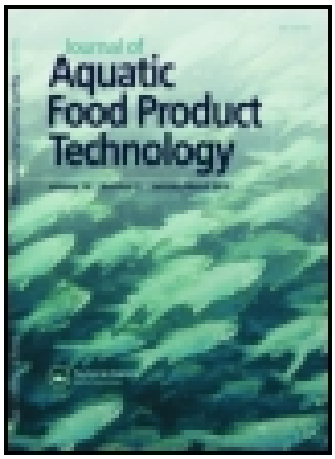

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Reduction of salt in haddock mince: Effect of different salts on the solubility of proteins

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Abstract

Due to negative health effects of high sodium intake, it is recommended to reduce the daily salt intake by around 50%. To reduce the sodium content, sodium salts can be exchanged with potassium or magnesium salts. The effect of sodium, potassium, and magnesium chlorides on extractability of proteins from fresh and frozen haddock muscle and minces was studied. Salting with KCl and $MgCl_2$ instead of NaCl changed protein extractability. The highest solubility of the proteins was achieved using Na^+. However, at low concentrations, extractability in K^+ and Mg^{2+} is on the same level as Na^+, showing that partial substitution of NaCl with KCl or $MgCl_2$ is possible. Freezing affected the structure of tissue and protein properties, resulting in decreased amount of salt soluble proteins.

Key words

Fish muscle, protein solubility, salt, ion substitution, mince, sodium reduction.

1 Introduction

Salting is one of the oldest and cheapest methods of food preservation used to prolong shelf life of food by reducing water activity as well as for improvement of functional properties such as water holding, formation of matrix-structure, and gelling (Albarracín et al., 2011; Nguyen et al., 2011). The daily intake of salt in the western world is around 8 - 11 g/day (Brandsma, 2006; EFSA, 2005), which is more than twice the amount needed. Industrially prepared foods contribute 70 – 80% of the daily intake of salt. Due to negative health effects of high sodium

intake, health organizations have strongly recommended a reduction from the present levels to values of around 6 g/day (Desmond, 2006; EFSA, 2005; FSA, 2003). To achieve this, it is necessary to develop products with reduced content of sodium chloride (Ruusunen and Puolanne, 2004). This can be done by reducing the salt content (Mitchell et al., 2011; Ugawa, 2003) or by replacing part of the sodium chloride with compounds having salty taste (Floury et al., 2009a, 2009b). Both Na^+ and Cl^- affect the taste of sodium chloride; the presence of Cl^- ion stimulates the receptor cells of the tongue. Even though the size of anions affects the perception of the salty taste and stimuli of the reception system, NaCl can be exchanged with KCl, $MgCl_2$, or other salts (Albarracín et al., 2011; Delwiche et al., 1999; Murphy et al., 1981; Åsli and Mørkøre, 2012). However, substitutions of Na^+ with other ions such as Mg^{2+} and K^+ often have negative effect both on the flavor and water holding capacity and texture (Åsli and Mørkøre, 2012). To achieve comparable properties in terms of structure and functional properties, exchange of cations in muscle foods should be based on equal quantity in moles. Since K^+ has a higher molar weight than Na^+, more KCl (in grams) is needed to substitute an equal number of ions, compared to NaCl. Since KCl has a bitter taste, substitution may lead to off taste (Desmond, 2006; Rössner et al., 2009).

In manufacturing minced fish products such as fish cakes, puddings, balls, and patés, the functional properties of the muscle proteins, including water holding capacity and gelling properties, are very important, directly influencing the final product quality (Bertram et al., 2003; Martínez-Alvarez and Gómez-Guillén, 2005). The molecular size and structure, charge distribution, and ability to interact with other ingredients affect the functional properties of the food components. Environmental factors such as pH, ionic strength, composition of salting

mixtures, temperature, and shear stress are therefore important for gelling and emulsifying properties, water retention, and fat binding of proteins (Kinsella, 1982). Addition of NaCl improves the mechanical properties of restructured fish, affecting the quality of the fish products (Costa-Corredor et al., 2010; Munasinghe and Sakai, 2006; Nguyen et al., 2011; Ramírez et al., 2002); therefore, reducing the sodium chloride content may affect the functional properties and thereby change the eating quality of the products (Rössner et al., 2009).

The proteins in muscle are usually divided into three main groups: proteins soluble at low ionic strength, the sarcoplasmic proteins; proteins soluble at high ionic strength, the contractile or myofibrillar proteins; and the insoluble proteins, the stroma or connective tissue proteins (Haard, 1992). Among these it is the proteins soluble at high ionic strength, myosin and actin, that are considered to be most important for the functional properties of the muscle (Jafarpour and Gorczyca, 2012; Kinsella, 1976; Stefansson and Hultin, 1994). Different buffers, salts, and pH have been used to study solubility properties of muscle proteins (Kelleher and Hultin, 1991; Munasinghe and Sakai, 2006; Xiong and Brekke, 1991). The phrase water soluble protein (WSP) is commonly used for the proteins soluble at low ionic strength, while salt soluble protein (SSP) is commonly used for the proteins soluble at high ionic strength (Hultmann and Rustad, 2002; Kinsella, 1976).

