Effects of Phosphate on Yield, Quality, and Water-Holding Capacity in the Processing of Salted Cod (*Gadus morhua*)

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ABSTRACT: Fillets of cod (*Gadus morhua*) were salted in a traditional way and with the addition of polyphosphates. After 3 weeks of storage, the fillets were rehydrated and desalted. Changes in weight and chemical content were followed through the process. The addition of polyphosphates resulted in poorer quality, when considering the entire process, although differences were not observed in sensory evaluation after rehydration and steam boiling. Improvements in yield were observed in the fillets containing polyphosphate, after dry-salting and storage (P < 0.05). However, the increase in weight during rehydration was far less by those fillets than by the control group, where no phosphate was used.

Keywords: salted cod, polyphosphates, yield, quality, water-holding capacity

Introduction

SALTING IS ONE OF THE OLDEST FOOD-PRESERVATION METH-ods, although many other methods are used now, of which freezing is the most common. Salting of fish remains a very important process for Iceland, where the products are primarily exported. In 1998, the quantity of exported salted fish was about 49,500 metric tons, of which salted cod was 84.5%. The traditional markets for Icelandic salted fish have been in Spain, Portugal, and Latin America. However, one of the largest growing markets is the United States, with 1998 imports approaching 10,000 metric tons.

In the production of salted fish, the raw fish is filleted or "butterfly" split and then heavily salted (Thorarinsdottir and others 2000a). During the salting process, the fish muscle takes up salt and loses water. Salting and dehydration result in decreased water activity, rendering less water accessible for bacteria and molds. Chemical and physical changes that affect the structure of the muscle occur mainly through denaturation of the proteins and dehydration of the muscle (van Klaveren and Legrende 1965; Bjarnason 1986). Many factors affect the quality of the final product, including the condition of the raw material, the salt used, and the curing method (Akse 1995). After the salting process is completed, the product is relatively shelf-stable and may be stored for months or even years given the right conditions.

Before consumption, the fish is submerged in water, which results in uptake of water, rehydration, and a decrease in salt content. This changes the texture of the fish, making the final salted fish product firmer and more compact, which is preferred by consumers. Taste and flavor characteristics are also altered.

Changes in water-holding capacity (WHC) and denaturation of proteins are affected by the concentration and composition of the salt in the muscle. Only 4 to 5% of the total water in the muscle is thought to be tightly bound to the muscle proteins and not influenced by changes in the structure and charges of proteins. Most of the water is influenced by changes in the structure and charges of muscle proteins (Warrier and others 1975). Sodium chloride and phosphate affect the water-holding capacity in both meat and fish. At very low concentrations (0 to 0.1 M) an increase in salt concentration decreases filament spacing and causes lateral shrinkage of the fiber, due to salt screening effects. At salt concentration higher than 0.1 M, spacing of filaments increases with increasing ionic strength due to the binding of anions to filaments, greater negative charges, and stronger repulsive forces. Increased swelling can also be due to partial depolymerization of the thick filaments, which favors the dissociation of the actomyosin complex (Fennema 1990). At concentrations above 1 M, there is progressively less swelling, and above 4.5 M the muscle shrinks (Offer and Knight 1988). Divalent ions (Mg^{2+}, Ca^{2+}) are believed to affect the WHC by cross-linking myofibrils, which decreases the space for water in the cell (Hamm 1960).

In recent years, the use of additives in food production has been increasing. Addition of polyphosphates to meat and seafood products has induced water retention (WHC) during processing and thereby increased the weight of these products.

According to Lindsay (1996), the mechanisms by which alkaline phosphates and polyphosphates enhance meat hydration are not clearly understood. It may involve effects on pH and ionic strength, and specific interactions of phosphate anions with divalent cations and myofibrillar proteins. The effectiveness of phosphates on water retention properties of meat products depends on the type of phosphate, the amount used, and the specific food product. It has also been concluded that a combination of phosphates and NaCl increases moisture retention in meat more than NaCl alone. Paterson and others (1988) studied the effect of salt and pyrophosphate on isolated beef myofibrils. Phosphate enhanced the extractability of proteins, but did not affect maximal swelling. However, phosphate lowered the concentration of NaCl from 1 M to 0.7 M, needed to achieve maximum swelling.

