brined muscle of cod (*Gadus morhua*)

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Abstract

Fillets of Atlantic cod (*Gadus morhua*) were held for 36 h in brines of pH 6.5 and 8.5, respectively, containing various combinations of NaCl, KCl, $MgCl_2$ and $CaCl_2$. Proximate analyses and chloride content were determined. Soluble muscle protein extracted in distilled water and protein released into the brine following salting was determined by SDS–PAGE. The soluble fraction was not affected by the presence of divalent salts or KCl in the brines, but it was affected by the initial pH. Both actin and myosin heavy chain (MHC) were released in pH 6.5 brines after immersion of the fish. On the other hand, an initial pH of 8.5 did not favour the release of these proteins. There was a smaller variety of remaining soluble proteins in salted muscle with an initial pH of 6.5, than with 8.5. However, this effect of the lower pH was offset to the extent that the ionic strength of the brine was higher.

Keywords: Brined cod; Divalent salts; Protein solubility; pH; Brines

1. Introduction

Salting is one of the oldest techniques known to man for the conservation of fish. Salting is essentially intended to prolong the shelf life of the product by reducing its water activity. This is traditionally done by placing fish fillets or boned fish in book-fashion in layers with salt in between. The process can be a long one (1–8 weeks), in which the position of the fish is altered every so often to ensure uniform salting (Madrid, Madrid, & Madrid, 1994). The resulting product – known as "green fish" – is normally processed and dried before distribution.

Today, however, salting procedures are changing significantly, with new controlled processes designed not only to preserve the fish but also to improve its aroma and flavour (Sikorski, 1990). One such process is wet salting, in which the fish is immersed in solutions of salt water (brines) for a given length of time, sufficient to achieve an osmotic balance between the salt content in the muscle and the salt content in the brine. Wet salting offers a major advantage as compared to dry salting in that not only does it prevent rancidity by precluding contact with the air (Wheaton & Lawson, 1985), but it also increases the yield thanks to the intake of water. In this connection, both the salt content in the brine and the temperature are important. Brines with small concentrations of salt are known to promote better yield and water holding capacity than saturated brines (25% of more) (Barat, Rodríguez-Barona, Andrés, & Fito, 2002; Hamm, 1960). Some fish

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species, among them cod, not only take up salt, but also lose water when salt concentrations in the brine exceed 13–15% (Deng, 1977; Fougére, 1952; Madrid et al., 1994), especially in the early stages (Hamm, 1960). In the case of swordfish the threshold concentration can be as low as 6% (Del Valle & Nickerson, 1968). Water loss due to high salt concentrations and/or temperature is closely related to the denaturation of muscle proteins (Del Valle & Nickerson, 1967; Hamm, 1960).

The salting industry also seeks to improve the quality of final products by selecting the type of salt used (Madrid et al., 1994). The industry has traditionally used sea salt for this purpose. Sea salt contains sodium chloride, calcium sulphate, magnesium sulphate and magnesium chloride, and it may also contain calcium chloride and sodium sulphate. Added salts can alter the outcome of the salting process in desirable or undesirable ways. Thus, sodium chloride makes fish softer and produces slight yellowing; on the other hand, calcium salts promote firmness of salted fish up to a concentration of 0.3%, while higher concentrations render the fish excessively hard and compact (Beatty & Fougére, 1957; Lauritzen & Akse, 1995; Van Klaveren & Legendre, 1965). Calcium and magnesium salts help to inhibit enzymatic processes that contribute to spoilage while making the flesh of salted fish whiter and hence more attractive (Wheaton & Lawson, 1985). Nonetheless, very large concentrations of both salts can produce hardening of cell walls, prevent sodium chloride penetration and cause putrefaction of the fish (Wheaton & Lawson, 1985). Also, magnesium salt concentrations of more than 0.15% can lend a bitter taste (Arganosa & Marriot, 1990; Gillette, 1985). On the other hand, potassium chloride in concentrations of more than 50% can detract from flavour intensity and produce bitter tastes (Hand, Terrell, & Smith, 1982a, 1982b, 1982c). Potassium chloride may be recommended as a substitute for part of the sodium chloride in the salting of various products (Pilkington & Allen, 1994), since the amount of sodium in the diet generally far exceeds the minimum intake requirements of the population and can contribute to high blood pressure and other problems (Topp, 1981).