The water binding and holding behavior as well as the gelling properties of proteins in the food system have been shown to be related to the solubility properties of the proteins (Kinsella, 1982; Martínez-Alvarez et al., 2005; Nayak et al., 1996; Nguyen et al., 2011). Several studies have shown that different ions affect solubility properties of muscle proteins and functional

properties of muscle differently (Nayak et al., 1996; Nguyen et al., 2011), and this has been shown to be related to changes in water holding properties (Richardson and Jones, 1987). The effect on the water holding capacity may be attributed to preferential anion binding of chloride ions, which neutralize the positive charge of the protein at pH below the isoelectric point (Aliño et al., 2010; Nayak et al., 1996; Richardson and Jones, 1987). The composition of the soluble protein fraction in muscle depends on the pH. In brine salting, the initial pH of the brine appeared to be a more important factor in selective solubilization of the myofibrillar proteins than the actual combination of salts (Martínez-Alvarez et al., 2005). Na^+ increases the number of acidic groups resulting in an acidic shift of the iso-electric point of the proteins. Na^+ may also act as a weak acid (Lauritzsen et al., 2004; Rhee and Ziprin, 2001).

Both fresh and frozen raw materials are used in the production of minced fish products. Freezing leads to changes in raw material properties including protein denaturation resulting in loss of functional properties. It is therefore important to study how freezing will affect the raw material properties and, furthermore, how this will affect the influence of different salts on the product properties.

Haddock (*Melanogrammus aeglefinus)* was chosen as raw material for the study as haddock is widely consumed in West-European countries and both fresh and frozen haddock are often used in minced products (Martínez-Alvarez et al., 2005).

In order to produce high quality minced fish products with a lower level of salt, there is a need for more knowledge on how reduction of salt content and different ions affect the functional properties and the solubility properties of fish muscle proteins.

The main aim of this study was to evaluate the effect of different types of cations and different salt concentrations on the protein solubility of haddock muscle and minces with different salts (NaCl, KCl, $MgCl_2$) and concentrations (0.4 – 3.2 % w/w). A second aim was to evaluate how freezing affected these properties.

2 Materials and Methods

2.1 The raw material

Two different batches of haddock (*Melanogrammus aeglefinus*) were used. In the first part of the experiment, extractions were done on fillets, and in the second part, protein extractions were done on minces.

The haddock fillets (8 fillets from 4 fish) were bought in a local fish store. The fish was stored at +4 °C from catch until used, which was within one week after catch. Half the fillets was packed in plastic bags and frozen at –28 °C for two weeks. Before analysis, they were thawed in a cold room at +4 °C for 24 hours. The rest of the fillets were used for determination of protein solubility the day after purchase.

The haddock used for the mince experiment was caught by Danish seine on Vesterålsbanken, Northern Norway. The catch was pumped on board by a vacuum pump and stored alive on board before electro stunning, bleeding, and gutting. The gutted weight was 1.8 ± 0.6 kg. The fish were iced in Styrofoam boxes and transferred to Trondheim by the coastal steamer (in a cold room). The fish were generally of very good quality. Six days after catch, one

half of the fish was packed in plastic bags and frozen at –28 °C. After 67 days, the fish were thawed with drainage in a cold room (+4°C) for 67 hours. The thawed fish was hand filleted and minces were prepared as described below. The other half of the fish was hand filleted six days after catch, and minces were prepared the day after filleting. Coarsely chopped muscle was mixed together with tap water and salt in a food processor. The minces were made with three different salts: sodium chloride, potassium chloride, and magnesium chloride at three concentrations as given in section 2.2.2. The temperature of the fish was < +4 °C the entire time.

All salts and chemicals were of "pro analysis" grade.

2.2 The experimental setup

2.2.1 The first part of experiment

In this experiment, the effect of type of buffer (BisTris or phosphate), pH, and different salts (NaCl, KCl, $MgCl_2$) to extract the water and salt soluble proteins from fresh and frozen haddock muscle were studied. BisTris buffer was chosen because phosphate buffer cannot be used in combination with magnesium chloride, due to precipitation of $MgPO_4$. All extraction of proteins was done with two molarities of salts – 0.3 M and 0.6 M.

2.2.2 The second part of experiment

In this part, the effect of pure salts (NaCl, KCl, and $MgCl_2$) with different concentrations on the protein solubility properties in the salted mince was studied; and the amount of water- and salt-soluble proteins in fish mince made from fresh and frozen haddock was compared.

