Influence of mono- and divalent salts on water loss and properties of dry salted cod fillets

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ABSTRACT

Salted cod is a product highly appreciated by consumers, especially in Southern Europe and Latin America. In recent years there has been increasing consumer demand for products with low sodium content, and this has led the salting industry to seek new salt mixtures to help to reduce Na^+ levels without producing alterations in the properties of the final product. In this study, Atlantic cod (*Gadus morhua*) was initially brined with various mixtures of salts based on NaCl, at various pH levels and including KCl, MgCl_2 and/or CaCl_2 . After subsequent dry salting with NaCl, the Na^+ , K^+ , Ca^{2+} and Mg^{2+} cation contents were evaluated, and also their effect on water loss, salt uptake, entry of chloride, water holding capacity (WHC), water extractable protein (WEP) and hardness of the end product. A greater presence of K^+ in muscle was associated with greater water loss, salt uptake and hardness of the dry salted product. Moreover, incorporation of Ca^{2+} and Mg^{2+}

negatively affected water holding capacity. These changes were not dependent on brine pH and might significantly alter the acceptability of the final product.

Keywords: Salted cod, Brining, Divalent salts, Potassium chloride, Water holding capacity

1. Introduction

Salting played a fundamental part in human nutrition in the course of history until well into the twentieth century. Initially, the aim of salting was the preservation of fish. Now, despite the development of other preservation methods, its low production cost, the simplicity of the process and demand for the product have meant that salted products, and in particular salted fish (especially cod) continue to be sold in high quantities. Nowadays, the main countries that produce salted cod are Iceland and Norway (Thorarinsdóttir, Bjørkevold, & Arason, 2010b), whereas the main consuming countries are those of southern Europe (mainly Portugal and Spain) and some Latin American countries, such as Brazil.

The typical salting process is dry salting or “kench salting”, where the fish is filleted or “butterfly” split and stacked with alternating layers of salt (Thorarinsdóttir, Arason, Bogason, & Kristbergsson, 2004). In recent years, presalting followed by dry salting has become the most popular production method (Thorarinsdóttir et al., 2004; Martínez-Alvarez & Gómez-Guillén, 2006; Brás & Costa, 2010; Gudjónsdóttir, Arason, & Rustad, 2011). The aim of presalting is to increase both the water holding capacity and the yield of the salted product. Presalting can be carried out by brine injection and/or immersion in brine (Thorarinsdóttir, Arason, Thorkelsson, Sigurgisladóttir, & Tornberg,

2010a; Thorarinsdottir, Arason, Sigurgisladottir, Valsdottir, & Tornberg, 2011). Salting by injection consists in injecting a saline solution into the muscle, thus reducing the salting time, which in turn favours rapid, homogeneous distribution of salt in the muscle. Brining, on the other hand, consists in immersing the fish for 1–4 days in a solution of salt and water (brine). The final product, lightly salted cod (approx. 2 g NaCl /100 g of product), can be marketed as it is (Lauzon, Magnússon, Sveinsdóttir, Gudjónsdóttir, & Martinsdóttir, 2009; Gudjonsdottir et al., 2010; Thorarinsdóttir et al., 2010b), although usually it is then dry salted, losing water and gaining salt until an equilibrium is reached, and at the same time acquiring its characteristic organoleptic characteristics. The dry salted product may be subjected to a drying process prior to distribution, finally being rehydrated by the consumer before consumption. The advantages of a prior brining step are that the final product is less hard (Brás & Costa, 2010), salting is quicker and the yield is greater (Thorarinsdottir et al., 2004; Brás & Costa, 2010), although during the first 4–5 days of brining there is a loss of water accompanied by soluble protein (Del Valle & Nickerson, 1967; Martínez-Alvarez & Gómez-Guillén, 2005).

During brining there is an increase in the salt content of the muscle, owing to diffusion of the NaCl present in the brine solution. There is also diffusion of water into the brine, brought about by the difference in NaCl concentration between the muscle and the surrounding brine/salt (Thorarinsdottir et al., 2010a). When the salt concentration in the muscle is slightly higher than the physiological ionic strength (>0.15 M), the inter-fibrillar spaces become larger owing to electrostatic shielding effects from salt ions binding to charged parts of the filaments, increasing the water absorption capacity of the proteins (Offer & Trinick, 1983; Offer et al., 1989; Barat, Rodríguez-Barona, Andrés, & Fito,

2002). At concentrations >0.5 M, the thick filaments depolymerise, which leads to a significant swelling of myofibrils, reaching a maximum at salt concentrations of 0.8–1 M, in a process known as “salting in” (Offer & Knight, 1988; Fennema, 1990). At higher concentrations (>1 M), the muscle proteins begin to aggregate irreversibly, and this causes a lateral contraction of the myofibrillar compartment. As a result, the muscle hardens and retracts. The water holding capacity of the cells decreases and the muscle begins to lose water, in a process known as “salting out” (Kelleher & Hultin, 1991; Stefansson & Hultin, 1994). During the dry salting process, the entry of salt into the muscle increases as a result of a diffusion mechanism, and extraction of water from the tissues increases, which leads to dehydration of the fish, resulting from the greater difference in concentrations between the sample and the medium, the pressure effect due to the weight of the salt, and the capillary effect in the salt bed (Barat, Rodríguez-Barona, Andrés, & Fito, 2003). This water loss affects the conformation of muscle proteins, causing denaturation and aggregation, and therefore changes in functional properties.