The use of different types of salt for fish salting is therefore of particular interest for technological purposes. Nevertheless, there have been practically no studies on their effect on chloride penetration and their influence on the functional properties of fish muscle in brine, especially as regards protein solubility, in real systems. The objective of this work was to determine the effect of brines of varying ionic strength, at two different pH values (6.5 and 8.5), on chloride penetration of cod muscle (*Gadus morhua*) and their influence on the solubility of the principal myofibrillar proteins.

2. Materials and methods

2.1. Samples

Following capture at the coast of Iceland by a commercial fishing boat, cod (*G. morhua)* specimens were headed, gutted, washed and placed in bins, covering with ice. The bins were immediately transported to the Icelandic Fish Processing School. After onset of rigor mortis, the cods were cut lengthwise into two parts, each weighing between 500 and 900 g, and salted by immersion in brine for 36 h: fish/brine ratio 1/1.4, temperature 4 °C. The salt concentration in all brines was the same (18% w/v). Brines consisted of distilled water and a mixture of salts containing variable quantities of sodium chloride, potassium chloride, calcium chloride and magnesium chloride (Table 1). The initial pH of each brine was adjusted to the level shown in Table 1 by addition of 0.1 N citric acid or sodium hydroxide. Sodium chloride (NaCl) was supplied by Supreme Salt Co., Ltd.; potassium chloride (KCl) was supplied by Saltkaup Ltd.; magnesium chloride ($MgCl_2$) hexahydrate and calcium chloride ($CaCl_2$) dihydrate were supplied by Merck.

On completion of salting, the fillets were frozen and sent by plane to Madrid, Spain. The transport to the laboratory was under refrigeration conditions. Then, in frozen state, each fillet was divided into two halves (back and tail). The backs were cut into small pieces (3 cm × 3 cm) for homogenization prior to analyses.

2.2. Determination of moisture

Moisture was determined on approximately 5 g of minced muscle, by oven drying at 110 °C to constant weight, following technique 950.46 (A.O.A.C., 2000). Results were means of three determinations and were expressed as grams of water/100 g of muscle.

2.3. Determination of ashes

A sample of minced muscle (approx. 5 g) was taken and heated at 500 °C in a Lenton muffle furnace (Leicestershire, England), following AOAC 923.03 (A.O.A.C., 2000). Results were means of five determinations and were expressed as g of ash/100 g of muscle.

2.4. Determination of chloride content

Five g of minced muscle was homogenized with 100 ml of 1% nitric acid in an Omnimixer-Homogenizer (model 17106, OMNI International, Waterbury, USA). Sample was filtered through No. 1 Whatman paper. The chloride content was measured at ambient temperature with constant stirring in an ORION 920A ion analyser (Barcelona, Spain), with an ORION 900200

Table 1
Composition and pH of brines used for cod brining

Brines composition	NaCl (%)	$CaCl_2$ (%)	$MgCl_2$ (%)	KCl (%)	Brines pH
NaCl	100.0	0.0	0.0	0.0	6.5
NaCl	100.0	0.0	0.0	0.0	8.5
NaCl + KCl	50.0	0.0	0.0	50.0	6.5
NaCl + KCl	50.0	0.0	0.0	50.0	8.5
NaCl + $CaCl_2$	99.2	0.8	0.0	0.0	6.5
NaCl + $CaCl_2$	99.2	0.8	0.0	0.0	8.5
NaCl + KCl + $CaCl_2$	50.0	0.8	0.0	49.2	6.5
NaCl + KCl + $CaCl_2$	50.0	0.8	0.0	49.2	8.5
NaCl + $MgCl_2$	99.6	0.0	0.4	0.0	6.5
NaCl + $MgCl_2$	99.6	0.0	0.4	0.0	8.5
NaCl + KCl + $MgCl_2$	50.0	0.0	0.4	49.6	6.5
NaCl + KCl + $MgCl_2$	50.0	0.0	0.4	49.6	8.5
NaCl + $CaCl_2$ + $MgCl_2$	98.8	0.8	0.4	0.0	6.5
NaCl + $CaCl_2$ + $MgCl_2$	98.8	0.8	0.4	0.0	8.5
NaCl + KCl + $CaCl_2$ + $MgCl_2$	50.0	0.8	0.4	48.8	6.5
NaCl + KCl + $CaCl_2$ + $MgCl_2$	50.0	0.8	0.4	48.8	8.5

reference electrode and an ORION 9417XXX chloride electrode. Results were means of three determinations and were expressed as g of salt/100 g of muscle.