The added salt was composed with equimolar concentrations of salts ($MgCl_2$, NaCl, KCl) at three levels: 0.1, 0.3, and 0.55 moles per kg mince (corresponding to 0.4-3.2 weight % salt). The compositional model variables were: molar concentration of salt and type of salt ($MgCl_2$, NaCl, KCl). The experiment was performed on both fresh and frozen / thawed fish. This was part of a larger experiment on minces salted with different salts, where the aim was to investigate the effect of cations Na^+, K^+, and Mg^{2+} on physicochemical properties of raw and cooked haddock mince.

The composition of the minces used for study of protein solubility is shown in Table 1:

Based on the result of the first experiment, BisTris buffer, pH 7.0 was chosen as extraction buffer, with extraction salts NaCl, KCl, $MgCl_2$ with concentrations of 0.3 M and 0.6 M. The salt soluble proteins were extracted from the minces using BisTris buffer, containing the same salt, which was added to the mince. In addition, all minces made from frozen raw materials were extracted with buffer containing 0.6 M KCl. KCl was chosen because it has been used for determination of extractability of salt soluble proteins in several earlier studies (Duun and Rustad, 2008; Hultmann and Rustad, 2002).

2.3 Determination of water and salt-soluble proteins

Extractions for investigation of protein solubility were performed in two steps, resulting in a water soluble and a salt soluble fraction by a modification of the methods of Licciardello (1982) as described by Hultmann and Rustad (2002). For the fillets, two different sets of buffers were used for the determination of the water and salt soluble proteins, 50 mM phosphate buffer at pH

6.0 and 7.0 and 50 mM BisTris buffer at pH 6.0 and 7.0. For the minces 50 mM BisTris buffer, pH 7.0 was used. Approximately 4 g of white muscle or mince was homogenized in 80 ml of BisTris or phosphate buffer, pH 6 or 7 at +4 °C using an Ultra Turrax and centrifuged (20 min, 9700 g, +4 °C). The supernatant was decanted through glass wool, and the volume was made up to 100 ml with the corresponding buffer. This is the water soluble fraction. The sediment was re-homogenized in 80 ml of the same buffer, with the relevant salt and re-centrifuged. The supernatant was decanted through glass wool, and the volume was made up to 100 ml with the corresponding buffer. Two parallels were extracted for each sample.

The amount of protein in the extracts was determined by the BioRad protein assay (Bradford, 1976), using bovine serum albumin as a standard (0.1 – 1.0 mg/ml).

2.4 Sodium Dodecyl Sulphate Polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE was performed according to Laemmli (1970), using PhastGel® Gradient 4 – 15 gels, SDS buffer strips, and High & Low Molecular Weight Standards, by PhastSystem with programmable power and temperature conditions for separation and staining (10 mA/gel). The gels were stained with Coomassie Brilliant Blue or Silver Staining. All equipment for electrophoresis was delivered by GE Healthcare UK Ltd (Buckinghamshire, UK). The analysis was carried out according to the instructions of the manufacturer (PhastSystem™ Separation Technique File № 130 & 200). The samples were mixed with denaturing buffer (0.5M Tris-HCl, pH 6.8, 4.4% SDS, 300mM Mercaptoethanol, 10mg/ml Bromophenol Blue) in a 1:1 ratio and

boiled for 5 minutes. The high molecular weight standard contained the following proteins: rabbit muscle myosin heavy chain (220,000), bovine plasma α_2- macroglobulin (170,000), E.coli β- galactosidase (116,000), human transferrin (76,000), and bovine liver glutamic dehydrogenase. The low molecular weight standard contained the following proteins: rabbit muscle phosphorylase b (97,000), bovine serum albumin (66,000), chicken egg white ovalbumin (45,000), bovine erythrocyte carbonic anhydrase (30,000), soybean trypsin inhibitor (20,100), and bovine milk α–lactalbumin (14,400).

2.5 Determination of pH

Determination of pH was done by mixing approximately 2 g of minced sample with an equal amount of 0.15 M KCl (Duun and Rustad, 2007; Mackie, 1993). The pH was measured by a pH meter (Mettler Toledo MP 220). Mean values were calculated from six replicates.

2.6 Statistical analyses

Results are presented as mean value ± standard deviation. The number of parallels is given for each analysis.

The two tailed Student-t test has been used to determine the significance of effects of NaCl, KCl, $MgCl_2$ on extractability of the proteins and equality of means. Unless otherwise stated, the level of significance has been set at $p = 0.05$.

Statistical analyses were carried out in addition to comparative analysis to achieve a better understanding of the possible relationships between compositional and functional properties of

the proteins in salted haddock minces. The aim was to identify significant differences in effects by simple analysis of variance (ANOVA) using the program Unscrambler X 10.1.

3 Results and discussion

3.1 Solubility properties of protein in fresh and frozen haddock muscle

No significant differences were found between the extractability of fresh tissue water soluble proteins extracted in the different buffers (Figure 1). Freezing did not result in a statistically significant difference in WSP; this is in accordance with earlier studies (Kelleher and Hultin, 1991; Lowry, 1951; Markwell, 1978). Except for phosphate buffer at pH 6.0, there was a small but not significant decrease in water soluble proteins after freezing in all the buffers. This could be due to drip loss during thawing.