In recent years, the relationship between excessive consumption of sodium and hypertension (Winter, Tuttle & Viera, 2013) has increased demand for low-salt products, and this has encouraged the food industry to reduce the sodium content in processed products. The alternatives to salting of fish include partial substitution of NaCl by KCl, the incorporation of other salts such as calcium chloride or magnesium chloride, or the use of salt solutions with a different pH during brining (Aliño, Fuentes, Fernández-Segovia, & Barat, 2011; Lee, 2011; Braschi, Gill, & Naismith, 2009; Rodrigues, Ho, López-Caballero, Bandarra, & Nunes, 2005; Martínez-Alvarez, Borderías, & Gómez-Guillén, 2005). However, the incorporation of KCl or divalent salts in the salt mixtures used could cause

93 changes in the sensory properties of the product (Gelabert, Gou, Guerrero, & Arnau, 2003;
94 Lauritzsen & Akse, 1995; Reddy & Marth, 1991; Arganosa & Marriott, 1990). Also, these
95 salts could alter the functional properties of muscle proteins (Kinsella, 1982; Morrissey,
96 Mulvihill, & O'Neill, 1987), affecting the quality of the final product to a greater or lesser
97 extent. Thus, the replacement of NaCl with other salts such as KCl, MgCl₂ or CaCl₂ may
98 have a negative effect on water holding capacity (Weinberg, Regenstein, & Baker, 1984),
99 and/or on texture (Martínez-Alvarez et al., 2005). The effect of salts on the conformational
100 stability and solubility of proteins is a strong function of the ionic species present (Zhang
101 & Cremer, 2006; Baldwin, 1996). Thus, interactions of protein with water are correlated
102 with the size of the hydrated radius of the ion, so that the greater the radius, the greater the
103 dehydration caused in the protein and vice versa. It is likely that these effects are
104 influenced by surface load intensity, which is in turn influenced by atomic radius
105 (Kinsella, 1982; Eagland, 1975). In general, in accordance with the Hofmeister series,
106 cations determine effectiveness in protein hydration, at a given (low) ionic strength, in the
107 following decreasing order: $\text{Ca}^{2+} > \text{Mg}^{2+} > \text{Li}^{+} > \text{Cs}^{+} > \text{Na}^{+} > \text{K}^{+} > \text{NH}_4^{+}$ (Baldwin, 1996;
108 Morrissey et al., 1987; Von Hippel & Wong, 1964). During the salting of the fish, the
109 entry of salts causes conformational changes in the proteins, which give rise to changes in
110 solubility to a greater or lesser extent, depending on the salts used, the pH of the brine and
111 the degree of salting achieved, among other factors. Furthermore, the pH of the medium
112 can also cause changes in the functional properties of the muscle proteins in the salted
113 product. Alteration of the net load of the protein molecule may increase or reduce protein–
114 water and protein–protein interactions (Stefansson & Hultin, 1994), consequently
115 affecting protein functionality (Lauritzsen, Akse, Gundersen, & Olsen, 2004).

The aim of this study was to relate muscle Na^+ , K^+ , Ca^{2+} and Mg^{2+} contents with parameters such as water loss, salt uptake and chloride content, and with physical and physicochemical properties, such as hardness, water-holding capacity (WHC) and water-extractability protein (WEP) of dry salted cod fillets (*Gadus morhua*).

2. Materials and methods

Following capture off the coast of Iceland by a commercial fishing boat, 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 hours: fish/brine ratio 1/1.4, temperature 4 °C. The salt concentration in all brines was the same (18 g of salt in 100 mL of water). The 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 the brines was adjusted to the level shown in Table 1 by addition of citric acid (19.2 g/L) or sodium hydroxide (4 g/L). 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) dehydrated were supplied by Merck.

The fillets were then dry salted for 25 days by covering in Torrevieja salt (99.4 g NaCl/100 g of Torrevieja salt, max. 0.1 g Ca^{2+} /100 g, max. 0.09 g Mg^{2+} /100 g, max. 0.45 g sulphate/100 g) from Unión Salinera de España (Barcelona, Spain). The temperature throughout the process was 4 °C. The fillets were then shipped to Spain by refrigerated

transport. The final water content of the dry salted samples was 53.82–57.46 g/100 g of sample. Protein content was 20.88–23.66 g/100 g, and ash content was 19.69–23.08 g/100 g. The dorsal part of each skinless fillet was chopped into 150–200 gram portions (approximate dimensions: length 9 ± 2 cm, width 5 ± 1 cm, and thickness 3 ± 1 cm). All these portions were then mixed together and stored at low temperature until used.

2.1. Determination of water loss

Moisture of samples before and after brining or dry salting was firstly determined on approximately 5 g of minced muscle, by oven drying at 110 °C to constant weight, following technique 950.46 (AOAC, 2000). Results were expressed as water loss (g/100 g) in brined (WLb) and salted (WLs) fillets.

$WLb = Mb - Mr$, where Mb = Moisture of brined sample and Mr = Moisture of raw sample.

$WLs = Ms - Mb$, where Ms = Moisture of dry salted sample and Mb = Moisture of brined sample.

2.2. Determination of ions: Sodium, potassium, calcium and magnesium

Approximately 5 g of muscle was reduced to ashes and homogenised in 5 mL suprapure nitric acid diluted with Milli-Q water (final concentration of 30 mL/100 mL of water). Samples were topped 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.

2.3. Determination of chloride content

Five g of minced muscle was homogenised with 100 mL of nitric acid (1 mL/100 mL of water) in an Omnimixer-Homogenizer (model 17106, OMNI International, Waterbury, USA). Sample was then 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/100 g of muscle.