2.5. Determination of protein

Samples of minced muscle (1.5 g) and brine (5 ml) were heated to 1050 °C following AOAC 992.15 (A.O.A.C., 2000) in a LECO model FP-2000 protein/nitrogen analyser calibrated with EDTA (Dumas method). The nitrogen-to-protein conversion factor considered was 6.25%. Results were means of four determinations and were expressed as grams of protein/100 grams of muscle or per 100 ml of brine.

2.6. Soluble protein

Soluble protein was obtained by a variation on the method used by Stefansson and Hultin (1994). Soluble protein was extracted in distilled water (low ionic strength), making it possible to see which proteins were solubilized as a result of adding salt to the muscle.

Two g of minced muscle was homogenized at low temperature for one minute in 50 ml of distilled water in an Omnimixer-Homogenizer (model 17106, OMNI International, Waterbury, USA), setting 6. The homogenates of these solutions were stirred constantly for 30 min at 2 °C then centrifuged (6000*g*) for 30 min in a Sorvall model RT 6000B centrifuge (Du Pont Co., Delaware, USA) at 3 °C. Protein concentration was determined in the supernatant by the colorimetric method of Lowry, Rosebrough, Farr, and Randall (1951). Optic density was measured at 750 nm in a Perkin–Elmer UV/VIS spectrophotometer (model Lambda 15, MA, USA). The standard curve was determined with various known concentrations of bovine serum albumen.

2.7. SDS–PAGE

The protein concentration in the resulting supernatants was adjusted to 0.20 mg/ml. The supernatants were treated with a denaturing solution composed of 5% 2-β-mercaptoethanol, 2.5% sodium dodecyl sulphate (SDS), 10 mM Tris–HCl, 1 mM ethylendiamine tetra acetic acid (EDTA) and 0.002% bromophenol blue, as described by Hames (1985).

The protein from the brines was desalted in PD-10 columns with Sephadex G-25 M (Amersham Pharmacia Biotech) then treated as described above with the concentration adjusted to 0.22 mg/ml. All the samples were then heated for five minutes at 100 °C.

Electrophoresis was performed by the method of Laemmli (1970), on a Mighty Small II SE250 unit (Hoefer Pharmacia Biotech. Inc, San Francisco, USA), using 10% polyacrylamide gels in the presence of sodium dodecyl sulphate (SDS), at 20 mA per gel and 250 V at 2 °C.

Protein bands were stained with Coomassie brilliant blue tablets (Phastgel Blue R., Pharmacia). Destaining was performed in an aqueous solution of 30% methanol and 10% acetic acid. Samples were conserved in a solution of 5% glycerol and 10% acetic acid.

The standard was a high molecular weight calibration kit (Amersham Pharmacia Biotech) consisting of: one sub-unit of ferritin (220 kDa), albumin (67 kDa), one sub-unit of catalase (60 kDa), one sub-unit of dehydrogenase lactate (36 kDa) and one sub-unit of ferritin (18.5 kDa). The molecular weight of each band was determined by the 1-D Manager (version 2.0) image analysis and quantification tool (Tecnología para diagnóstico e Investigación, S.A., Spain).

2.8. Statistical analyses

The significance of differences between mean value pairs was evaluated using one-way ANOVA. Tukey

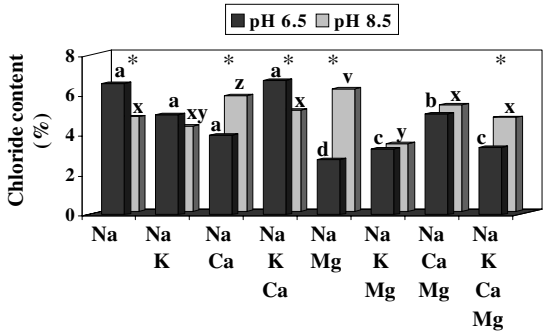

Fig. 1. Chloride content in cod muscle after brining with different brines for 36 h. a, b, c,... indicate significant differences among samples brined at pH 6.5. x, y, z,... indicate significant differences among samples brined at pH 8.5. (*) indicate significant differences between each sample brined at pH 6.5 and the corresponding brined at pH 8.5.