Offer and Knight (1988) discussed the effects of salt and phosphates on myofibrils and WHC in meat. They explained the action of phosphates in 3 ways. First, phosphates are good buffers, which may assist in the depolymerization of thick filaments and increase water uptake and retention. Second, in the presence of Mg^{2+}, pyrophosphate and triphosphate bind to the myosin molecule. Pyrophosphate acts as

an analog to ATP and binds to the myosin heads, thus promoting the dissociation of actomyosin. Third, polyphosphate can bind to the myosin tails and promote disassociation of the myosin filaments to myosin molecules. Trout and Schmidt (1984) also explained the effects of salt and phosphate in muscle, dissociation of actin and myosin, and the tendency of the thick filament shafts to depolymerize. Factors like concentration of ions (Mg^{2+}, Ca^{2+}, Cl^-) and phosphate, temperature, and pH were believed to affect how phosphates interact with the muscle.

The effects of phosphates in the processing of salted cod were studied by Arnesen and Dagbjartsson (1973, 1974). The fish were either dipped into a solution of phosphates or the phosphates were added to the brine. The use of phosphate resulted in increased total weight of the fish after salting. However, no references were found about the effects of phosphate after rehydration. Additives like phosphates have not been used for the production of salted cod, but statements about increased profits have led to our study, the objectives of which were to determine the effects of polyphosphates on yield, quality, and sensory characteristics of salt-cured cod during salting and after rehydration.

Materials and Methods

COD (*GADUS MORHUA*) WAS CAUGHT BY LINE NEAR THE South West of Iceland by commercial fishing boat. In a preliminary experiment, which was performed for optimization of the methods, the fish used were caught at the end of July. In the following (main) experiment, the fish were caught at the end of January. The fish were placed in bins with ample ice immediately after gutting. The bins were transported to the SIF Ltd. Development Center (Hafnarfjordur, Iceland), where the experiments were conducted.

Commercial grade salt, from the same sack, was used throughout all experiments. The salt used for the preliminary experiments was imported from Torrevieja, Spain; the salt used for the main experiment was imported from Almeria, Spain. All materials used for chemical analysis were of analytical grade.

Salting

After rigor mortis had set in, filleting was done by hand using standard industrial procedures. Each fillet was identified with a numbered plastic marker, and the left and right fillet of each fish were randomly put into either a test group or a control group for paired comparison. Both groups were salted in brine of 16 °Baumé (analyzed as 17.5% w/w NaCl in water). Polyphosphate blend (Brifisol B512; BK Giulini Chemie, Germany) was added to the brine of the test group. Brifisol 512 consisted of a blend of sodium polyphosphates with different chain lengths and a pH value of 9,0 ± 0,3. The fish-to-brine ratio was 1:1.6 for both experiments.

Preliminary experiment (I)

The fish was submerged in 16 °Baume brine for 42 h, with 2.5% of polyphosphate (PP-Test I). After removal from the brine, the fillets were placed in plastic tubs and piled into stacks with alternating thin layers of salt, then kept for 12 d for dry-salting. After dry-salting, the fillets were packed into waxed cardboard boxes and kept at 3 to 5 °C for approximately 3 wks, after which the salted fillets were desalted and rehydrated. Approximately 6-cm-wide pieces were made by cutting across the whole fillet from both the thick section at the head and the thin tail section of the fillet. These pieces were then submerged in water, with a fish-to-water ratio of 1:18, and allowed to rehydrate for 72 h at 4 ± 1 °C.

Main experiment (II)

The main experiment was performed in nearly the same way as the preliminary experiment, except that only 2.0% (PP-Test II) of polyphosphate was added to the brine instead of 2.5% (PP-Test I), since some quality defects had been observed in the preliminary experiment thought to be due to excessive use of polyphosphates. Dry-salting took 14 d and the rehydration process was different from that in the preliminary experiment. The fillets were cut into 3 pieces which were rehydrated by submerging the fish in a 1:5 (fish-to-water) ratio for 30 h, after which the water was replaced with fresh water and the fish allowed to rehydrate to an additional 80 h submerged in a 1:4 ratio water bath. The environmental temperature during rehydration was 4 ± 1 °C.