2.4. Determination of salt uptake

Ash content of each sample was determined following AOAC 923.03 (AOAC, 2000). Results were expressed as salt uptake (g/100 g) in brined (SUB) and salted (SUs) fillets.

$SUB = S_b - S_r$, where S_b = Ash content in brined sample and S_r = Ash content in raw sample.

$SUs = S_s - S_b$, where S_s = Ash content of dry salted sample and S_b = Ash content of brined sample.

2.5. Water holding capacity, Water extractable protein and Shear strength (hardness)

WHC, WEP in distilled water and shear strength of salted samples were determined as described in Martínez-Alvarez and Gómez-Guillén (2006).

For WHC, 2 g of chopped cod muscle were placed in a centrifuge tube with 3 dried pipette filters (Gilson, Viliers-le bel, France), previously weighed, to act as absorbents. Each sample was centrifuged for 10 min at 6000g (Sorvall, RT6000B; Du-Pont Co., Wilmington, Del., USA) at room temperature, and the pipette filters were further weighed. Results were means of three determinations and were expressed as g of water retained per g of muscle protein.

WEP 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 were homogenized at low temperature in 50 mL of distilled water. The homogenates of these solutions were stirred constantly for 30 min at 2 °C then centrifuged (6000g) for 30 min (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), and the standard curve was determined with various known concentrations of bovine serum albumin (BSA). Results were means of three determinations and were expressed as g of soluble protein per g of total protein in muscle. 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 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 were means of three determinations and were expressed as Newtons per g of muscle at the point of maximum load before sample breaking.

2.6. Statistical analyses

The significance of differences between mean value pairs was evaluated using one-way ANOVA. Tukey HSD test was used to identify significant differences among main effects. The level of significance setting was $P \leq 0.05$. The data from the different variables analysed in dry salted cod were used as the data matrix in a principal component analysis (PCA). Further discriminant analyses were also performed. All statistical analyses were performed with Statgraphics Plus software.

3. Results

The brining process caused a water loss of approximately 2–5 g/100 g of muscle. This water loss was dependent on the pH of the brine, and also on the combination of salts used, in agreement with what has been reported previously (Martínez-Alvarez et al., 2005). The dry salting process also caused a decrease in the water content in all the samples, losses reaching approximately 20–23 g/100 g with respect to the brined cod (Figure 1). This decrease in the water content has been explained by a loss of myofibril water holding capacity owing to salting out of proteins (Offer & Trinick, 1983; Xiong, 2005). The pH of the brine used for brining did not affect the water loss during the dry salting process to a greater or lesser extent. However, the incorporation of KCl, MgCl₂ and/or CaCl₂ in the brines caused a significantly ($p \leq 0.05$) greater loss of constituent water in most cases, the most pronounced changes being observed at pH 6.5 (Fig. 1a).

Brining produced a noticeable increase in salt uptake, in some cases close to 8 % with respect to the unsalted cod (Figure 2). This increase was associated with the increase in chloride content, as reported previously (Martínez-Alvarez et al., 2005). The subsequent dry salting process caused a significant increase in salt uptake, especially in the samples

brined at pH 6.5, owing to the massive entry of NaCl into the muscle. The incorporation of KCl, MgCl₂ and/or CaCl₂ in the pH 6.5 brines was associated with a significant increase in salt uptake after dry salting. In the case of the dry salted cod previously brined at pH 8.5, replacement of part of the NaCl in the brines with KCl caused an increase in salt uptake, significant in some cases ($p \leq 0.05$).

The dry salting process noticeably increased the muscle chloride content, reaching values of 16.90–19.87 g/100 g of sample (Table 1). In most cases, the muscle salt content was higher in the samples previously brined at pH 6.5. The differences attributable to pH were significant when the brining was done with NaCl and CaCl₂ or else with NaCl, KCl and CaCl₂ (Table 1). However, when the wet salting was done with mixtures that included MgCl₂, the salt content was almost always less at pH 8.5, although this behaviour might be due to the lesser degree of salting of these samples after brining.

The Na⁺, K⁺, Ca²⁺ and Mg²⁺ ion contents (Table 1) increased strikingly in comparison with the contents reported for brined cod (Martínez-Alvarez et al., 2005). In general, dry salting with common salt from Torrevieja led to an increase of 40 -75 % in Na⁺ content, which was attributed not only to the treatment with that salt, which is very rich in NaCl, but also to the evident dehydration that the muscle had undergone. The muscle Na⁺ content, very variable in the case of brined cod (Martínez-Alvarez et al., 2005), was fairly uniform after dry salting, although a smaller incorporation of Na⁺ was observed when NaCl was partially replaced with KCl in the preliminary brining. This decrease, however, was not statistically significant.

The K⁺ content was higher in the samples previously salted with brines that included KCl. This is important, since an increase in potassium intake has been associated

with a decrease in arterial tension (Martin & Fischer, 2012; He & MacGregor, 2008). In general, the cod previously brined at pH 6.5 with mixtures that included KCl gave a final product with a lower K^+ content in comparison with the cod previously brined at pH 8.5 (Table 1), these differences being significant ($p \leq 0.05$) in all the cases in which they were present, accompanying KCl, $CaCl_2$ and/or $MgCl_2$.