Significant differences were found for the extractability of salt soluble proteins in fresh haddock fillets depending on type of buffer, ionic strength, and type of salt (Figure 2). This is in agreement with earlier studies (Martínez-Alvarez and Gómez-Guillén, 2005; Stefansson and Hultin, 1994). The amount of salt soluble proteins was significantly higher in 0.6 M solutions than in 0.3 M solutions, except for the samples extracted with $MgCl_2$ at pH 6.0. The ionic strength of 0.3 M and 0.6 M $MgCl_2$ is 0.9 and 1.8, respectively; while the ionic strength for 0.3 M and 0.6M KCl and NaCl is 0.3 and 0.6, respectively. The ionic strength of 0.3 M $MgCl_2$ is therefore high enough to solubilize the salt soluble proteins.

Except for samples extracted with 0.6 M NaCl in phosphate buffer at pH 7.0, the amount of SSP in BisTris buffer is higher than in phosphate buffer ($p<0.05$).

In phosphate buffer with 0.3 M salt (both NaCl and KCl), there is a small but significant effect of pH, while no significant effect of pH was found for BisTris buffer.

For 0.6 M NaCl and 0.6 M KCl, the extractability of the salt soluble proteins in phosphate buffer was significantly higher at pH 7.0 than at pH 6.0. For BisTris buffer, no effect of pH was found for NaCl, while there was a large and significant effect of pH for KCl. When pH is further from the isoelectric point, the protein network is more open, influencing the extractability of the proteins (Koľodziejska and Sikorski, 1980). However, for both 0.3 M and 0.6 M $MgCl_2$, there is a significantly lower extractability at pH 7.0 compared to pH 6.0; this could be due to more formation of cross linkages at higher pH.

The type of ion plays a significant role both for swelling and for the formation of cross-linkages between proteins, the high electronegativity of the Mg^{2+} ion leading to a strong binding to the polar groups of proteins, thereby strengthening interactions between the proteins and reducing the pH (Koľodziejska and Sikorski, 1980; Martínez-Alvarez and Gómez-Guillén, 2005; Thorarinsdottir et al., 2002). In the second part of the experiment, a reduction in pH was found for minces salted with $MgCl_2$ (Table 2):

Potassium chloride may reduce the protein solubility in fish muscle (Thorarinsdottir et al., 2002), probably because KCl has the ability to aggregate myosin (Aliño et al., 2010; Martínez-Alvarez et al., 2005). K^+ has a lower charge density (0.026 units of charge/molecular weight)

compared to Na^{+} and Mg^{2+} (0.043, 0.082). If KCl is used for salting of tissue, it may have some difficulty in penetrating inside the muscle, compared to sodium and magnesium ions (Mackie, 1993). Comparing KCl, NaCl, and LiCl, the highest extractability was found at a concentration of 0.8M for all salts, and NaCl gave the highest extractability (Aliño et al., 2010).

The extractability of salt soluble proteins was significantly lower in frozen tissue compared to fresh (Figures 2 and 3). This is in accordance with other studies on frozen fish and is due to conformational changes in the myofibrillar proteins caused by freezing and frozen storage (Mackie, 1993). In general, the molarity of the extraction buffer has less influence on extractability of salt soluble proteins from frozen fillets than for fresh fillets. The salt concentration and type of salt may therefore have less influence on physiochemical properties of mince from frozen raw material. A small but significant difference in extractability of salt soluble proteins was found between BisTris and phosphate at salt concentrations of 0.3 M. For NaCl and KCl, the amount of proteins extracted in BisTris buffer was lower than the amount extracted in phosphate buffer; while the opposite was the case for fresh fillets. Except for $MgCl_2$, the increase in extractability with increase in salt concentration from 0.3 M to 0.6 M is smaller than for the fresh fillets.

Both for fresh and frozen tissue, the type of buffer influences the amount of salt soluble proteins extracted in 0.3 M NaCl and KCl. When extraction of proteins was performed with 0.6 M NaCl and KCl, both the type of buffer and pH influenced amount of salt soluble proteins.

The molecular weight distribution of the extracted proteins was analyzed using SDS gel electrophoresis. The molecular weight distribution of water soluble proteins from fresh and

frozen haddock extracted in 50 mM phosphate or BisTris buffer, pH 6.0 and 7.0, were similar (results not shown).

The results for the salt soluble proteins show clear bands for myosin heavy chain (MHC) for samples extracted with NaCl, both for BisTris and phosphate buffers (Figures 4 a and b.)