HSD test was used to identify significant differences among main effects. Statistical processing was done by the SPSS computer program™ (SPSS Inc., Chicago, IL, USA). The level of significance setting was $P \leqslant 0.05$.

3. Results and discussion

The chloride content of unsalted cod muscle was $0.22 \pm 0.03\%$. As a result of osmosis, immersion in the various brines increased the percentage of chlorides in the cod fillets, leading to some loss of moisture as compared to unsalted muscle (Sikorski, 1990). The addition of 0.4% $MgCl_2$ to the brines reduced the uptake of chlorides in most cases, especially at pH 6.5 (Fig. 1). Several authors have also noted lower final salt contents in muscle samples containing small amounts of $MgCl_2$ (and $CaCl_2$) (Iyengar & Sen, 1970; Moody, Flick, Martin, & Correa, 2000). Moreover, this effect appears to be related to higher moisture and protein percentages in salted muscle (Table 2). The replacement of approximately half of the NaCl by KCl appeared to hinder the uptake of chlorides by the muscle at both pH (Fig. 1). In pH 8.5 brines, this effect occurred also with the different brines including divalent salts, correlating with increased moisture content following brining and a slightly reduced protein content (Table 2).

Brining also caused solubilisation of some of the muscle proteins, which were released into the brines (Del Valle & Nickerson, 1967; Hamm, 1960). As shown in Fig. 2, the release was higher in the case of cod fillets salted at pH 8.5. It is important to note that, taking into account the moderate salt content in the muscle, the final salt concentration in the extraction medium with distilled water varied between 0.1% and 0.27%. The higher rate of protein extraction in more alkaline solutions – that is, those furthest from the isoelectric point of the proteins – which is consistent with previous findings (Damodaran & Kinsella, 1982; Hermansson, 1973; Kinsella, 1982; Stefansson & Hultin, 1994), generally coincided with increased chloride uptake by the muscle, especially where the alkaline-pH brines included salts of magnesium (Fig. 1). The added chlorides clearly had a major effect on muscle protein solubility and on water holding properties, as described by Hamm (1977, 1982) and Fennema (1977). At the same time, the cations introduced could have also induced a number of conformational changes in the protein molecule, which according to the initial pH of the brine, might have affected the protein/water interactions and hence solubility.

Fig. 3 shows the electrophoretic profiles of the proteins released into the various pH 6.5 brines after 36 h

Table 2
Proximate analyses of brined cod

Brines composition	Brines pH	Moisture in muscle (%)	Protein in muscle (%)	Ash in muscle (%)
NaCl	6.5	76.0 ± 0.23^{a}	17.6 ± 0.35^{de}	8.67 ± 0.74^{a}
NaCl	8.5	76.7 ± 0.73^{abc}	16.1 ± 1.23^{bcd}	6.82 ± 1.10^{abc}
NaCl + KCl	6.5	77.4 ± 1.02^{abcde}	15.6 ± 0.52^{bc}	7.49 ± 0.63^{abc}
NaCl + KCl	8.5	76.6 ± 0.18^{abc}	15.4 ± 0.15^{bc}	6.09 ± 1.17^{bc}
NaCl + $CaCl_2$	6.5	78.1 ± 0.40^{abcde}	16.0 ± 0.36^{bcd}	6.26 ± 0.59^{cd}
NaCl + $CaCl_2$	8.5	75.5 ± 0.16^{a}	16.5 ± 0.38^{bcd}	8.33 ± 0.32^{ab}
NaCl + KCl + $CaCl_2$	6.5	80.1 ± 0.70^{de}	15.1 ± 0.46^{bc}	8.10 ± 0.37^{ab}
NaCl + KCl + $CaCl_2$	8.5	78.1 ± 0.24^{abcde}	15.5 ± 0.78^{bc}	7.30 ± 0.59^{abc}
NaCl + $MgCl_2$	6.5	80.4 ± 0.28^{e}	16.0 ± 0.17^{bcd}	4.56 ± 0.53^{d}
NaCl + $MgCl_2$	8.5	77.4 ± 0.51^{abcde}	15.9 ± 0.17^{bcd}	7.71 ± 0.64^{abc}
NaCl + KCl + $MgCl_2$	6.5	77.4 ± 0.74^{abcde}	18.0 ± 0.68^{e}	6.21 ± 0.51^{cd}
NaCl + KCl + $MgCl_2$	8.5	79.1 ± 0.54^{bcde}	14.8 ± 0.74^{ab}	7.34 ± 0.68^{abc}
NaCl + $CaCl_2$ + $MgCl_2$	6.5	77.2 ± 0.49^{abcd}	16.8 ± 0.10^{cde}	6.25 ± 0.42^{cd}
NaCl + $CaCl_2$ + $MgCl_2$	8.5	76.7 ± 0.00^{abc}	15.7 ± 0.22^{bc}	8.16 ± 0.49^{ab}
NaCl + KCl + $CaCl_2$ + $MgCl_2$	6.5	78.9 ± 0.61^{bcde}	17.6 ± 0.08^{de}	6.02 ± 0.76^{cd}
NaCl + KCl + $CaCl_2$ + $MgCl_2$	8.5	77.9 ± 0.13^{abcde}	15.6 ± 0.38^{bc}	6.75 ± 0.33^{bc}
Not salted	–	81.8 ± 0.09	17.6 ± 0.02	1.21 ± 0.15