In the samples previously brined with mixtures that included $CaCl_2$ there was generally a greater muscle Ca^{2+} content, as observed in the samples brined with the same salt mixtures (Martínez-Alvarez et al., 2005), which might indicate that in general the interactions of the cation with the muscle protein were maintained during the dry salting. However, the differences in comparison with the samples brined without $CaCl_2$ were not always statistically significant (Table 1). The quantity of Ca^{2+} present in the dry salted muscle also did not produce significant differences as a function of the pH of the brine used. In most cases the Mg^{2+} content was higher in the dry salted samples previously brined with mixtures that included $MgCl_2$ (Table 1). This fact was also observed in the brined muscle before salting (Martínez-Alvarez et al., 2005), although these authors did not describe the increment in Mg^{2+} content in muscle as significant, and shows that the differences observed in the Mg^{2+} content in muscle were maintained after the dry salting. The Mg^{2+} content was significantly higher ($p \leq 0.05$) in the case of the samples previously brined with mixtures with $MgCl_2$ and without $CaCl_2$ at pH 6.5. This fact was not observed before salting (Martínez-Alvarez et al., 2005), and suggests that the presence of Ca^{2+} in muscle negatively affected retention of Mg^{2+} in muscle during the dry salting. In contrast, the incorporation of $CaCl_2$ in basic brines including $MgCl_2$ resulted in a higher retention

of Mg^{2+} in dry salted muscle, being this effect significant when both divalent salts were accompanied by KCl and NaCl.

The composition of the brines used for the salting prior to dry salting caused substantial changes in the functional properties of the muscle proteins in the dry product, expressed as water holding capacity and water extractable protein, as discussed in a previous paper (Martínez-Alvarez & Gómez-Guillén, 2006). In that study there is also a description of the distribution of molecular weights in the soluble fraction, and the differences observed in the shear strength (hardness) of the samples.

4. Discussion

In a previous study we showed that the brining process at various pH levels and with various combinations of salts conditioned the quality of the brined product (Martínez-Alvarez et al., 2005). Although the product may then be ready for consumption, the brining step is normally accompanied by dry salting with NaCl. In another study (Martínez-Alvarez & Gómez-Guillén, 2006), we reported that the composition of the brines can produce substantial changes in the functional properties of the muscle protein of dry salted cod, especially in its composition. In the present study, we have shown that the main cations (Na^+ , K^+ , Ca^{2+} and Mg^{2+}) employed during the preliminary brining process are incorporated in the muscle, and that they affect the water loss and salt uptake of the cod after dry salting. Equally, the incorporation of these cations in the muscle may affect the functional properties of the muscle protein, and the hardness, to a greater or lesser extent after dry salting.

The obtained results were subjected to a principal component analysis of factors. These analyses were performed separately for samples previously brined at pH 6.5 and at pH 8.5. The aim here was to identify significant differences that might have been overlooked previously in simple ANOVA. From these analyses, it was possible to gain a better understanding of the possible relationships of the muscle cation contents with both the salt uptake and the water loss produced, and also with the alterations in the functional properties of the muscle protein produced during the dry salting. The corresponding principal component analyses (PCA) data matrix are shown in Fig. 3 and 4.

In the case of the cod previously salted at pH 6.5, four principal components (PCs) were extracted. Together they account for 89.9 % of the variability in the original data. Of these factors, Figure 3 shows only the first two, which together account for 68.9 % of the total explained variance.

Factor analysis (Figure 3a and Table 2a) showed that the contents in muscle of various cations correlated strongly with water loss and salt uptake, and also with the functional properties and hardness of the dry salted cod. The first component (PC 1), which explained 45.3 % of the total variance, showed an inverse relationship between muscle Na^+ content and water loss. In other words, a greater muscle Na^+ content, within values of 67–75 mg/g of Na, was associated with a smaller constituent water loss. Equally, Na^+ content correlated negatively with hardness and salt uptake. These last two components correlated positively, given that the salting (Barat et al., 2002) and drying (Lauritzsen et al., 2004) steps are known to increase hardness, as well as protein denaturation.

Muscle K^+ content correlated negatively with muscle Na^+ content, and positively with the hardness of the dry salted product. This result has also been described in ham (Aliño, Grau, Fuentes, & Barat, 2010a; Aliño, Grau, Toldrá, & Barat, 2010b) and is probably the result of greater water loss and poor functional properties of the muscle protein (Martínez-Alvarez et al., 2005; Brás & Costa, 2010), this latter characteristic being the result of the greater capacity of K^+ , with respect to Na^+ , to change the native protein–water structure (Kolodziejaska & Sikorski, 1980). Interestingly, in a previous study we showed that the effect of KCl on the hardness of brined cod was the opposite (Martínez-Alvarez et al., 2005), which seems to indicate that subsequent salting with NaCl counteracts this effect. Muscle K^+ content correlated positively with salt uptake. Moreover, muscle Mg^{2+} content, and to a lesser extent muscle Ca^{2+} content, also correlated positively with salt uptake, and negatively with Na^+ content, possibly because of the capacity of divalent salts to reduce the penetration of NaCl (Iyengar & Sen, 1970). Moreover, the Ca^{2+} and Mg^{2+} contents correlated negatively with WHC. These cations reduce the hydrating capacity of proteins because they cause compacting of the structure, forming bridges between the peptide chains of a given component (actin, myosin) or else between chains of actin and myosin (Stanley & Hultin, 1968; Haurowitz, 1950). Equally, in the first component the factor analysis also showed a direct correlation between hardness and water loss, similar to what was observed in brined cod at the same pH prior to dry salting (Martínez-Alvarez et al., 2005).