Very few bands are visible for salt soluble proteins, extracted by 0.3 M KCl in BisTris buffer at pH 7.0, and there is no visible band for myosin heavy chain. This might be due to the ability of KCl to aggregate myosin (Aliño et al., 2010), as mentioned above. For proteins extracted by 0.3 M magnesium chloride, visible MHC bands are found, but this is not the case for extracts made in 0.6 M $MgCl_2$. The disappearance of MHC bands in extracts made in 0.6 M $MgCl_2$ is probably due to cross linkage and aggregation, due to the high electronegativity of the magnesium ions. Only weak bands for myosin light chains are visible for samples extracted in KCl and $MgCl_2$, while clear bands are visible for samples extracted in NaCl. For the extracts made from frozen samples, weak bands for MHC are seen for the extracts made with 0.3 M and 0.6 M NaCl and 0.3 M KCl. No bands for actin are visible; but for samples extracted with $MgCl_2$, bands for myosin light chains are visible. The disappearance of the myosin heavy bands is caused by conformational changes and aggregation of myosin during frozen storage (Mackie, 1993). The molecular weight of the separated proteins was affected by the state of the raw material (fresh/frozen), and no effect was seen due to the type of buffer and pH. The results of SDS gel electrophoresis confirm that using BisTris buffer to extract the salt soluble proteins has no adverse effect on the qualitative characteristics of the extracted proteins. It is therefore possible

to substitute phosphate buffer with BisTris for determination of the extractability of water- and salt- soluble proteins.

3.2 Solubility properties of proteins in salted mince

No significant differences were found in amount of extracted water soluble proteins for the minces salted with different salts. In the minces made from frozen and thawed raw material, the amount of water soluble proteins was approximately on the same level as in the fresh mince (results not shown). For mince made from fresh haddock, the extractability of salt soluble proteins in buffer containing 0.6 M of the corresponding salt (Figure 5) was around 5 % w/w for all three salts except for mince salted with 0.55 M $MgCl_2$. As discussed earlier, the drop in extractability in minces with 0.55 M $MgCl_2$ could be due to formation of cross linkages between proteins (Barat et al., 2002; Martínez-Alvarez and Gómez-Guillén, 2005, 2006; Tahergorabi et al., 2012). Except for mince salted with 0.55 M $MgCl_2$, significant differences were found between amount of salt soluble proteins extracted in buffers with salt concentrations of 0.3 M and 0.6 M for all the three salts. An increase in SSP extractability with increasing salt concentration (from 0.11 to 0.55 M in the mince) was found for minces extracted with 0.6 M NaCl. No significant differences were found for minces extracted with 0.6 M KCl. The highest extractability of SSP was found for minces with 0.55 M NaCl. The extractability in 0.3 M NaCl decreases with increasing salt concentration in the mince, while the opposite was found for KCl. This could be due to differences in charge density of the ions (Kinsella, 1982; Perisik et al., 2011). This and salt concentration are the main factors for secondary structural changes in

proteins (Barat et al., 2002; Martínez-Alvarez and Gómez-Guillén, 2005, 2006; Tahergorabi et al., 2012).

The range of molarities used in this study was from 0.11 to 0.55 (Table 1). This is low compared to studies on brine salted fish (Duerr and Dyer, 2011; Dyer et al., 1950; Thorarinsdottir et al., 2004), but the concentrations chosen are close to the ones used in the industry for minced fish products. While high salt concentrations led to denaturation of proteins, at low concentrations ≈ 0.3 – 0.5 M NaCl, up to 95% of fish muscle proteins may be extracted (Costa-Corredor et al., 2010).

Figure 5 shows extractability of myofibrillar proteins from the fresh salted minces, which were extracted with the salts used in the mince. To enable comparison of extractability in one standard solution, all the minces made from frozen and thawed fillets were also extracted in 0.6 M KCl. The results show that the amount of extracted proteins depends not only on the salt added into the raw material, but also on the salt in the extraction buffer. There is a small but significant difference between amount of SSP in mince salted with 0.11 M NaCl and extracted in KCl and amount of SSP in mince salted with 0.11 M KCl and extracted with KCl (1.0 vs 0.8). For all three concentrations of $MgCl_2$, a significant difference was found between extractability in $MgCl_2$ and KCl. At $MgCl_2$ concentrations of 0.11 M and 0.55 M in the mince, the amount of SSP extracted in 0.6 M $MgCl_2$ is higher than the amount extracted in 0.6 M KCl (1.13 % vs 0.38 % w/w and 0.40 % vs 0.16 % w/w correspondingly); but at a $MgCl_2$ concentration of 0.28 M, KCl gives a higher yield of proteins compared to $MgCl_2$.

The largest differences between extraction in 0.3 and 0.6 molar salts were found in minces made from fresh raw material containing 0.28 M NaCl, 0.55 M NaCl, 0.11 M KCl, 0.11 M, and 0.28 M $MgCl_2$. For the other minces, no significant differences were found. For minces made from frozen raw material containing 0.28 M salt, small, but significant differences were found between amount extracted in buffers with 0.3 M and 0.6 M salt (Figure 5).

For minces made from frozen tissue, no significant differences were found between amount of SSP extracted in buffer with 0.6 M salt corresponding to the salt used in the mince and buffer with 0.6 M KCl; the only exception was minces containing 0.55 M NaCl (Figure 5).