The ratio of water to fish was very high in the preliminary experiment but was decreased in the main experiment for ease of future scale-up of the process.

Sampling

In the preliminary experiment, 2 fillets were collected as samples from the raw material and after each processing step, but in the main experiment 3 fillets were collected as samples. A piece of about 6 cm was cut from the middle of the fillets for determination of water-holding capacity (WHC). For chemical analysis, 2 pieces, approximately 6 cm each, were cut from the fillets, from the tail part and near the head, next to the parts taken for measurement of WHC.

Preparation and storage of samples

The samples (fish and brine) collected for chemical analysis were frozen immediately and stored at –24 °C until measurements were made. Prior to chemical analysis, the fish were skinned by hand and minced in a Braun mixer (type 4262; Braun, Kronberg, Germany). Commercial quality rating was performed on the fish without further preparation. The fish were steam-boiled prior to sensory analysis.

Weight determination and yield calculations

The weight and length of each gutted fish were recorded. The fillets were weighed as raw material and after each processing step; yield was determined by the observed changes in weight with respect to the weight of the raw fillets.

Determination of water, salt, and protein contents and pH

Moisture content (g/100 g) was calculated as the loss in weight during drying at 105 °C for 4 h (ISO 1983). Salt content was determined by the method of Volhard (JAOAC 1937, 1940). The nitrogen/protein content of the fish muscle and brine was obtained with the Kjeldahl method (ISO 1979), with the aid of Digestion System 40 (1026 Digester, Tecator, Hoganas, Sweden). Protein recovery (%) was calculated from the protein content of samples, using the content in the raw material as reference value. The pH of the muscle was measured by inserting a combined glass electrode ('Red rod' C2401-7; Radiometer, Copenhagen, Denmark) directly into the cod mince. This method was a modification of the procedure by Kramer and Peters (1981), who measured pH in fillets by inserting the electrode directly in the approximate middle of the fillets.

Determination of phosphate

Phosphorus was estimated colorimetrically as phosphovanadomolybdate (Hanson 1950; Sutton and Ogilvie 1967) by the spectrometric method (vanadum phosphomolybdate) which is based on the reaction of orthophosphate in an acid-

ic solution with ammonium molybdate and ammonium vanadate in nitric acid.

Approximately 1 to 5 g of cod mince was weighted accurately into a silica dish and 1 g CaO added. The sample was heated over a low flame until thoroughly charred and then placed in a furnace at 550 °C for 3 h. After cooling, the sample was transferred to a 250 mL beaker and diluted with approximately 10 mL of distilled water. Then approximately 12 mL of concentrated HCl and approximately 5 mL of HNO_3 were added. The solution was heated, cooled, transferred to a 250-mL volumetric flask, and diluted to volume with distilled water. The solution was then filtered and the first 10 to 20 mL of the filtrate was discarded. The absorbance of this solution was determined at 420 nm and samples were compared to a calibration curve where the phosphate content was determined as mg P_2O_5/g sample.

A series of a standard solution (vanado-molybdate reagent) was prepared and the absorption measured at 420 nm. The calibration curve obtained was used for the determination of phosphate content in the samples.

Determination of water-holding capacity (WHC)

The salted cod samples (n = 3) were coarsely minced with a Braun mixer (type 4262) for approximately 20 s at speed 4. Approximately 10 g of the minced cod muscle was weighed accurately and immediately centrifuged at 210 × g (1500 rpm) for 15 min; a temperature of 2 to 5 °C was maintained (Akse and others 1993; Ofstad and others 1996). The weight loss after centrifugation was divided by the moisture content of the fillet and expressed as % WHC.

Quality rating

Commercial quality rating was performed by a trained grader on the cod after the dry-salting and storage period. Three quality grades were used for assessing the cod fillets: A, B, and C. Some basic rules are reported here, but the knowledge of the grader was gained by training and experience. In the evaluation of salted cod fillets, appearance or color is of great importance:

Grade A: Fillets were to be light in color, thick, without gaping or blood stains, where gaping appeared as openings or ruptures between the myotomes because of weakening of the connective tissue.

