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Sodium replacement in the cod (*Gadus morhua*) muscle salting process

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Abstract

Cod fillets were salted in brines with different pHs (6.5 and 8.5) and saline compositions. Water-holding capacity, protein extractability, dry matter, ion content and hardness in muscle were assessed to determine the effect of replacing NaCl with varying combinations of KCl, $CaCl_2$ and/or $MgCl_2$. Discriminant and principal component analyses were performed to better understand the relationships between brine composition and functional properties of brined cod. Partial replacement of 50% NaCl with 50% KCl reduced penetration of Na^+ into muscle, as did the addition of small amounts of $CaCl_2$ (0.8%) and/or $MgCl_2$ (0.4%) to pH 6.5 brines. The use of 0.4% $MgCl_2$ at pH 6.5 negatively affected functional properties and further hindered salt penetration into the muscle. The use of KCl in pH 8.5 brines increased hardness, negatively affecting protein water-extractability. Moreover, the addition of divalent salts, at basic pHs, slightly decreased water-holding capacity.

Keywords: Cod; pH; Potassium chloride; Divalent salts; Brining

1. Introduction

Salting is a time-honoured method of preserving fish, especially cod, and traditionally comprises a salting step, followed by air-drying. Salted cod is widely consumed in Spain, Portugal and Latin America, although the largest producers are North Atlantic countries, such as Norway and Iceland (Bjornsson, 2000). These two countries exported over 40,000 tonnes of salted cod to the principal consumer countries in 1999, which gives an idea of the economic importance of the product (Gallart-Jornet, Rodríguez-Barona, Barat, Andrés, & Fito, 2003).

In the typical salting process (dry or *kench* salting), the fish is filleted or "butterfly" split, and piled into stacks where layers of fish and dry salt alternate (Thorarinsdottir, Arason, Bogason, & Kristbergsson, 2004). An alternative to dry-salting is immersion in solutions of salt and water. There are several advantages to brining: higher weight yield (Beraquet, Iaderoza, Jardim, & Lindo, 1983; Bogason, 1987) caused by uptake of water; protection against oxidative rancidity (Wheaton & Lawson, 1985); and faster salting due to a higher rate of salt penetration in the fish muscle (Akse, Gundersen, Lauritzen, Ofstad, & Solberg, 1993). To achieve higher yields and good water-holding capacity – and hence better rehydration capacity – it is important that the brine should not be saturated (Barat, Rodríguez-Barona, Andrés, & Fito, 2002; Hamm, 1960) and that the process temperature not exceed 25 °C (Torry Research Station, 1962).

The gradual increase in salt concentration in cod muscle results in changes in functional properties. The presence of high concentrations of salt in muscle gradually increases the water-holding capacity (WHC), obtaining a maximum at an ionic strength of 1 M (~5.8% salt) (Offer & Knight, 1988). At high ionic strengths, water-holding capacity decreases, apparently by a salting-out effect due to water binding by the salt

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and concurrent dehydration of the protein. Furthermore, the effect of salt concentration is dependent, in turn, on the pH of the medium. At pH values higher than the isoelectric point of the muscle proteins (~pH 5), the protein net charge is increased and the muscle swells. The reason is a repulsion between protein groups with the same charge and, in this way, the space between the peptide chains is enlarged and therefore more water can penetrate (Hamm, 1960). Taken together, salt and pH can alter the net charge of the protein molecule, affecting protein functionality to a greater or lesser extent and reducing protein-water and protein-protein interactions (Kinsella, 1982; Morrissey, Mulvihill, & O'Neill, 1987; Stefansson & Hultin, 1994).

On the other hand, the type of salt used can also influence the salting process to a greater or lesser extent. Calcium and magnesium salts, for example, hinder the proper penetration of salt into fish tissues (Moody, Flick, Martin, & Correa, 2000), but small amounts can be beneficial in that they whiten the fish and halt enzymatic processes that can spoil the product. At the same time, the presence of more than 50% KCl can attenuate the flavour and produce bitterness (Hand, Terrell, & Smith, 1982a, 1982b, 1982c; Seman, Olson, & Mandigo, 1980). The additional use of KCl to partially replace NaCl (Gillette, 1985; Pilkington & Allen, 1994) could be helpful in reducing sodium content, which would render the product more acceptable, given the growth of demand for low-sodium foods in recent years.