The ionic strength of minces with $MgCl_2$ is approximately three times higher than in minces salted with NaCl and KCl. Reducing the amount of magnesium chloride to obtain the same ionic strength may lead both to change in taste but also to reduced water activity and therefore reduction of shelf life. The fish puddings made from salted minces with $MgCl_2$ were less hard and had a lower water holding capacity compared to puddings made from minces salted with NaCl and KCl (Aliño et al., 2010).

The composition of salt soluble proteins in the salted minces studied by SDS page electrophoresis (Figures 6 and 7) shows that minces salted with NaCl have the clearest bands for MHC. For minces salted with 0.11 M and 0.55 M KCl, more intense bands for myosin heavy chain were seen in extracts made with 0.3 M KCl; while very weak bands were seen for minces with 0.55 M KCl, when extracted by 0.6 KCl (Figure 6). For samples extracted with NaCl, clear bands for actin and myosin light chains are visible; while for samples extracted with KCl, clear bands for actin are visible, but the myosin light chains are weak. For samples extracted with

$MgCl_2$, bands for actin are not visible. As discussed earlier, this is in agreement with the ability of KCl to aggregate myosin. For minces salted with $MgCl_2$, MHC bands were not visible. As earlier mentioned in section 3.1, the high electronegativity of the Mg^{2+} ion may lead to the strong binding to the polar groups of proteins and lead to cross linkages and myosin aggregation.

Only minor differences were observed between SSP extracted by KCl and extracted by the corresponding salts as found with SDS-gel electrophoresis (Figure 7). Thus to compare protein extractability in minces and fillets salted with different salts, it is possible to use one extraction agent for determination of the salt soluble proteins for all the studied salts.

Conclusion

The type of buffer and pH did not influence amount of water soluble proteins in fresh or frozen haddock fillets. Significant differences were found for the extractability of salt soluble proteins in fresh haddock fillets depending on type of buffer, ionic strength, and type of salt. Except for extracts in $MgCl_2$, the amount of salt soluble proteins was significantly higher in buffers with 0.6M salt than in buffers with 0.3 M salt. The type of buffer influences the extractability of the salt soluble proteins, extracted by 0.3 M NaCl and KCl; while pH matters for phosphate buffer only. When extraction of proteins was performed with 0.6 M NaCl and KCl, both the type of buffer and pH affected extractability. The extractability of salt soluble proteins was significantly lower in frozen tissue compared to fresh. For frozen raw materials, the highest extractability was found in 0.6M $MgCl_2$, but the molarity of the buffer had less influence on the extractability of salt soluble proteins from frozen raw materials. Extractability of SSP for fresh and frozen fillets

is the same or better in BisTris buffer compared to phosphate buffer, showing that BisTris buffer can be used as an extraction buffer for salt soluble proteins.

For minces salted with NaCl, an increase in extractability of salt soluble proteins at 0.6 M NaCl was found with increasing salt concentration in the mince,

while no difference was found for minces salted with KCl and extracted with 0.6 M KCl. For minces salted with $MgCl_2$, the extractability in minces with 0.55 M $MgCl_2$ was significantly lower than for minces with 0.11 M and 0.28 M $MgCl_2$.

Extractability of salt soluble proteins in minces made from frozen raw material was significantly lower than in minces made from fresh raw material. For frozen raw material, use of $MgCl_2$ increased solubility properties of the proteins, except for the highest concentration (0.55 M).

All the effects (molarity of extracting salt, kind of salt, storage conditions) performed and analyzed by ANOVA test show insignificance of interactions. Effect of conditions presents significant difference at 95% confidence level for the salt soluble proteins, extracted by 0.6M salts only. This may mean that the nature of the salt at low concentrations does not significantly influence the extractability of the proteins.

Based on these results, it seems possible to exchange part of the NaCl in minced fish products with KCl or $MgCl_2$ without affecting protein solubility. However, this should be further investigated.

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Table 1. Ionic strength, molar concentration, and salt concentration in the salted minces (I and M) * was calculated corresponding to the mass of mince without added water.

Sample	I *	M *	Amount of salt, g	Salt %
NaCl	0.11	0.11	3.0	0.4
NaCl	0.28	0.28	7.5	1.0
NaCl	0.55	0.55	15.0	2.0
KCl	0.11	0.11	3.8	0.5
KCl	0.28	0.28	9.6	1.3
KCl	0.55	0.55	19.2	2.5
$MgCl_2$	0.33	0.11	10.4	0.7
$MgCl_2$	0.83	0.28	26.1	1.6
$MgCl_2$	1.66	0.55	52.2	3.2

Table 2. pH in minces made from fresh and frozen-thawed haddock fillets; values are given as means ± STDEV, n=6.