Grade B: Fillets that because of small defects were not of Grade A quality. They could be darker in color, thinner, and with long gaps in the length direction of each fillet.

Grade C: Fillets that had defects like gaping or other mechanical defects of the flesh. The color of the fillets was too dark to be graded as B quality. Slight red discoloration of fillets (caused by the growth of halophilic bacteria) or yellow staining (caused by copper-catalyzed oxidation of fat) was classified as C quality.

Sensory analysis

A trained in-house sensory panel at the Icelandic Fisheries Laboratories (IFL) evaluated the fish. In the preliminary experiment, a duo-trio difference test was used for comparison of fillets from the test group and the control group. In the main experiment, a paired difference test was used for pair II together with quantitative descriptive analysis (QDA). The software used in the sensory evaluation, was Hypersense 1.6 designed by IFL.

Statistical analysis

Statistical analysis was performed by Microsoft Excel 8.00 (Microsoft Inc., Redmond, Wash., U.S.A.). Student's t-test and ANOVA were performed on the means of values. The significance level was $P \leq 0.05$.

Results and Discussion

Weight determination and yield calculations

The average weight of the gutted fish used in the main experiment (II) was 1.77 kg (± 0.36) and the average length was 63.06 cm (± 4.82). The yield after filleting was 51.6% (± 2.61), with an average fillet weight of 0.46 kg. Yield was calculated as a function of raw fillets after each processing step. During brine-salting the weight of fillets increased, resulting in a yield of 106.2% and 105.9% for the polyphosphate-treated (PP-treated) and control fillets, respectively. The addition of polyphosphate to the brine improved yield significantly ($P < 0.05$) both after dry-salting and storage. However, the opposite was true for the rehydration where the PP-treated fillets increased their weight less than fillets from the control group (Figure 1). For example, the yield was 98% for the PP-treated fillets, but 100% for the fillets from the control group (P > 0.05). The conclusion was that Brifisol 512 polyphosphate blend in the concentrations used could improve yield during salting but not after rehydration (P < 0.05). The results supported the findings of Arnesen and Dagbjartsson (1973, 1974), who stated that phosphate would improve yields after salting. However, they did not test the rehydration of the salted cod.

Water and salt content in fillets.

The water and salt content (% w/w) was determined after each step in the process (Figure 2), but no significant differences were observed between fillets from the test group containing polyphosphate and the control group (P > 0.05). The recovery of water (calculated from mass balance) in the PP-treated fillets was found to be slightly higher after dry-salting and storage (PP-test II - 54.7% and control II - 51.8%), but the difference was not statistically significant (P > 0.05). After rehydration, water recovery was 98.4% for the PP-treated fillets and 102.3% for the fillets from the control group which had gained slightly in weight. The results indicated a slight reduction in water content in the PP-treated fillets.

Phosphate content of fillets

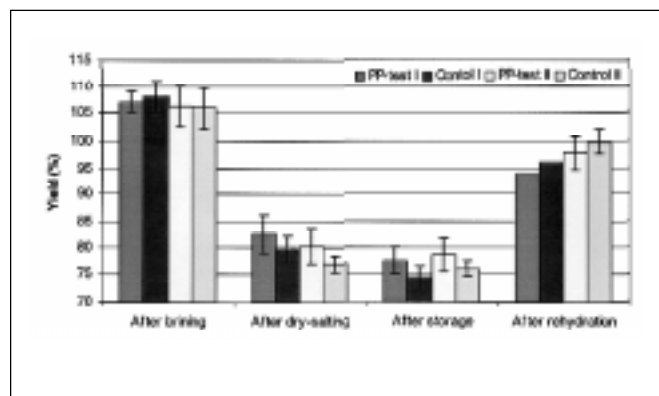Polyphosphates were absorbed by the cod muscle during

Figure 1—Changes in yield during salt-curing and rehydration (PP-test stands for polyphosphate addition, I and II, for the preliminary and main experiment, respectively)

Table 1—Phosphate content (mg P₂O₅ /g sample) in raw material and cured fillets

	Raw material	After brine-salting	After storage	After rehydration
PP-test (II)	4.41 ± 0.42	5.98 ± 1.02	4.17 ± 0.40	1.02 ± 0.26
Control (II)	4.41 ± 0.42	3.33 ± 0.86	3.39 ± 0.49	0.99 ± 0.24