The object of this work was to examine the relationship between ionic composition and functional quality of lightly salted cod, using brines for salting at two different pHs (6.5 and 8.5) in which NaCl was partially replaced by KCl, $MgCl_2$ and/or $CaCl_2$.

2. Materials and methods

2.1. Material

Following capture off the coast of Iceland in April, cod (*Gadus morhua*) specimens were headed, gutted, washed and placed in bins covered with ice. The bins were immediately transported to the Icelandic Fish Processing School. Cod specimens 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.

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	6.5
NaCl	100	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

The fillets were then shipped to Spain by refrigerated transport. The back part of each fillet was chopped into 150–200 g portions (approximate dimensions: length 9 ± 2 cm, width 5 ± 1 cm, and thickness 3 ± 1 cm). All these portions were randomised, and stored at −24 °C.

2.2. Determination of dry matter

Moisture was determined on approximately 5 g of minced muscle, by oven-drying at 110 °C to constant weight, following technique 950.46 (AOAC, 2000). Results of dry matter were means of three determinations and were expressed as 100 minus moisture (in g of water/100 g of muscle).

2.3. Determination of chloride content

Five grams of minced muscle was homogenised 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 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.

2.4. Determination of ions: sodium, potassium, calcium and magnesium

Approximately 5 g of muscle was reduced to ash and homogenised in 5 ml of 60% suprapure nitric acid di-

luted 1:1 with Milli-Q water. Samples were made up to 100 ml, also with Milli-Q water. A Perkin-elmer model 5100 Pc atomic absorption spectrophotometer (Massachusetts, USA) was used to determine calcium, (Ca^{2+}), magnesium (Mg^{2+}), sodium (Na^{+}) and potassium (K^{+}) cations in these solutions. Results were expressed in mg/g of muscle.

2.5. Water-holding capacity

Water-holding capacity (WHC) was determined by the method described by Montero, Gómez-Guillén, & Borderías (1996). Two grams of muscle was chopped and weighed, then placed in a centrifuge tube, along with three pipette filters (Gilson pipetman, france) as absorbents. Samples were centrifuged for 10 min at 6000*g* in a sorvall RT60008 centrifuge (Dupont Co., Delaware, USA) at room temperature. Results are averages of three determinations and are expressed as g of water/g of protein in the muscle.

2.6. Water-extractable protein

Water-extractable protein (WEP) was obtained by a variation on the method used by Stefansson & Hultin (1994). Soluble protein was extracted in distilled water, with 0.1–0.27% chloride content in the homogenates (low ionic strength), making it possible to see which proteins were solubilised as a result of adding salt to the muscle.

Two g of minced muscle was homogenised 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, & Randall (1951). Optical density was measured at 750 nm in a Perkin elmer UV/VIS spectrophotometer (model Lambda 15, Massachusetts, USA). The standard curve was determined with various known concentrations of bovine serum albumen. WEP was expressed as percent protein remaining in the supernatant with respect to total protein in the homogenate prior to centrifuging, which was previously determined following AOAC 992.15 (AOAC, 2000) in a LECO model FP-2000 protein/nitrogen analyser (St. Joseph, MI, USA).

2.7. Shear strength

Shear strength was determined on a bone-free muscle sample, 4 cm long and 2 cm wide. This was divided in half lengthwise and spread on a Kramer cell (Kramer, Burkhardt, & Rogers, 1951) with the myotomes perpendicular to the cell. A computer-controlled Instron Universal texturometer model 4501 was used (Instron Engineering Corp., Canton, MA, USA), with a cell load of 5 kN at a setting of 100 mm/min. Results are the means of three determinations and are expressed as Newtons/g of muscle at the point of maximum load before sample breaking.

2.8. Statistical analyses

The significance of differences between mean value pairs was evaluated using one-way ANOVA. Tukey's HSD test was used to identify significant differences among main effects. The data from the different variables analysed in brined cod were used as the data matrix in a principal component analysis (PCA). Further discriminant analyses were also performed. The computing programme used was SPSS (SPSS Inc., Chicago, Ill., USA).