Different letters (a, b, c,...) indicate significant differences ($p \leqslant 0.05$) between samples.

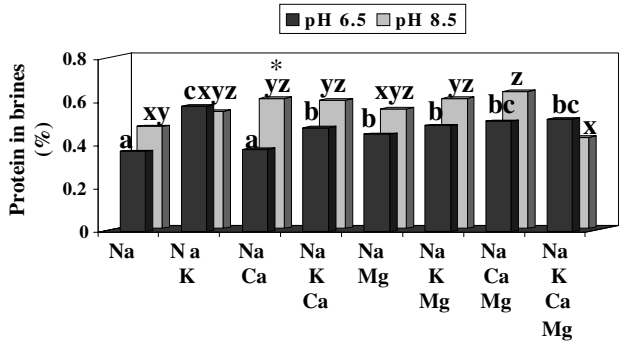

Fig. 2. Protein content in brines after brining for 36 h. a, b, c,... indicate significant differences among samples brined at pH 6.5. x, y, z,... indicate significant differences among samples brined at pH 8.5. (*) indicate significant differences between each sample brined at pH 6.5 and the corresponding brined at pH 8.5.

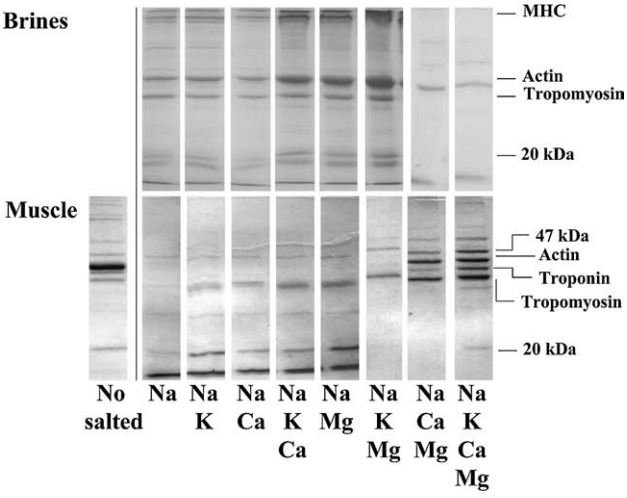

Fig. 3. SDS–PAGE banding pattern of (a) protein released into the brine at pH 6.5 following salting; (b) protein in brined muscle at pH 6.5 extracted in distilled water.