Table 2—Quality rating in the preliminary experiment - % of fillets in each of 3 categories

	A	B	C
After dry-salting			
PP-test I	82.6	17.4	0.0
Control I	91.3	8.7	0.0
After storage			
PP-test I	19.0	61.9	19.0
Control I	100.0	0.0	0.0

the initial brining, but the content decreased during dry-salting, storage, and rehydration. Before the salting process, the phosphate content of the fillets was 4.41 mg P_2O_5/g sample. After brine-salting, the PP-treated fillets contained 5.98 mg P_2O_5/g sample and the control fillets 3.33 mg P_2O_5/g sample (Table 1). The difference between phosphate content in the test and control groups was only significant after brine-salting ($P < 0.05$). After rehydration, the fillets from both groups contained similar amounts ($P > 0.05$) of phosphate, approximately 1 mg P_2O_5/g sample. That correlated with results of the preliminary experiment, where control fillets and the thinner parts (tail end) of the PP-treated fillets contained 1.2 mg P_2O_5/g sample after rehydration. The thicker parts of the PP-treated fillets contained 1.7 mg P_2O_5/g sample, which could be explained by the shorter rehydration period in the preliminary experiment than was used in the main experiment. The results suggest leakage of polyphosphate during processing.

Protein content and recovery

Protein content in the raw fillets was 17.5% 0.44. During the salting process, the ratio of protein in the muscle increased due to dehydration of the muscle (Figure 3). Changes in protein content of the PP-treated fillets and the control fillets were similar throughout the process. After rehydration, it was slightly higher in the PP-treated fillets 15.7% 0.62 than in the control fillets 15.0 0.73, but not significantly ($P > 0.05$). This was also true for the protein recovery, which was 86.7% for the PP-treated fillets and 85.8% for the control fillets after rehydration.

It has been stated that phosphates have a stabilizing effect on proteins (Linko and Nikkila 1961; Kijowski and Mast 1988). However, it must be kept in mind that changes in salt and water content during salting of cod were extreme and resulted in protein denaturation determined with SDS-phage and DSC analysis (Thorarinsdottir and others 2000b). As suggested above, the polyphosphates leaked out of the muscle during the process, but whether they affected the protein recovery could not be answered in this trial. Linko and Nikkila

(1961) stated that phosphates have a stabilizing effect on myosin at relatively low salt concentration (2%). However, the effect was dependent on the type of phosphate. Kijowski and Mast (1988) studied the effects of salt and phosphates on chicken meat proteins. They stated that addition of phosphate (0.5% pyrophosphate) stabilized myosin, but destabilized actin. Anion (Cl^-) binding to potential sites on proteins was thought to cause displacement of water molecules that stabilized the protein structure.

Changes in pH during the process

The pH in fish muscle decreased during the salting process. With added polyphosphate, a slight change was observed after brine-salting, from 6.9 in the raw material to 6.6 in the fillets. The pH of the brine (17.5% NaCl w/w) with added polyphosphate was 6.6 before fillets were placed into the brine, but 8.1 in the brine of the control group. After brining, the pH of brine for the two groups was the same 6.6. A more rapid drop was observed after dry-salting where the pH dropped to 6.1 and 6.2 in the fillets, with and without polyphosphates, respectively (Figure 4). During rehydration the pH rose again, to pH 6.6 and 6.7 for the test and control group, respectively. The use of polyphosphate did not affect the pH in the muscle significantly ($P < 0.05$); however, its average was slightly lower in the PP-treated fillets.

The changes observed in pH during processing might be affected by protein conformational changes. It has been suggested that increases in pH of pressure-treated (Angsupanich and Ledward 1998) and cooked cod (Poulter and others 1985) were due to a decrease in available acidic groups in the muscle.