3. Results and discussion

Table 2 presents the results of salt content (expressed as percent total chlorides) and dry matter of salted cod muscle after 36 h in the different brines. Dry matter content in unsalted muscle was 18.18 ± 0.09%. After brine salting, values varied slightly ($p \leqslant 0.05$) among samples, ranging from 19.64% in sample Na–Mg-6.5 to 24.53% in sample Na–Ca-8.5. These results indicate that the degree of osmotic dehydration, as a consequence of salting, may depend, to some extent, on the composition and pH of the brine solutions. The salt content in brined cod differed considerably from one sample to another. The lowest values ($p \leqslant 0.05$) were recorded in the Na–Mg-6.5 sample, which also presented the lowest dry matter content. It therefore seems clear that the lower degree of dehydration attained in these brine conditions was directly related to lower penetration of salt into the muscle. Various authors have reported a reduction in the final salt content of muscle in samples salted with small amounts of $MgCl_2$ (Iyengar & Sen, 1970; Moody et al., 2000). One possible explanation is that, because the Mg^{2+} cation is the most electronegative of all those assayed, it binds strongly to the protein polar groups, strengthening protein interactions (Xiong & Brekke, 1991) and thus hindering the penetration of salt.

As regards the effect of brine pH, the salt content of the muscle was highest at alkaline pH (Na–Mg-8.5) with same mixture of salts (Na–Mg). This is a clear example of how initial pH and the nature of the salts influence important quality parameters of brined salted cod, such as the salt content and the degree of muscle dehydration. The pH of the salt has also been reported to influence the final characteristics of pickle salted cod (Lauritzsen,

Table 2
Dry matter and chloride content of brined cod (means and standard deviations)

Brines composition	Brine pH	Dry matter (%)	Chloride content (%)
No salted		18.18 ± 0.09	0.22 ± 0.03
NaCl	6.5	23.99 ± 0.23^{de}	6.63 ± 0.95^{f}
NaCl	8.5	23.28 ± 0.73^{cde}	4.78 ± 0.07^{cde}
NaCl + KCl	6.5	22.56 ± 1.02^{bcde}	5.05 ± 1.08^{cde}
NaCl + KCl	8.5	23.40 ± 0.18^{cde}	4.31 ± 0.09^{bcd}
NaCl + $CaCl_2$	6.5	21.89 ± 0.40^{abcde}	4.00 ± 0.70^{bc}
NaCl + $CaCl_2$	8.5	24.53 ± 0.16^{e}	5.83 ± 0.05^{ef}
NaCl + KCl + $CaCl_2$	6.5	19.89 ± 0.70^{ab}	6.77 ± 0.12^{f}
NaCl + KCl + $CaCl_2$	8.5	21.86 ± 0.24^{abcde}	5.11 ± 0.10^{cde}
NaCl + $MgCl_2$	6.5	19.64 ± 0.28^{a}	2.18 ± 0.10^{a}
NaCl + $MgCl_2$	8.5	22.65 ± 0.51^{bcde}	6.98 ± 0.06^{f}
NaCl + KCl + $MgCl_2$	6.5	22.61 ± 0.74^{bcde}	3.32 ± 0.02^{ab}
NaCl + KCl + $MgCl_2$	8.5	20.94 ± 0.54^{abc}	3.42 ± 0.07^{b}
NaCl + $CaCl_2$ + $MgCl_2$	6.5	22.81 ± 0.49^{cde}	5.08 ± 0.04^{cde}
NaCl + $CaCl_2$ + $MgCl_2$	8.5	23.29 ± 0.00^{cde}	5.39 ± 0.08^{de}
NaCl + KCl + $CaCl_2$ + $MgCl_2$	6.5	21.13 ± 0.61^{abcd}	3.41 ± 0.03^{ab}
NaCl + KCl + $CaCl_2$ + $MgCl_2$	8.5	22.12 ± 0.13^{abcde}	4.74 ± 0.10^{cde}

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

Akse, Gundersen, & Olsen, 2004). As Table 2 indicates, salt penetration into the muscle was greater at pH 8.5 than at pH 6.5 in most of the brines containing divalent cations (Ca^{2+} and/or Mg^{2+}). Because calcium and magnesium cations are divalent, at pH 6.5 they bind more strongly to the outermost layers of the muscle proteins, compacting the surface of the fish and delaying the penetration of sodium chloride (Iyengar & Sen, 1970). On the other hand, an alkaline pH in brines, much more remote from the isoelectric point of the muscle proteins, may cause the proteins to unfold to some extent, thus favouring penetration of the muscle by salt ions according to Hamm (1960). This process presumably takes place during the early stages of brining, given that, in all cases, the pH of the brine was buffered by the fish muscle up to values around 6.6–6.8. It should be said, however, that Lauritzsen et al. (2004) found no evidence of buffering by the fresh muscle during pickle salting, presumably due to massive penetration of salts into the muscle.