In the second component (PC 2), which explained approximately 24 % of the variance, it was seen that chloride content and K^+ cation content correlated inversely with WEP (Figure 3a and Table 2a). The massive entry of chloride into the muscle during dry

salting must have caused contraction of myofibrils (Offer & Knight, 1988) and dehydration of proteins, causing them to be in a highly aggregated state owing to salting out (Thorarinsdottir et al., 2011; Thorarinsdottir et al., 2010a; Stefansson & Hultin, 1994; Kelleher & Hultin, 1991; Offer & Trinick, 1988). This highly aggregated state is probably responsible for the lower solubility of the protein in the studies conducted. However, it is important to emphasise that after brining but before dry salting a positive correlation was observed between chloride content and WEP (Martínez-Alvarez et al., 2005), which might be because the chloride concentration in the muscle that had only been brined was low, and not high enough to cause protein denaturation, resulting in cross-linking between proteins increasing shrinkage and consequently loss of water from the muscle. Finally, the second component also showed a negative correlation between water loss and WEP, which could be explained by the fact that the water released during dry salting might have been accompanied by soluble protein.

Figure 3b shows the principal component analysis for the samples brined at pH 8.5 and then dry salted with NaCl. Four principal components (PCs) were extracted, together accounting for 87.6 % of the variability in the original data. The contents in muscle of the various cations analysed also caused significant changes in water loss and salt uptake, and in functionality of the muscle protein after dry salting.

The first component (PC 1), which explains 43.4 % of the total variance, showed a strong direct correlation between Na^+ content and chloride content, and an inverse correlation between Na^+ content and hardness, water loss, salt uptake, K^+ Ca^{2+} and Mg^{2+} contents, similar to what was observed in the samples previously brined at pH 6.5 (Figure 3b and Table 2b). An inverse correlation between K^+ and chloride content was also

observed, similar to what was observed in cod that had only been brined (Martínez-Alvarez et al., 2005), and so it seems that this effect was maintained during the dry salting. Moreover, K^+ content, muscle hardness and salt uptake were positively correlated, as previously observed in Fig. 3a.

The second component (PC 2), which explains 20.9 % of the total variation, showed an inverse correlation between WHC and Ca^{2+} and Mg^{2+} contents (Figure 3b and Table 2b), similar to that observed in dry salted cod previously brined at pH 6.5. It is also important to note the slight direct correlation between divalent cation content and WEP, and the absence of a clear relation between WEP and hardness. Better functional quality of muscle protein is usually associated with less hardness, but this relationship was not reflected in the cod previously brined at pH 8.5. In this case, greater hardness seems to be related more with salt uptake, as the first component showed. It is also important to emphasise that the loss of constituent water was not correlated with a loss of water extractable protein. The reason could be the physical and chemical nature of the muscle surface of these samples, which may affect drainage of soluble protein during the salting process (Lauritzsen et al., 2004).

Finally, discriminant analyses were performed to confirm the effect of the ion contents on the properties of the final product. Two kinds of discriminant analysis were performed: (i) as a function of the absence or presence of KCl at pH 6.5 or pH 8.5; (ii) as a function of the absence or presence of $CaCl_2$ and/or $MgCl_2$.

Analysis as a function of the presence of KCl at both pHs (Figure 4) produced three canonical discriminant functions (canonical correlations: 0.998, 0.813 and 0.711),

the first of which explained 99.04 % of the variance and was statistically significant at the 95.00 % confidence level. One hundred per cent of the cases were correctly classified.

The first component (99.04 % of total explained variance) clearly differentiated between the groups previously brined in the presence of KCl and those brined without KCl. The second canonical function was of minor importance and tended to separate the groups brined in the presence of KCl according to the pH of the brine. Therefore, this analysis clearly shows the influence of the incorporation of KCl into the brines on the functional and compositional characteristics of the cod after subsequent dry salting. This effect has been clearly shown in brined cod (Martínez-Alvarez et al., 2005) and demonstrates that the global effect of KCl on brined cod cannot be reverted by a dry salting process with NaCl.

Analysis as a function of the presence/absence of divalent salts in the brines (Figure 5) produced three canonical discriminant functions (canonical correlations: 0.980, 0.954 and 0.769), the first of which explained 67.82 % of the variance and was statistically significant at the 95.00 % confidence level. One hundred per cent of the cases were correctly classified.

The first canonical function (67.82 % of total explained variance) clearly differentiated the samples previously brined with mixtures including CaCl_2 from the ones brined with mixtures that included MgCl_2 as the only divalent salt (Figure 5). This helps to confirm that the effect of the two divalent salts is different. Furthermore, the second canonical function (28.19 % of total explained variance) distinguished the samples brined with mixtures including both divalent salts (CaCl_2 and MgCl_2) from the other samples. As shown before, this effect was observed in brined cod (Martínez-Alvarez et al., 2005) and

demonstrates that the incorporation of MgCl_2 and CaCl_2 together into the brines also affects the characteristics of cod after subsequent dry salting with NaCl . However, it can be concluded that dry salting with NaCl seems partly to counteract the clear differences in the characteristics of brined cod, already described in a previous paper (Martínez-Alvarez et al., 2005), attributable to the presence or absence of divalent salts in the brines.

5. Conclusions

To sum up, this study confirms that salt uptake, water loss and functional properties of myofibrillar proteins of salted cod may be influenced by the content in muscle of cations derived from the salt mixtures included in the brines used for presalting. Thus, a greater presence of K^+ in muscle is associated with an increase in the hardness of the product, and with an increase in salt uptake, especially if the pH of the brine used previously was 8.5. Its incorporation also causes a decrease in the muscle sodium content in the dry salted product. Equally, the incorporation of small quantities of CaCl_2 and MgCl_2 in the brines used previously seems to give rise to a greater constituent water loss and a smaller entry of Na^+ into the muscle, and also has an effect on salt uptake, differing as a function of the pH of the brine used previously.