Salt concentration in minces:	NaCl			KCl			$MgCl_2$			Unsalted minces
	0.1 M	0.3 M	0.55 M	0.1 M	0.3 M	0.55 M	0.1 M	0.3 M	0.55 M	
Fresh	6.51	6.46	6.34	6.63	6.54	6.49	6.09	5.71	5.52	7.0
Frozen	6.48	6.44	6.24	6.55	6.52	6.48	6.16	5.97	5.63	7.2

Figure 1. Extractability of water soluble proteins in fresh and frozen haddock fillets (% wet weight) in 0.05M BisTris and phosphate buffer, pH 6.0 and 7.0. Values are given as mean ± stdev, n=6.

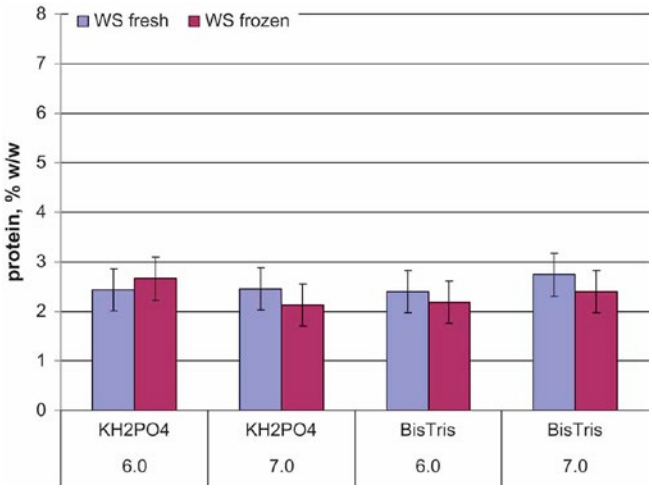

Figure 2. Extractability of salt soluble proteins (% of wet weight) in fresh haddock fillets in 0.3 M and 0.6 M NaCl, KCl, $MgCl_2$ in phosphate and BisTris buffers at pH 6.0 and pH 7.0. Values are given as mean (n=2) ± uncertainty of the method (7.5 %).

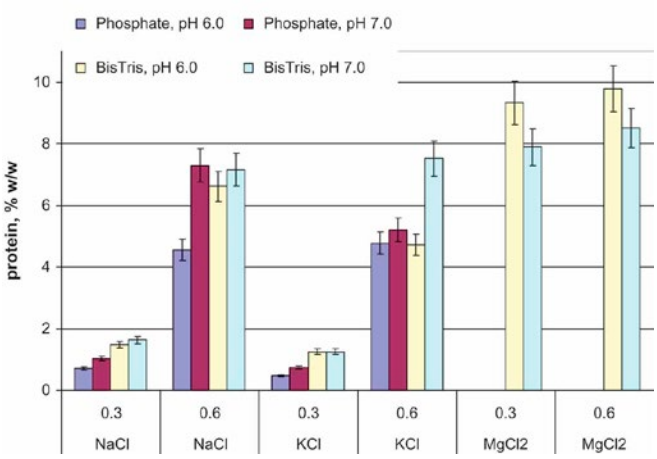

Figure 3. Extractability of salt soluble proteins (% of wet weight) in frozen haddock fillets in 0.3 M and 0.6 M NaCl, KCl, $MgCl_2$ in phosphate and BisTris buffers at pH 6.0 and pH 7.0. Values are given as mean (n=2) ± uncertainty of the method (7.5 %).

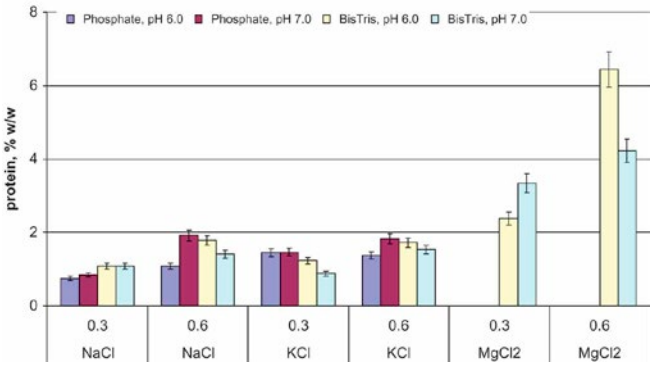

Figure 4 (a,b). Composition of salt soluble proteins extracted in BisTris and phosphate buffers with 0.3 M and 0.6 M NaCl, KCl, $MgCl_2$, pH 7.0, analyzed by SDS PAGE. Molecular weight standards are presented as: LMW – low and HMW – high.

4 a wells:

1-0.3M NaCl in BisTris; 2-0.6M NaCl in phosphate; 3-0.6M NaCl in BisTris; 4-0.3M KCl in phosphate; 5-0.3M KCl in BisTris; 6-0.6M KCl in phosphate; 7-0.6M KCl in BisTris; 8-0.3M $MgCl_2$ in BisTris; 9-0.6M $MgCl_2$ in BisTris.