The residual content of the different cations used for brining was determined in salted muscle (Table 3). Brine salting produced a noticeable increase in concentrations of Na^+, K^+ and Ca^{2+} cations, varying according to the nature of the brine. In the particular case of Mg^{2+}, where only a small amount of $MgCl_2$ was added to the brine, the increases recorded in residual concentrations in the muscle were not significant ($p \leqslant 0.05$). When NaCl was partially replaced by KCl in the brines, the concentration of Na^+ in the muscle was much lower than in muscle salted with 100% NaCl, the sample with the highest sodium content (37–43 mg/g muscle). In many cases the reduction in residual Na^+ was greater than 50%. As Table 3 shows, the residual K^+ content of these samples invariably increased but in no case exceeded 20–21 mg/g of muscle. Addition of small amounts of divalent salts ($CaCl_2$ and/or $MgCl_2$) significantly reduced the sodium content in samples where the initial brine pH was 6.5. This did not occur where the initial pH was 8.5, which confirms that an alkaline pH in the presence of divalent salts enhanced the diffusion of ions inside the muscle, particularly Na^+ ions, which are the most abundant. The amount of residual Ca^{2+} increased significantly ($p \leqslant 0.05$) in muscle salted in brines containing 0.8% $CaCl_2$; however, residual Ca^{2+} did not differ significantly as a function of the pH of the brine in pickle salted cod as reported by Lauritzsen et al. (2004). The cited authors found that the pH of the salt and the muscle correlated negatively with Ca^{2+} and Mg^{2+} contents, presumably because these ions are less soluble at higher pH values and because, in adjusting the pH, the CO_3^{2-} ions caused increased precipitation. The observed differences between dry-salted and brine-salted muscle may be due to the buffering effect of the brined muscle; the smaller amounts of $CaCl_2$ and $MgCl_2$ in the brines, and/or non-addition of CO_3^{2-} ions to the brines.

Functional quality of salted cod was evaluated in terms of water-holding capacity (WHC), water-extractable protein (WEP) and muscle hardness (Table 4). WHC increased slightly from 3.3 g water retained/g protein in unsalted cod, to around 3.5–4.4 g water retained/g protein in brined samples, although, in many cases, the differences were not significant ($p \leqslant 0.05$). This was attributed chiefly to increasing salt concentration in the muscle without salting out (Hamm, 1982; Offer & Trinick, 1983; Parson & Knight, 1990; Thorarinsdottir et al., 2004).

In most cases, the brining process induced a significant decrease in water-extractable protein (WEP) as compared to the unsalted muscle, with the exception of the Na-8.5 sample where values were slightly higher

Table 3
Sodium, potassium, calcium and magnesium content in brined muscle cod

Brines composition	NaCl (%)	KCl (%)	$CaCl_2$ (%)	$MgCl_2$ (%)	Brines pH	Na^+ (mg/g)	K^+ (mg/g)	Ca^{2+} (mg/g)	Mg^{2+} (mg/g)
No salted						0.862^{a}	2.89^{a}	0.433^{a}	0.279^{a}
NaCl	100				6.5	43.1^{h}	2.07^{a}	0.757^{abcde}	0.270^{a}
NaCl	100				8.5	37.1^{gh}	2.24^{a}	0.709^{abcd}	0.202^{a}
NaCl + KCl	50	50			6.5	18.8^{bcd}	19.8^{fg}	0.866^{defg}	0.220^{a}
NaCl + KCl	50	50			8.5	15.9^{bc}	12.2^{bcd}	0.546^{ab}	0.207^{a}
NaCl + $CaCl_2$	99.2		0.8		6.5	25.6^{def}	2.20^{a}	0.974^{bcdefg}	0.219^{a}
NaCl + $CaCl_2$	99.2		0.8		8.5	36.6^{gh}	1.84^{a}	1.19^{efg}	0.185^{a}
NaCl + KCl + $CaCl_2$	50	49.2	0.8		6.5	20.00^{bcd}	20.3^{g}	1.3^{fg}	0.179^{a}
NaCl + KCl + $CaCl_2$	50	49.2	0.8		8.5	17.0^{bc}	20.4^{g}	1.09^{defg}	0.210^{a}
NaCl + $MgCl_2$	99.6			0.4	6.5	21.9^{bcde}	2.37^{a}	0.622^{abc}	0.305^{a}
NaCl + $MgCl_2$	99.6			0.4	8.5	33.3^{fg}	2.12^{a}	0.812^{abcde}	0.276^{a}
NaCl + KCl + $MgCl_2$	50	49.6		0.4	6.5	15.7^{bc}	16.9^{efg}	0.628^{abc}	0.299^{a}
NaCl + KCl + $MgCl_2$	50	49.6		0.4	8.5	18.1^{bcd}	12.7^{bcd}	0.606^{abc}	0.264^{a}
NaCl + $CaCl_2$ + $MgCl_2$	98.8		0.8	0.4	6.5	27.0^{ef}	2.170^{a}	1.01^{cdefg}	0.296^{a}
NaCl + $CaCl_2$ + $MgCl_2$	98.8		0.8	0.4	8.5	36.9^{gh}	1.79^{a}	1.41^{g}	0.275^{a}
NaCl + KCl + $CaCl_2$ + $MgCl_2$	50	48.8	0.8	0.4	6.5	14.5^{b}	16.0^{def}	0.92^{bcdef}	0.267^{a}
NaCl + KCl + $CaCl_2$ + $MgCl_2$	50	48.8	0.8	0.4	8.5	15.6^{bc}	17.0^{efg}	1.00^{bcdefg}	0.292^{a}

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

Table 4
Water-holding capacity (WHC), water-extractable protein (WEP) in distilled water and hardness of brined cod (means and standard deviations)

Brines Composition	Brines pH	WHC (g water retained/g protein)	WEP (%)	Hardness (N/g)
No salted		3.32 ± 0.21^{a}	17.9 ± 0.53^{hi}	19.95 ± 1.59^{b}
NaCl	6.5	3.62 ± 0.18^{abcd}	13.7 ± 0.95^{def}	11.19 ± 2.19^{a}
NaCl	8.5	4.42 ± 0.07^{e}	18.6 ± 0.80^{i}	9.67 ± 1.91^{a}
NaCl + KCl	6.5	3.87 ± 0.12^{abcde}	14.7 ± 0.74^{ef}	11.61 ± 3.26^{a}
NaCl + KCl	8.5	3.85 ± 0.04^{abcde}	13.7 ± 0.90^{def}	13.92 ± 1.86^{ab}
NaCl + $CaCl_2$	6.5	4.00 ± 0.20^{bcde}	16.6 ± 0.56^{gh}	15.74 ± 3.60^{ab}
NaCl + $CaCl_2$	8.5	4.01 ± 0.15^{bcde}	16.4 ± 0.18^{gh}	11.99 ± 1.86^{a}
NaCl + KCl + $CaCl_2$	6.5	4.14 ± 0.07^{cde}	13.1 ± 0.50^{de}	9.65 ± 0.81^{a}
NaCl + KCl + $CaCl_2$	8.5	3.93 ± 0.04^{abcde}	12.9 ± 0.43^{d}	14.19 ± 2.35^{a}
NaCl + $MgCl_2$	6.5	3.89 ± 0.30^{abcde}	12.9 ± 0.68^{d}	10.95 ± 1.09^{a}
NaCl + $MgCl_2$	8.5	3.96 ± 0.00^{abcde}	14.9 ± 0.34^{fg}	9.21 ± 2.19^{a}
NaCl + KCl + $MgCl_2$	6.5	3.45 ± 0.04^{ab}	10.5 ± 0.68^{c}	11.16 ± 1.88^{a}
NaCl + KCl + $MgCl_2$	8.5	4.19 ± 0.07^{de}	10.2 ± 0.12^{bc}	12.94 ± 1.86^{a}
NaCl + $CaCl_2$ + $MgCl_2$	6.5	3.62 ± 0.12^{abcd}	8.35 ± 0.05^{a}	12.53 ± 1.47^{a}
NaCl + $CaCl_2$ + $MgCl_2$	8.5	3.69 ± 0.07^{abcd}	15.1 ± 0.55^{fg}	13.23 ± 2.85^{ab}
NaCl + KCl + $CaCl_2$ + $MgCl_2$	6.5	3.48 ± 0.07^{abc}	7.44 ± 0.52^{a}	11.48 ± 1.21^{a}
NaCl + KCl + $CaCl_2$ + $MgCl_2$	8.5	4.01 ± 0.15^{bcde}	12.9 ± 0.37^{d}	14.40 ± 2.45^{ab}