Acknowledgments

This research was supported by the European Union under project FAIR-CRAFT CT 98-9109. We wish to thank Dr. S. Sigurgisladottir (ICETEC, Reykjavik, Iceland) and Dr G. Stefánsson (SIF, Ltd., Fjargardata, Iceland) for supplying the salted cod fillets.

References

- Aliño, M., Fuentes, A., Fernández-Segovia, I., & Barat, J. M. (2011). Development of a low-sodium ready-to-eat desalted cod. *Journal of Food Engineering*, 107(3–4), 304–310.
- Aliño, M., Grau, R., Fuentes, A., & Barat, J. M. (2010a). Characterisation of pile salting with sodium replaced mixtures of salts in dry-cured loin manufacture. *Journal of Food Engineering*, 97(3), 434–439.
- Aliño, M., Grau, R., Toldrá, F., & Barat, J. M. (2010b). Physicochemical changes in dry-cured hams salted with potassium, calcium and magnesium chloride as a partial replacement for sodium chloride. *Meat Science*, 86(2), 331–336.
- AOAC (2000). *Official Methods of Analysis* (17th ed.). Gaithersburg, Maryland: 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.
- Baldwin, R. L. (1996). How Hofmeister ion interactions affect protein stability. *Biophysical Journal*, 71(4), 2056–2063.
- Barat, J. M., Rodríguez-Barona, S., Andrés, A., & Fito, P. (2003). Cod salting manufacturing analysis. *Food Research International*, 36(5), 447–453
- Barat, J. M., Rodríguez-Barona, S., Andrés, A., & Fito, P. (2002). Influence of increasing brine concentration in the cod-salting process. *Journal of Food Science*, 67(5), 1922–1925.

455 Brás, A., & Costa, R. (2010). Influence of brine salting prior to pickle salting in the
 456 manufacturing of various salted-dried fish species. *Journal of Food Engineering*,
 457 100(3), 490–495.

458 Braschi, A., Gill, L., & Naismith, D. J. (2009). Partial substitution of sodium with
 459 potassium in white bread: Feasibility and bioavailability. *International Journal of*
 460 *Food Sciences and Nutrition*, 60(6), 507–521.

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

463 Eagland, D. (1975). Protein hydration. Its role in stabilizing the helix conformation of the
 464 protein. In Duckworth, R. B., *Water Relations of Foods* (pp. 73–80). New York:
 465 Academic Press.

466 Fennema, O. R. (1990). Comparative water holding properties of various muscle foods. A
 467 critical review relating to definitions, methods of measurement, governing factors,
 468 comparative data and mechanistic matters. *Journal of Muscle Foods*, 1, 363–381.

469 Gelabert, J., Gou, P., Guerrero, L., & Arnau, J. (2003). Effect of sodium chloride
 470 replacement on some characteristics of fermented sausages. *Meat Science*, 65,
 471 833–839.

472 Gudjónsdóttir, M., Arason, S., & Rustad, T. (2011). The effects of pre-salting methods on
 473 water distribution and protein denaturation of dry salted and rehydrated cod – A
 474 low-field NMR study. *Journal of Food Engineering*, 104(1), 23–29.

475 Gudjonsdottir, M., Gunnlaugsson, V. N., Finnbogadottir, G. A., Sveinsdottir, K.,
 476 Magnusson, H., Arason, S., & Rustad, T. (2010). Process control of lightly salted

477 wild and farmed Atlantic cod (*Gadus morhua*) by brine injection, brining, and
 478 freezing – A low field NMR study. *Journal of Food Science*, 75(8), E527–E536.

479 Haurowitz, F. (1950). *Chemistry and Biology of Proteins* (p. 86). New York: Academic
 480 Press, New York.

481 He, F. J., & MacGregor, G. A. (2008). Beneficial effects of potassium on human health.
 482 *Physiologia Plantarum*, 133(4), 725–735.

483 Iyengar, J. R., & Sen, D. P. (1970). The Equilibrium Relative Humidity Relationship of
 484 Salted Fish (*Barbus carnaticus* and *Rastelliger canagurta*): The effect of calcium
 485 and magnesium as impurities in common salt used for curing. *Journal of Food*
 486 *Science and Technology*, 9, 17–19.

487 Kelleher, S. D., & Hultin, H. O. (1991). Lithium chloride as a preferred extractant of fish
 488 muscle proteins. *Journal of Food Science*, 56, 315–317.

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

492 Kolodziejska, I., & Sikorski, Z. E. (1980). Inorganic salts and extractability of fresh and
 493 frozen fish products. *International Journal of Refrigeration*, 3(3), 151–155.

494 Lauritzsen, K., & Akse, L. (1995). *Quality of salt and salted fish*. Report no. 1/1995.
 495 Tromsø (Norway): Fiskeriforskning.

496 * Lauritzsen, K., Akse, L., Gundersen, B., & Olsen, R. L. (2004). Effects of calcium,
 497 magnesium and pH during salt curing of cod (*Gadus morhua* L.). *Journal of the*
 498 *Science of Food and Agriculture*, 84(7), 683–692.

499 Lauzon, H. L., Magnússon, H., Sveinsdóttir, K., Gudjónsdóttir, M., & Martinsdóttir, E.
 500 (2009). Effect of brining, modified atmosphere packaging, and superchilling on the
 501 shelf life of cod (*Gadus morhua*) loins. *Journal of Food Science*, 74(6), M258–
 502 M267.

503 Lee, G.-H. (2011). A salt substitute with low sodium content from plant aqueous extracts.
 504 *Food Research International*, 44(2), 537–543.