Water holding capacity (WHC)

The reason for using the polyphosphate was to improve the WHC in the muscle and thereby increase the yield. The addition of polyphosphate did not improve ($P > 0.05$) the
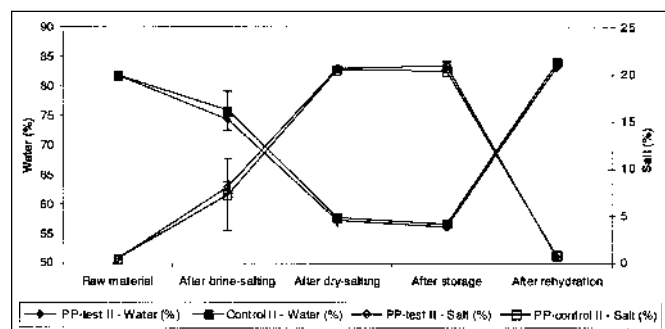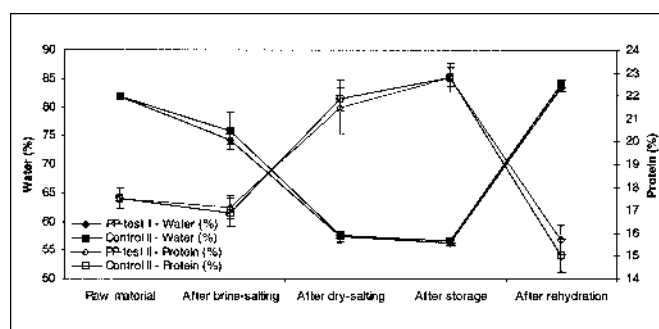

Figure 2—Changes in water and salt exchange during salt-curing and rehydration (PP-test II stands for polyphosphate addition for the main experiment)

Figure 3—Changes in protein recovery during salt-curing and rehydration (PP-test II stands for polyphosphate addition for the main experiment)

Table 3—Quality rating - % of fillets in each of 3 categories

	A	B	C
After dry-salting			
PP-test II	50.0	50.0	
Control II	52.4	45.2	2.4
After storage			
PP-test II	48.7	51.3	
Control II	64.1	33.3	2.6

WHC of the salted cod at any stage of the process (Figure 5). The WHC for the raw material was approximately 86%. After brining, it was approximately 95% for both PP-treated fillets and the fillets from the control group. Further processing led to decreased WHC (Figure 5).

The salt concentrations in fillets from the polyphosphate treatment and the fillets from the control group were 8.05% and 7.24%, respectively, and both had higher WHC than the raw material (Figure 5). After dry-salting and storage, the salt concentration was above 20% in both groups. There was a significant loss in WHC after dry-salting (Figure 5), which was believed to be due to protein denaturation at higher salt concentration. Lower WHC after rehydration may have been due to some irreversible degradation of proteins in the salting process.

The polyphosphates were expected to affect the hydration of the fish, due to influences on pH, ionic strength and specific interactions with the myofibrillar proteins, as was discussed in the Introduction. It must be kept in mind that denaturation of proteins in heavily salted cod is much higher than in lightly salted products where the use of phosphate seems to be more effective in improving WHC (Thorarinsdottir and others 2000b).

Several authors have reported on the effect of pH on WHC in fish and meat (Offer and Knight 1988; Fennema 1990; Foegeding and others 1996). The mean isoelectric point of the major myofibrillar proteins is about pH 5. Minimum water-holding capacity and swelling of meat has been observed around the isoelectric point, but it increases again with either decreasing or increasing pH, which results in stronger electrostatic repulsion forces and increased space for water to be held in the muscle (Offer and Knight 1988). Addition of salt affects the isoelectric point of proteins. The chloride ions selectively neutralize positively charged sites on the protein molecules, thereby effectively shifting the isoelectric point to a lower value and thus enhancing the protein solubility at the existing pH (Foegeding and others 1996).