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

(Table 4). In this connection, Stefansson & Hultin (1994) also reported greater protein solubilisation at alkaline pH; this was attributed to increased protein unfolding, caused by an increase of the pH to values further removed from the isoelectric point of the myofibrillar proteins. Alkaline pH has been reported to favourably affect protein solubility and WHC (Hamm, 1982; Martínez-Alvarez, 2003), due to a change in the protein conformation induced by repulsive forces between filaments, which increased swelling capacity of the muscle and space for water (Honikel, 1989; Offer & Trinick, 1983). In the case of Na–Ca samples, the decrease in WEP was not significant ($p \leqslant 0.05$). The lower WEP in the other brined samples could have been caused, at least in part, by the release of some muscle protein into the brines during salting (Martínez-Alvarez, 2003). WEP was lowest in pH 6.5 brines containing both Ca^{2+} and Mg^{2+}. This was attributed to the high capacity of divalent cations to interact with myofibrillar protein at this pH. Nevertheless, as also shown in Table 3, this slight protein aggregation was not associated with any muscle hardening. On the contrary, all brined samples presented lower hardness values than unsalted muscle, although these differences were not significant in all cases ($p \leqslant 0.05$).

Because of the complexity involved in determining the effect of brines with different pH levels (6.5 and 8.5) and different salt components (NaCl, KCl, $CaCl_2$ and $MgCl_2$), further statistical analyses were carried out in addition to comparative analysis so as to achieve a better understanding of the possible relationships between compositional and functional properties of brined

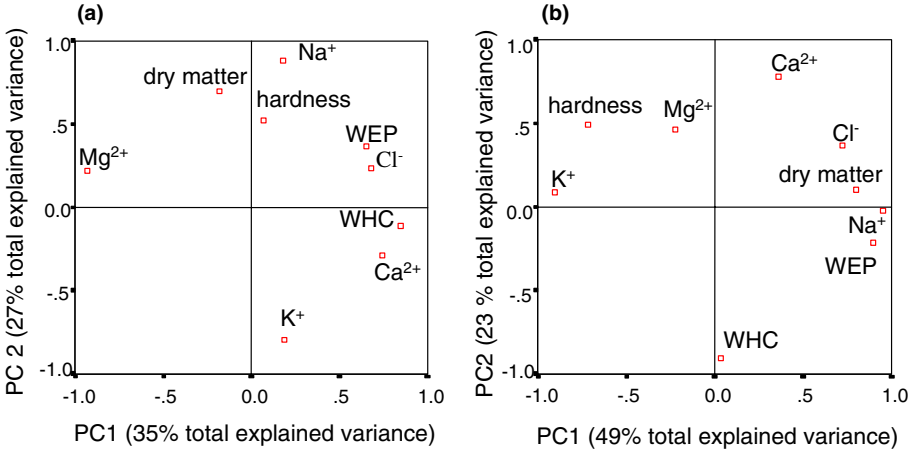

Fig. 1. Principal component analysis of ion concentrations and different variables analysed in cod brined at pH 6.5 (a) or pH 8.5 (b).

fish. The aim here was to identify significant differences that might have been overlooked previously in simple ANOVA. These further analyses were performed separately for samples brined at pH 6.5 and at pH 8.5; the corresponding principal component analysis (PCA) data matrix is shown in Fig. 1.