505 Lowry, O.H., Rosebrough, N.J., Farr, A. L., & Randall, R. J. (1951). Protein measurement
 506 with the Folin phenol reagent. *Journal of Biological Chemistry*, 193(1), 265-275.

507 Martin, T. P., & Fischer, A. N. (2012). Sodium, potassium, and high blood pressure.
 508 *ACSM's Health and Fitness Journal*, 16(3), 13–21.

509 Martínez-Alvarez, O., & Gómez-Guillén, C. (2005). The effect of brine composition and
 510 pH on the yield and nature of water-soluble proteins extractable from brined
 511 muscle of cod (*Gadus morhua*). *Food Chemistry*, 92(1), 71–77.

512 Martínez-Alvarez, O., Borderías, J., & Gómez-Guillén, C. (2005). Sodium replacement in
 513 cod (*Gadus morhua*) muscle salting process. *Food Chemistry*, 93(1), 125–133.

514 * Martínez-Alvarez, O., & Gómez-Guillén, C. (2006). Effect of brine salting at different
 515 pHs on the functional properties of cod muscle proteins after subsequent dry
 516 salting. *Food Chemistry*, 94, 123–129.

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

520 Offer, G., & Knight, P. (1988). The structural basis of water-holding in meat. Part 1:
 521 General principles and water uptake in meat processing. In R. Lawrie (Ed.),

522 *Developments in Meat Science* (Vol. 4, pp. 63–171). London: Elsevier Applied
 523 Science.

524 * Offer, G., & Trinick, J. (1983). On the mechanism of water holding in meat: the
 525 swelling and shrinking of myofibrils. *Meat Science*, 8, 245–281.

526 Offer, G., Knight, P. Jeacocke, R., Almond, R., Cousins, T., Elsey, J., Parsons, N., Sharp,
 527 A. Starr, R., & Purslow, P. (1989). The structural basis of the water-holding,
 528 appearance and toughness of meat and meat products. *Food Microstructure*, 8,
 529 151–170.

530 Reddy, K. A., & Marth, E. H. (1991). Reducing the sodium content of foods: A review.
 531 *Journal of Food Protection*, 54, 138–150.

532 Rodrigues, M. J., Ho, P., López-Caballero, M. E., Bandarra, N. M., & Nunes, M. L.
 533 (2005). Chemical, microbiological, and sensory quality of cod products salted in
 534 different brines. *Journal of Food Science*, 70(1), M1–M6.

535 Stanley, D. W., & Hultin, H. O. (1968). Mechanism of emptying of skeletal muscle cell
 536 segments. *Journal of Cell Biology*, 39, C1–C4.

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

539 Thorarinsdottir, K. A., Arason, S., Bogason, S. G., & Kristbergsson, K. (2004). The
 540 effects of various salt concentrations during brine curing of cod (*Gadus morhua*).
 541 *International Journal of Food Science and Technology*, 39(1), 79–89.

542 * Thorarinsdottir, K. A., Arason, S., Sigurgisladottir, S., Valsdottir, T., & Tornberg, E.
 543 (2011). Effects of different pre-salting methods on protein aggregation during
 544 heavy salting of cod fillets. *Food Chemistry*, 124(1), 7–14.

545 * Thorarinsdottir, K. A., Arason, S., Thorkelsson, G., Sigurgisladottir, S., & Tornberg, E.
 546 (2010a). The effects of presalting methods from injection to pickling, on the yields
 547 of heavily salted cod (*Gadus morhua*). *Journal of Food Science*, 75(8), E544–
 548 E551.

549 Thorarinsdóttir, K. A., Bjørkevoll, I., & Arason, S. (2010b). *Production of salted fish in*
 550 *the Nordic countries. Variation in quality and characteristics of the salted*
 551 *products*. Mátis ohf / Icelandic Food and Biotech R&D. Report number 46-10.
 552 <http://www.moreforsk.no/default.aspx?menu=794&id=732> (last accessed 3-4-
 553 2013).

554 Von Hippel, P. H., & Wong, K-Y. (1964). Neutral salts: The generality of their effects on
 555 the stability of macromolecular conformations. *Science*, 145, 577–580.

556 Weinberg, Z. G., Regenstein, J. M., & Baker, R. C. (1984). Effects of different salts on
 557 water-retention and heat initiated binding-properties of comminuted cod muscle.
 558 *Journal of Food Biochemistry*, 8, 215–227.

559 Winter, K.H., Tuttle, L.A. & Viera, A.J. (2013). Hypertension. *Primary Care - Clinics in*
 560 *Office Practice*, 40, 179-194.

561 Xiong, Y. L. (2005). Role of myofibrillar proteins in water-binding in brine-enhanced
 562 meats. *Food Research International*, 38(3), 281–287.

563 Zhang, Y. & Cremer, P.S. (2006). Interactions between macromolecules and ions: The
 564 Hofmeister series. *Current Opinion in Chemical Biology*, 10 (6), 658–663.

565

Table 1: Sodium, potassium, calcium, magnesium and chloride content in dried salted cod previously brined with different brines.