of fillets immersion (Fig. 3(a)), and of the resulting fraction of muscle protein extracted in distilled water (Fig. 3(b)). Except in samples containing the two divalent salts together, when cod samples were immersed in pH 6.5 brines, an appreciable amount of myosin heavy chains (MHC) (Squire, 1994) was released into the brine (Fig. 3(a)). This presumably occurs in the early stages of salting, given that the rapid denaturation and aggregation of myosin, which is dependent on salting time and salt concentration (Ito, Kitada, Yamada, Seki, & Arai, 1990; Tambo, Yamada, & Kitada, 1992), would hinder such loss at later stages. Therefore, the absence or scant presence of the MHCs in the soluble fraction of the muscle following brining (Fig. 3(b)) is presumably due not only to their poor solubility in low ionic strength conditions (Morrissey, Mulvihill, & O' Neill, 1987), but also to immersion in brine. Other factors that must be borne in mind are the instability of MHCs during handling and processing (Hasting, Rodger, Park, Matthews, & Anderson, 1985), aggregation in response to salt input (Morrissey et al., 1987), and possible degradation during salting (Thorarinsdottir, Arason, Geirsdottir, Bogason, & Kristbergsson, 2002). Regarding the latter, slight traces of what are possibly products of myosin degradation, of about 170 and 70 kDa (presumably LMM) (Niwa, Suzuki, Nowsad, & Kanoh, 1993; Wright & Wilding, 1984) and not present in the soluble fraction of the salted cod, were found in the protein fraction of the brines (Fig. 3(a)). These brines also took up appreciable quantities of other proteins, tentatively identified as actin (42 kDa), tropomyosin (35 kDa) (Squire, 1994), and troponin C, troponin J and/or myosin light chains (MLC), and other low molecular weight compounds (around 20 kDa). The tropomyosin, the other low molecular weight compounds mentioned, and a protein of about 47 kDa (of which there were traces in the brines) also formed part of the protein profile of the salted cod muscle (Fig. 3(b)). Actin, however, was notably absent.

In the case of brines containing NaCl, $MgCl_2$ and $CaCl_2$ with or without KCl, clearly less actin was released to the brine, much of it remaining soluble in the salted muscle. The same was true of the 47 and 70 kDa proteins (LMM) (Wright & Wilding, 1984). Tropomyosin and troponin T (38 kDa) were also noticeable in the soluble fraction.

These results indicated that there was a notable loss of soluble muscle proteins by osmosis in salting at pH 6.5; in particular, myosin (MHC) and especially actin, as well as other minority proteins, were released into the brine. However, this did not occur when the divalent salts Ca^{2+} and Mg^{2+} were added both together to the brine, probably because they promoted more muscle protein interactions at pH 6.5.

The soluble protein fraction of cod muscle salted in brines at pH 8.5 was noticeably different from that generally obtained when salting at pH 6.5 and was largely similar to that of unsalted cod (Fig. 4(b)). Consequently, the protein composition of alkaline brines differed from that of slightly acidic brines (Fig. 4(a)). Unlike the case of pH 6.5, no great ionic strength-dependent differences were observed; the protein composition of the brine after removal of the fish consisted largely of a single band, tentatively identified as sub-units of tropomyosin (35 kDa), which was also detected in the soluble fraction of the brined muscle. A protein of about 47 kDa was also observed, though less intense, in the brines and in the soluble fraction of the brined muscle. Bands corresponding to MHCs and their degradation products were faintly visible in the muscle fraction, but only in samples brined without magnesium chloride (Fig. 4(b)). Actin was noticeably present in the muscle protein profile and absent from the brines, unlike in samples salted at pH 6.5. In this connection, moreover, the presence of

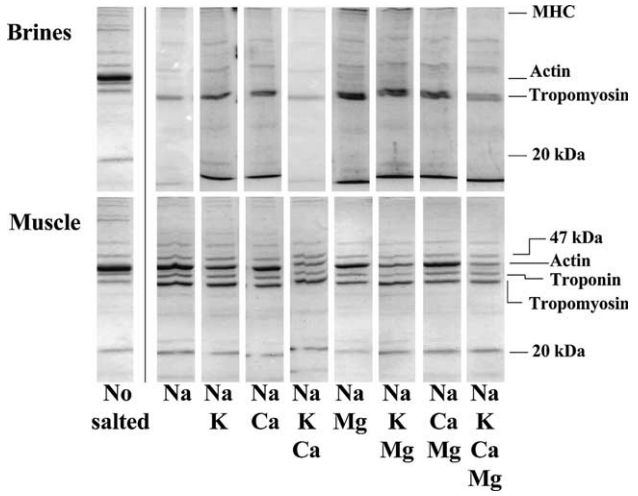

Fig. 4. SDS–PAGE banding pattern of (a) protein in brines with pH 8.5 (b) protein in brined cod at pH 8.5 extracted by distilled water.

KCl in the brines seems to have reduced the solubility of actin, whose band was less intense in these samples. As in the case of actin, a protein of about 38 kDa (possibly troponin T) and other less intensely visible proteins of about 58 and 20 kDa were absent from the brines but present in the muscle soluble fraction. Coinciding with the presence of certain low molecular weight peptides, Stefansson and Hultin (1994) also found a 25 kDa peptide in their studies of fresh cod muscle proteins in low ionic strength media.