In the case of samples brined at pH 6.5, the global data matrix was reduced to 6 principal components (PC); of these, the figure shows only the first two, which together account for 62% of the total explained variance (Fig. 1(a)). The analysis of PC1 showed that WHC and WEP correlated positively with muscle salt content (Cl^-). At the same time, these three parameters correlated positively with the residual Ca^{2+} concentration and inversely with the Mg^{2+} concentration. These results suggest that Mg^{2+} hindered salt penetration in the muscle, hence negatively influencing WEP and WHC (Kolodziejska & Sikorski, 1980; Stefansson & Hultin, 1994). The concentration of $MgCl_2$ (0.4% in the brine) was high in terms of the studies reported by Xiong & Brekke (1991), who found that this salt only had a solubilising effect at very low concentrations, below 0.05%. According to Von Hippel & Wong (1964), the increased instability of protein conformation produced by the size of the cation could be sufficient to account for its effect on the myofibrillar proteins. However, given that the Ca^{2+} cation is larger than the Mg^{2+}, the electronegativity of the cation cannot be ruled out as a cause. The Mg^{2+} cation is the most electronegative of all those assayed; this means that it can bind more strongly to the protein polar groups, thus strengthening protein interactions (Xiong & Brekke, 1991) and increasing aggregation at increasing concentrations (Nayak, Kenney, & Slider, 1996).

Na^+ and K^+ ion concentrations, which correlate inversely with one another, are essentially reflected by PC2; they correlate chiefly with dry matter content, and to a lesser extent, with hardness and WEP. Thus, when the sodium content was reduced and the potassium content increased, the overall tendency was for the product to be less dehydrated; in other words, moisture was higher but hardness and WEP were lower. Loss of hardness could be a direct consequence of increased moisture, while reduced WEP could be associated with higher K^+ content. KCl has been shown to decrease protein solubility (Grishchenko, 1958; Kolodziejska & Sikorski, 1980), possibly because KCl has a greater capacity to aggregate myosin (Thorarinsdottir, Arason, Geirsdottir, Bogason, & Kristbergsson, 2002).

In pH 8.5 brine samples, multivariate analysis again produced a data matrix of 6 principal components, of which the first two explained 72% of the total variance (Fig. 1(b)). PC1 showed a high positive correlation between salt content and WEP, as in the case of samples brined at pH 6.5. The relationship between WEP and penetration of salts in the muscle has also been reported in similar studies by Thorarinsdottir et al. (2004). However, unlike sample brined at pH 6.5, where salt content and WEP correlated strongly with the concentration of divalent cations, in samples brined at pH 8.5 salt content and WEP correlated more strongly with variations in Na^+ content (positively) and K^+ (negatively). As in the pH 6.5 sample, reduction of the Na^+ content and the consequent increase of K^+ content made for a more hydrated product (lower dry matter) with lower WEP. In this case there was some tendency for muscle hardness to increase due to increased potassium content, possibly as a result of the protein aggregation effect of KCl noted above. Frye, Hand, Calkins, & Mandigo (1986) also reported increased hardness in ham as a result of salting in the presence of KCl. Again, as in the case of sample brined at pH 6.5, the effect of divalent cations was independent of that of monovalent ions; this is chiefly apparent in the second PC (PC2), where they present a significant negative correlation with WHC. At pH 8.5, the concentrations of both divalent cations correlated

positively with each other, unlike the case of pH 6.5 brines, which indicates that the salts were better able to penetrate the muscle together at alkaline pH.

Finally, discriminant analysis was performed to determine which of the target properties most clearly distinguish the influence of the treatment on the final product. Three types of discriminant analysis were conducted: (i) as a function of pH; (ii) as a function of the absence or presence of KCl, at pH 6.5 and 8.5; and (iii) as a function of the absence or presence of $CaCl_2$ and/or $MgCl_2$.

Statistical analysis, based on the pH of the treatment, produced a single discriminant canonical function (canonical correlation: 0.773) which allowed correct classification of 87.5% of the cases in the original grouping. According to the correlations established between the target variables and the discriminant canonical function, WHC and WEP were the characteristics that best differentiated between samples brined at pH 6.5 and samples brined at pH 8.5. This shows that, despite the buffering effect of the muscle, the increase of pH in the early stages of brining at pH 8.5 influenced the functional properties of the myofibrillar proteins to some extent. It is possible that, in the early stages of brining, the basic pH induces unfolding of the myofibrillar proteins, and therefore these proteins are not as folded as they would be in cod brined at pH 6.5 (Martínez-Alvarez, 2003). This has also been reported in connection with salting of vegetables in basic brines (Hye-Hyun-Yoon, Eun-Jae-Jeon, Soon-Jung-Sung, & Dong-Man-Kim, 2000a, 2000b).