Changes in muscle tissue of fish during salting have been discussed by many authors who have based their findings on studies of the effect of salt during fish and meat processing (Hamm 1960; Borgstrom 1968; Offer and Trinick 1983; Wilding and others 1986; Honikel 1989; Akse and others 1993). The general conclusions have been that WHC was increased at low concentrations of salt (0,5 to 5,0%). Very low salt concentrations 0 to 0.1 M or less than 0.5% induced shrinkage in the fiber due to screening effects by the salt and a reduction in natural repulsive charges. At approximately 0.1 M salt concentration or at the pI filament, spacing has been shown to be at a minimum. When the salt concentration was increased to 0.5 to 5%, filament spacing increased with increasing ionic strength. This may have been due to salt ions that bind to the proteins, causing them to assume a greater negative charge and become mutually more repulsive, or by less structured constraints to swelling and partial depolarization of the thick filaments, inducing dissociation of the actinomyosin complex (Fennema 1990). At higher salt concentrations in the muscle, above 9 to 10%, the proteins were believed to denaturate, causing shrinkage and dehydration of the muscle (Duerr and Dyer 1952). Repulsive forces between the proteins decreased and protein-protein bonds became stronger, resulting in less space for water in the muscle and less WHC (Offer and Trinick 1983; Honikel 1989). The dehydrating effect at higher salt concentrations has also been attributed to the precipitation of myosin (Lawrie 1998).

Quality rating and sensory analysis

The fillets were quality-rated after dry-salting and storage. The PP-treated fillets were difficult to classify into specific quality groups and could be described as wetter or having a "rawer" surface with a white precipitate forming small dots excreting from the flesh. The proportion of fillets in class A (Tables 2 and 3) was higher in the control group than in the test group, indicating a negative effect on quality by the polyphosphate. The difference between the test group and the control group was greater in the preliminary experiment, where the concentration of polyphosphate in the brine was 2.5% compared to 2% in the main experiment. Again, this indicated a negative effect of the polyphosphates used. These negative quality attributes noted in the dry-salted fish were not observed in the sensory analysis after cooking. No difference ($P < 0.05$) was found between test and control samples with the duo-trio test in the preliminary experiment nor with the paired difference test with the aid of discriminate analysis in the main experiment for the sensory analysis of the cooked fish.
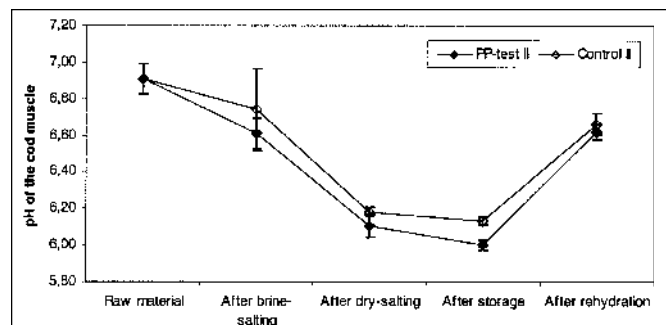
Figure 4—Changes in pH with salt-curing and rehydration (PP-test II stands for polyphosphate addition for the main experiment)
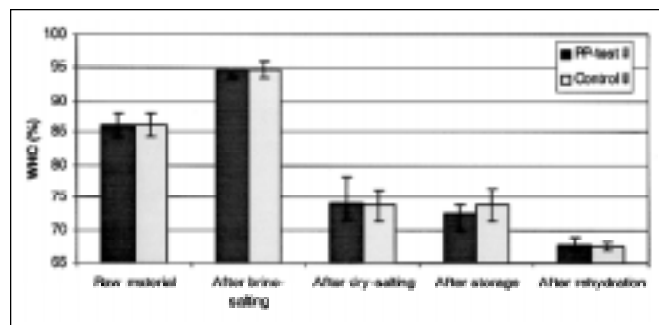

Figure 5—Changes in water holding capacity (WHC) in raw material, during salt-curing and rehydration (PP-test II stands for polyphosphate addition for the main experiment)

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Conclusion

THE PP-TREATED (BRIFISOL 512) FILLETS LOST LESS WEIGHT than the control fillets after dry-salting and storage up to 3 wks. However, the quality of the PP-treated fillets was poor compared to the untreated fillets. Salted cod is generally sold in its dry-salted form, so any profits made by gain in weight may be lost by the poorer appearance of the fillets. The weight gain of the PP-treated fillets over the control was lost during rehydration, a step normally performed by the consumer. However, the visual decrease in quality manifested by the quality rating was not carried over to the sensory attributes after cooking of the salted cod. Considering the entire process, the use of this polyphosphate blend at the concentrations tested cannot be recommended. Further studies are needed to evaluate the effects of different types of polyphosphates used at various concentrations.

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