4. Conclusions

The use of 0.4% $MgCl_2$ for brining of Atlantic cod (*G. morhua*) fillets, especially in brines with an initial pH of 6.5, reduced the transfer of chlorides to the muscle; the effect was similar when approximately half the NaCl was replaced by KCl in brines of pH 8.5. The pH of the brines itself influenced the transfer of soluble protein to the brines, being easier at alkaline pH. In addition, the variety of peptides found in brines after salting differed from one pH to another. Similarly, the composition of the muscle soluble protein fraction depended on the pH of the brine used; thus, there were a smaller variety of remaining soluble proteins with an initial pH of 6.5 than with one of 8.5. However, this effect of the lower pH was offset to the extent that the ionic strength of the brine was higher. Both actin and myosin heavy chains were released in 6.5 pH brines after immersion of the fish. To the contrary, an initial pH of 8.5 did not favour the release of these proteins. The initial pH of the brine therefore appears to be a more important factor in selective solubilization of myofibrillar proteins than the actual combination of salts.

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References

A.O.A.C. (2000). *Official methods of analysis* (17th ed.). Maryland, EEUU: Association of Official Analytical Chemistry.

Arganosa, G. C., & Marriott, N. G. (1990). Salt substitutes in canned luncheon meat. *Journal of Muscle Food, 1*, 207–216.

Barat, J. M., Rodríguez-Barona Andrés, A., & Fito, P. (2002). Influence of increasing brine concentration in the cod-salting process. *Journal of Food Science, 67*(5), 1922–1925.

Beatty, S. A., & Fougére, H. (1957). *The processing of dried salted fish* (Bulletin No. 112). Ottawa: Fisheries Research Board of Canada.

Damodaran, S., & Kinsella, J. E. (1982). Effects of ions on protein conformation and functionality. In J. P. Cherry (Ed.), *Protein structure deterioration. ACS symposium series* (206, p. 13). Washington, DC: American Chemcal Society.

Del Valle, F. R., & Nickerson, J. T. R. (1967). Studies on salting and drying fish. I. Equilibrium considerations in salting. *Journal of Food Science, 32*, 173–179.

Del Valle, F. R., & Nickerson, J. T. R. (1968). A quick-salting process for fish. 1. Evolution of the process. *Food Technology, 22*(8), 1036–1038.

Deng, J. C. (1977). Effect of freezing and frozen storage on salt penetration into fish muscle inmersed in brine. *Journal of Food Science, 42*(2), 348–351.

Fennema, O. R. (1977). Water and protein hydration. In J. R. Whitaker & S. R. Tannenbaum (Eds.), *Food proteins* (pp. 50–90). Westport, CT: Avi Publishing Company.

Fougére, H. (1952). The water transfer in codfish muscle inmersed in sodium chloride solutions. *Fisheries Research Board of Canada, 9*(8), 388.

Gillette, M. (1985). Flavor effects of sodium chloride 56. *Food Technology, 39*(6), 47–52.

Hamm, R. (1960) Biochemistry of meat hydration. In C. O. Chichester, E. M. Mrak, & G. F. Stewart (Eds.), *Advances in Food Research*. N 10 (pp. 355–463) New York: Academic Press.

Hamm, R. (1977). Postmortem breakdown of ATP and glycogen in ground muscle: a review. *Meat Science, 1*, 15–39.

Hamm, R. (1982). Postmortem changes in muscle with regard to processing of hot boned beef. *Food Technology, 36*(11), 105–115.

Hammes, B. D. (1985). An introduction to polyacrylamide gel electrophoresis. In B. D. Hames & D. Rickwood (Eds.), *Gel Electrophoresis of Proteins. A Practical Approach*. England: IRL Press, Oxford.

Hand, L. W., Terrell, R. N., & Smith, G. C. (1982a). Effects of chloride salt, method of manufacturing and frozen storage on sensory properties of restructured pork roasts. *Journal of Food Science, 47*, 1771–1772.

Hand, L. W., Terrell, R. N., & Smith, G. C. (1982b). Effects of complete or partial replacement of sodium chloride on processing and sensory properties of hams. *Journal of Food Science, 47*, 1776–1778.

Hand, L. W., Terrell, R. N., & Smith, G. C. (1982c). Effects of chloride salts on physical chemical and sensory properties of frankfurters. *Journal of Food Science, 47*, 1800–1802.

Hasting, R. J., Rodger, W., Park, P., Matthews, A. D., & Anderson, E. M. (1985). Differential scanning calorimetry of fish muscle: the

effect of processing and species variation. *Journal of Food Science, 50*, 503–510.

Hermansson, A.M. (1973). Technical report no. 2. Lund, Sweden: Chemical Centre.

Iyengar, J. R., & Sen, D. P. (1970). The equilibrium relative humidity relationship of salted fish (*Barbus carnaticus* and *Rastelliger canagurta*): The effect of calcium and magnesium as impurities in common salt used for curing. *Journal of Food Science and Technology, 9*, 17–19.

Ito, T., Kitada, N., Yamada, N., Seki, N., & Arai, K. (1990). Biochemical changes in meat of Alaska pollack caused by soaking in NaCl solution. *Bulletin of the Japanese Society of Scientific Fisheries, 56*, 687–693.

Kinsella, J. E. (1982). Relationships between structure and functional properties of food proteins. In P. F. Fox & J. J. Condon (Eds.), *Food proteins*. London: Aplied Science Publishers.

Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophase T4. *Nature, 227*, 680–685.

Lauritzen, K., Akse, L. (1995). Quality of salt and salted fish. Report no. 1/1995. Tromso (Norway): Pub. Fiskeriforskning.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951). Protein measurement with the Folin Phenol Reagent. *Journal of Biological Chemistry, 193*, 265–275.

Madrid, A., Madrid, J. M., & Madrid, R. (1994). *Tecnología del pescado y productos derivados*. Madrid, Spain: Mundiprensa Libros, S.A.

Moody, M. W., Flick, G. J., Jr., Martin, R. E., & Correa, A. I. (2000). Smoked, cured and dried fish. In R. E. Martin, E. P. Carter, G. J. Flick, Jr., & L. M. Davis (Eds.), *Marine and freshwater products handbook* (pp. 381). Lancaster, PA: Technomic Publishing Company, Inc.

Morrissey, P. A., Mulvihill, D. M., & O' Neill, E. M. (1987). Functional properties of muscle proteins. In B. J. F. Hudson (Ed.), *Developments in food proteins-5* (pp. 195). New York: Elsevier Applied Science.

Niwa, E., Suzuki, S., Nowsad, A., & Kanoh, S. (1993). Lower molecular fish proteins separated from the band around that of crosslinked myosin heavy chain. *Nippon suisan gakkaishi, 59*(6), 1013–1016.

Pilkington, D. H., & Allen, J. C. (1994). Substitution of potassium chloride for sodium chloride in commercially-produced dry-cured hams. *Journal of Food Protection, 57*(9), 792–795, 801.

Sikorski, Z. E. (1990). *Tecnología de los productos del mar: Recursos, composición nutritiva y conservación*. Zaragoza, Spain: Edit. Acribia, S.A.

Squire, J. M. (1994). The actomyosin interaction-shedding light on structural events: Plus ca change, plus c' est la meme chose. *Journal of muscle research and cell motility, 15*, 227–231.

Stefansson, G., & Hultin, H. O. (1994). On the solubility of cod muscle proteins in water. *Journal of Agricultural and Food Chemistry, 42*, 2656–2664.

Tambo, T., Yamada, N., & Kitada, N. (1992). Change in myofibrillar protein of fish muscle caused by soaking in NaCl solution. *Bulletin of the Japanese Society of Scientific Fisheries, 58*, 677–683.

Thorarinsdottir, K. A., Arason, S., Geirsdottir, M., Bogason, S., & Kristbergsson, K. (2002). Changes in myofibrillar proteins during processing of salted cod (*Gadus morhua*) as determined by electrophoresis and differentical scanning calorimetry. *Food Chemistry, 77*, 377–385.

Topp, N. B. (1981). You are what you eat. *Journal of Food Protection, 44*, 556.

Van Klaveren, F. W., & Legendre, R. (1965). Salted cod. In G. Borgstorm (Ed.). *Fish as Food* (Vol. III, pp. 133–163). New York: Edit. Academic Press Inc.

Wheaton, F. W., & Lawson, T. B. (1985). *Processing Aquatic Food Products*. New York: Wiley.

Wright, D. J., & Wilding, P. (1984). Differential scanning calorimetric study of muscle and its proteins: myosin and its subfragments. *Journal of Science of Food and Agriculture, 35*, 357–372.