Analysis as a function of the presence KCl at both pHs produced three discriminant canonical functions (canonical correlations: 0.992, 0.785, 0.764), of which the first explained 95.5% of variance and the second 2.4%. Ninety-three point eight per cent (93.8%) of the cases in the initial grouping were correctly classified. As Fig. 2 shows, the first canonical function (CF1) clearly differentiated between the groups brined in the presence of KCl and those brined without KCl; the principal discriminant variables were the residual concentrations of sodium ions and potassium ions. The second discriminant function, which is of minor importance, tended to separate the groups according to the initial pH, but in different ways depending on whether or not the brine contained KCl.

Discriminant analysis, as a function of the divalent salt concentrations in the brine ($CaCl_2$ and/or $MgCl_2$), produced three canonical functions: the first explained 98.7% of total variance, with canonical correlations of 0.971 and 0.948, respectively (Fig. 3). All of the cases in the original grouping were correctly classified. The first canonical function (CF1, 66% explained variance) clearly distinguished lots brined without divalent ions from those brined with both together; the principal discriminant variable was magnesium content, followed by

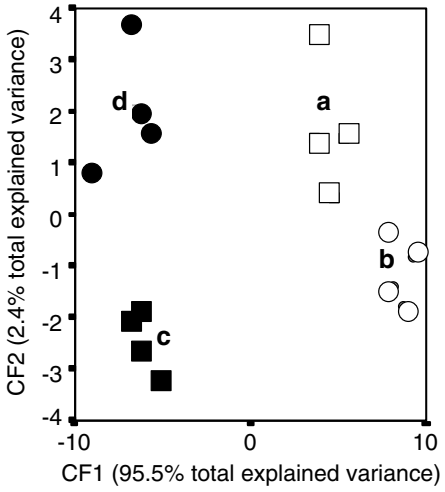

Fig. 2. Discriminant analysis of different variables analysed in brined cod as a function of the absence or presence of KCl in brines with two different pH levels (6.5 and 8.5). (a) pH 6.5 without KCl; (b) pH 8.5 without KCl; (c) pH 6.5 with KCl; (d) pH 8.5 with KCl.

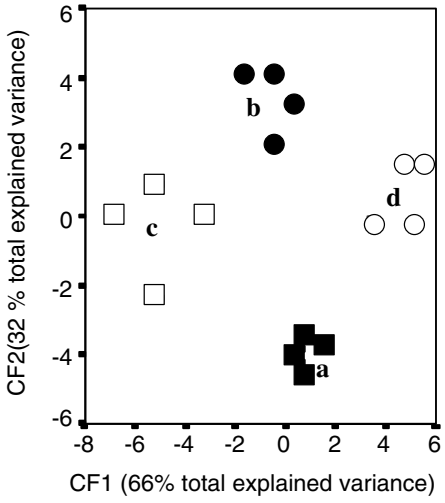

Fig. 3. Discriminant analysis of the different variables analysed as a function of the divalent salt concentrations in the brines. (a) With Ca; (b) with Mg; (c) with Ca and Mg; (d) without Ca and Mg.

calcium content and WEP. The second canonical function (CF2, 32% variance) preferentially distinguished between samples brined with $MgCl_2$ and samples brined with $CaCl_2$. Here again, the principal discriminant variables were magnesium and calcium content, followed in this case by hardness.

4. Conclusion

The results of this study confirm the influence of the pH and the mix of salts in the brine on various compositional and protein functionality characteristics of brined muscle. The replacement of around 50% NaCl

by KCl in brines considerably reduced the sodium content of the product. In pH 6.5 brines, the presence of KCl did not alter major functional properties to any great extent. However, when brine pH was 8.5, KCl negatively affected protein water-extractability, increasing hardness. Addition of small amounts of divalent salts ($CaCl_2$, $MgCl_2$) to the brines could help to significantly reduce the penetration of sodium ions into the muscle, provided that salting was carried out with pH 6.5 brines. The presence of magnesium in pH 6.5 brines tended to hinder the general penetration of chlorides into the muscle, thus negatively affecting water-holding capacity and water-extractable protein.

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