4 b wells:

1-0.3M NaCl in BisTris; 2-0.6M NaCl in phosphate; 3-0.6M NaCl in BisTris; 4-0.3M KCl in phosphate; 5-0.3M KCl in BisTris; 6-0.6M KCl in phosphate; 7-0.6M KCl in BisTris; 8-0.3M $MgCl_2$ in BisTris; 9-0.6M $MgCl_2$ in BisTris.

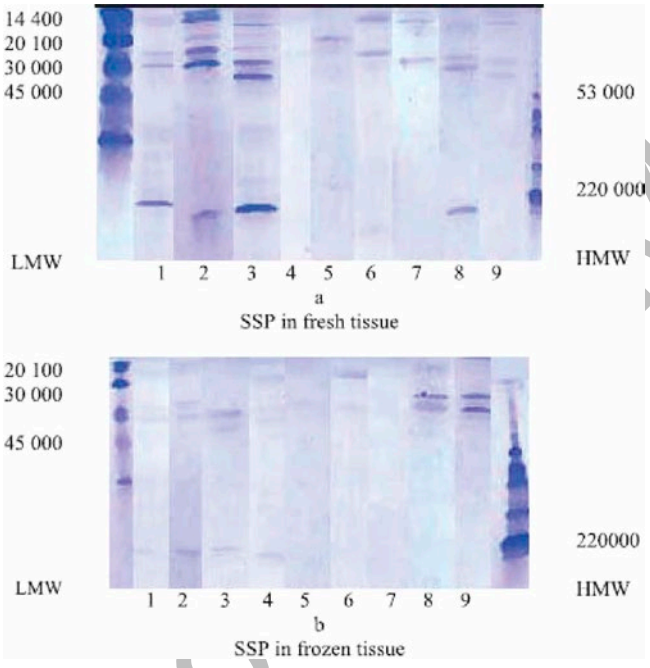

Figure 5. Extractability of salt soluble proteins in salted minces made from fresh and frozen haddock. Extraction was done in 0.3 M and 0.6 M salt corresponding to the salt in the mince. Results are given in % w/w of muscle in the sample.Values are given as mean (n=2) ± uncertainty of the method (7.5 %).

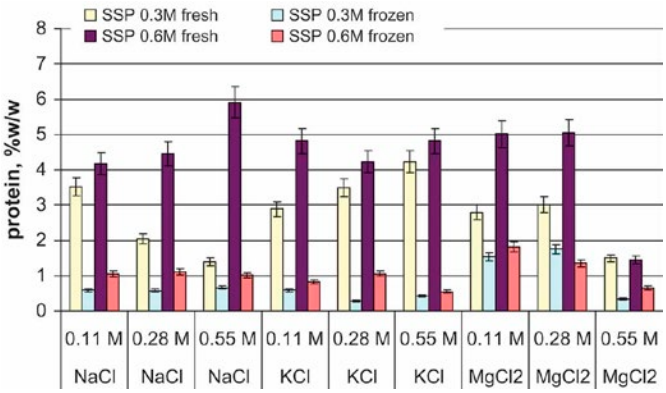

Figure 6. Composition of salt soluble proteins from minces made from fresh haddock, salted with NaCl, KCl, $MgCl_2$ in 3 different concentrations (0.11M, 0.28M, 0.55M), extracted in BisTris buffer, pH 7.0 by 0.3 M and 0.6 M salts, analyzed by SDS-PAGE. Molecular weight standards are presented as: L (LMW) – low and H (HMW) – high.

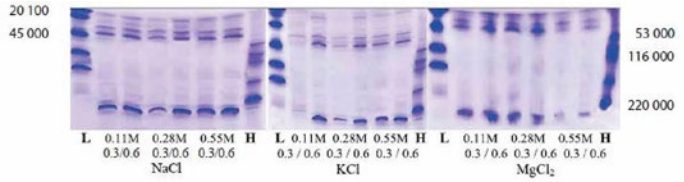

Figure 7. Composition of salt soluble proteins from frozen minces using SDS-PAGE, made from frozen raw material, salted with NaCl, KCl, $MgCl_2$ in 3 different concentrations (0.11M, 0.28M, 0.55M), extracted by the 0.6 M corresponding salt and 0.6 M KCl for comparison, in BisTris buffer, pH 7.0. Molecular weight standards are presented as: L (LMW) – low and H (HMW) – high. Numbered:

Well NaCl 1,3,5 salted with 0.11 M, 0.28 M and 0.55 M NaCl, extracted with 0.6 M NaCl;

Well NaCl 2,4,6 salted with 0.11 M, 0.28 M and 0.55 M NaCl, extracted with 0.6 M KCl;

Well KCl 1,2,3 salted with 0.11 M, 0.28 M and 0.55 M KCl, extracted with 0.6 M KCl;

Well $MgCl_2$ 1,3,5 salted with 0.11 M, 0.28 M and 0.55 M $MgCl_2$, extracted with 0.6 M $MgCl_2$;

Well $MgCl_2$ 2,4,6 salted with 0.11 M, 0.28 M and 0.55 M $MgCl_2$, extracted with 0.6 M KCl.