<i>Brine composition</i>	<i>NaCl</i>	<i>KCl</i>	<i>CaCl₂</i>	<i>MgCl₂</i>	<i>Brine</i>	<i>Na⁺</i>	<i>K⁺</i>	<i>Ca²⁺</i>	<i>Mg²⁺</i>	<i>Cl⁻</i>
	(g/100 g salt)	(g/100 g salt)	(g/100 g salt)	(g/100 g salt)	pH	(mg/g)	(mg/g)	(mg/g)	(mg/g)	(g/100 g)
NaCl	100				6.5	74.00 ^a	3.03 ^a	1.77 ^a	1.01 ^a	18.40 ^{abcd}
NaCl	100				8.5	75.21 ^a	4.40 ^b	2.21 ^{ab}	1.40 ^{bcd}	17.40 ^{ab}
NaCl+KCl	50	50			6.5	69.32 ^a	15.39 ^{fgh}	3.43 ^{abc}	1.10 ^{ab}	18.87 ^{bcd}
NaCl+KCl	50	50			8.5	69.46 ^a	15.80 ^{gh}	2.55 ^{abc}	1.50 ^{bcd}	18.03 ^{abc}
NaCl+CaCl ₂	99.2		0.8		6.5	72.94 ^a	4.23 ^{ab}	3.27 ^{cd}	1.39 ^{abcd}	19.83 ^d
NaCl+CaCl ₂	99.2		0.8		8.5	73.01 ^a	4.13 ^{ab}	3.26 ^{bcd}	1.23 ^{abc}	18.03 ^{abc}
NaCl+KCl+CaCl ₂	50	49.2	0.8		6.5	70.05 ^a	13.30 ^d	3.10 ^{bcd}	1.38 ^{abcd}	19.87 ^d
NaCl+KCl+CaCl ₂	50	49.2	0.8		8.5	70.50 ^a	17.07 ⁱ	3.37 ^{cd}	1.21 ^{ab}	18.10 ^{abc}
NaCl+MgCl ₂	99.6			0.4	6.5	69.19 ^a	4.11 ^{ab}	2.45 ^{abc}	1.63 ^{cde}	19.10 ^{cd}
NaCl+MgCl ₂	99.6			0.4	8.5	72.87 ^a	4.41 ^b	2.61 ^{abc}	1.55 ^{bcd}	18.07 ^{abc}
NaCl+KCl+MgCl ₂	50	49.6		0.4	6.5	70.56 ^a	14.93 ^{efg}	2.55 ^{abc}	1.66 ^{cde}	1.757 ^{abc}
NaCl+KCl+MgCl ₂	50	49.6		0.4	8.5	70.15 ^a	16.45 ^{hi}	2.43 ^{abc}	1.44 ^{bcd}	18.10 ^{abc}
NaCl+CaCl ₂ +MgCl ₂	98.8		0.8	0.4	6.5	71.86 ^a	4.02 ^{ab}	4.04 ^d	1.41 ^{abcd}	16.90 ^a
NaCl+CaCl ₂ +MgCl ₂	98.8		0.8	0.4	8.5	69.82 ^a	3.84 ^{ab}	3.23 ^{bcd}	1.60 ^{cde}	17.50 ^{ab}
NaCl+KCl+CaCl ₂ +MgCl ₂	50	48.8	0.8	0.4	6.5	66.89 ^a	14.16 ^{def}	3.33 ^{cd}	1.36 ^{abcd}	17.60 ^{abc}
NaCl+KCl+CaCl ₂ +MgCl ₂	50	48.8	0.8	0.4	8.5	67.27 ^a	16.59 ^{hi}	3.31 ^{bcd}	1.74 ^e	18.40 ^{abcd}

Table 2: Tables of component weights. (a) pH 6.5; (b) pH 8.5.

a)	PC 1	PC 2	b)	PC 1	PC 2
Salt uptake	0.416	−0.176	K⁺	0.457	−0.165
Hardness	0.414	0.104	Hardness	0.450	−0.159
Water loss	0.370	0.213	Salt uptake	0.414	−0.286
Mg²⁺	0.364	−0.287	Water loss	0.298	0.208
K⁺	0.319	0.276	Mg²⁺	0.168	0.259
Ca²⁺	0.214	−0.288	WHC	0.142	−0.628
Cl[−]	−0.059	0.280	WEP	0.113	0.227
WEP	−0.087	−0.591	Ca²⁺	0.067	0.383
WHC	−0.241	0.432	Cl[−]	−0.329	−0.337
Na⁺	−0.412	−0.239	Na⁺	−0.395	−0.221

FIGURE CAPTIONS:

Figure 1. Water loss (Means $\pm$ SD, n = 3) in brined cod (grey bars) and in brined+dry salted cod (black bars). (a) Samples brined at pH 6.5; (b) samples brined at pH 8.5. Different letters (v, w, x... or a, b, c...) indicate significant differences between samples brined or between samples brined and dry salted, respectively. The level of significance setting was $P \leq 0.05$.

Figure 2. Salt uptake (Means $\pm$ SD, n = 3) in brined cod (grey bars) and in brined+dry salted cod (black bars). (a) Samples brined at pH 6.5; (b) samples brined at pH 8.5. Different letters (v, w, x... or a, b, c...) indicate significant differences between samples brined or between samples brined and dry salted, respectively. The level of significance setting was $P \leq 0.05$.

Figure 3. Principal component (PC) analysis of ion concentration and different variables analysed in salted cod previously brined at pH 6.5 (a) or 8.5 (b).

Figure 4. Discriminant analysis of the different variables analysed in salted cod as a function of the absence or presence of KCl in the brines previously used. (a) pH 6.5 without KCl; (b) pH 6.5 with KCl; (c) pH 8.5 without KCl; (d) pH 8.5 with KCl.

Figure 5. Discriminant analysis of the different variables analysed in salted cod as a function of the absence or presence of divalent salts in the brines previously used. (a) without MgCl₂ and CaCl₂; (b) with CaCl₂; (c) with MgCl₂; (d) with MgCl₂ and CaCl₂.

FIGURES:

- Figure 1:

- Figure 2:

- Figure 3:

- Figure 4:

- Figure 5: