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Phosphate treatment of light and heavy salted cod products

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Abstract

Project activities have been divided in a previous small scale trial, and a large scale trial with industrial production of salted cod. Analytical and sensorial evaluations have been carried out on the final products and after chilled storage. Both light and heavy salted products, and different salted cod production methods, were studied. The raw material presented large variability in quality which in addition to analytical uncertainty contributed to difficulties in interpretation some of the results.

Pre-salting steps represented by injection of phosphates solubilized in brine were the most effective method for the absorption of phosphates and better yield gains were obtained. The quality of the fish seems also to be improved in some cases, but more studies need to be carried out. All the obtained improvements in salted cod quality comply with the present legislation phosphate threshold. It has also been confirmed that important levels of natural phosphorus is lost during salting processes.

The effectiveness of phosphate additives in the reduction of the fish oxidation and stabilizing color has been rather diffuse. No induced metal chelation was detected in the experiments and sensorial evaluation showed contradictory patterns in some of the trial results. However, positive results were obtained in light salting. Further research is needed in testing the effects of phosphates in varying cod quality during large scale production.

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PREFACE

This report is a deliverable of the project ”Stabilizing quality of light salted and fully cured cod” which has been funded by The Norwegian Seafood Research Fund (FHF). Research consortium is composed by the participation of Møreforsking Marin, Nofima Marin and Anfaco-Cecopesca.

Polyphosphates are permitted additives in fish processing mainly because of its effect on water retention and reduction of drip loss during thawing. The application of phosphates during the salting processes is banned by international legislation, though in practice, salting companies from different countries avoid international prohibition based on the carry-over principle.

It has not been well defined if this group of compounds develops substantial benefits in the production of salted fish. This project aims to test the effects of phosphate additives in cod salting. Results will aid to determinate the positive and negative effects of using phosphate during the salting processes. Results will also help defining whether di- and triphosphates should be considered as additives or processing aids, opening a discussion within the international legal background this issue entails.

Ålesund 4.7.2012

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Scientist

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SUMMARY

Project activities have been divided in a previous small scale trial, and a large scale trial with industrial production of salted cod. Analytical and sensorial evaluations have been carried out on the final products and after chilled storage. Both light and heavy salted products, and different salted cod production methods, were studied.

The raw material presented large variability in quality which in addition to analytical uncertainty contributed to difficulties in the interpretation of some of the results.

Pre-salting steps represented by injection of phosphates solubilized in brine was the most effective method for the absorption of phosphates and better yield gains were obtained. There were indications that the quality of the fish was improved in some cases, but further investigations are required to confirm this. All the obtained improvements in salted cod quality comply with the present legislation phosphate threshold. It has also been confirmed that high levels of natural phosphorus are lost during the salting processes.

The effectiveness of phosphate additives in the reduction of the fish oxidation and stabilizing color has been rather diffuse. No induced metal chelation was detected in the experiments and sensorial evaluation showed contradictory patterns in some of the trial results. However, positive results were obtained in light salting.

Further research is needed in testing the effects of phosphates on varying raw material quality during large scale production.

1 INTRODUCTION

1.1 Background and objectives.

Møre Research Marin contributed with information regarding process development and process aids in the salt fish industry in a meeting between FHL and the Norwegian food safety authorities in late 2010. The purpose of the meeting was to discuss previous technology and process development in salt fish products, and inform about possibilities and limits for the use of phosphate in today's salt fish production.

Several media features on the use of phosphates in the fisheries sector have created a great interest in this subject from both the consumers, governments and commercial actors. Media elements show that consumers are sceptical of the use of phosphates, that there is confusion about what is allowed and how companies should interpret the regulations.

The meeting with the Norwegian food safety authority, FHL, commercial actors and Møre Research was an important arena to discuss legislation and enforcement of regulations, definitions and effects of additive and process aids, as well as determine further research needs within this theme. The results of the meeting between FHL and the Norwegian food safety authority was that Norwegian food safety authority clearly communicated that there was no room to define phosphate as a process aid after today's interpretation of the legislation. FHL, on its side, meant that the way they interpreted the legislation there was an opening for this, but took note that the Norwegian food safety authority had a different opinion.

One of the main explanations for how Iceland has taken over the Spanish market of salted fish has been associated with the use of phosphate and that Iceland has enforced the prohibition against the use of phosphate in light salted and fully salted fish differently from other European countries. Phosphate is said to give a whiter and thicker fish (Thorarinsdottir *et al.* 2010) and the water holding capacity increases. Furthermore, it is said that phosphate-treated fish are juicier and have better texture than other salted fish. Knowledge of the use of phosphate in the production of salted fish is largely based on trials carried out by producers and is in only few cases documented in controlled, scientific trials.

Some of the main challenges in the production of salted fish is blood in the raw material, the fish becomes yellow (discoloration) during production and storage, and that the fish loses varying amounts of liquids out into cardboard boxes during storage, transport and sale.

Through Møre Research´s work with the use of phosphate in salted fish in the fall of 2010, a review of the literature in this area has shown that very few scientific experiments have been carried out. Therefore there is a significant need for more work to examine the effects phosphates on salted fish. Different types of phosphates have different properties that can help to resolve or reduce the main problems listed above.

Some phosphates can bind iron (blood) and other metals that could potentially cause the fish to oxidize during storage, as well as the blood may be able to be extracted from the raw material during salting. Both of these effects will potentially give whiter salted fish. Moreover, phosphates could increase the WHC in salted fish. This can be used (but also abused) to increase the yield, but the yield/water content will largely be determined by what is acceptable in the various markets. By increasing the WHC may result in that fish releases smaller amounts of liquid during storage. It will be beneficial both to save weight, but also to avoid the brine from leaking out into the environment during storage, transport and sale. Phosphates can also affect the sensory characteristics such as texture and juiciness because the water binding properties can be affected (cooking loss is reduced).

There can be a significant potential for phosphates to have several positive effects on seafood and phosphates can help increase the quality of some of these products. To clarify whether these conditions also are applicable for salted fish, controlled experiments at both the small and industrial scale need to be conducted. Based on this a project on determining the effects of phosphates on quality and stability of light and heavy salted cod was carried out. The effects of the phosphate on the products, and the remaining levels of phosphates in the final products will largely determine whether phosphate should be regarded as a processing aid or additive.

1.2 Objectives

The main objective in this project is to **document the effects of phosphates on light salted and salt cured fish during processing and storage. We will approach this subject** through the following targets:

- **Target 1: To document how phosphates affect the amount of blood in the raw material and salted products**

- **Target 2: To map how phosphates affect the development in color during production and storage**

- **Target 3: Investigate how the storage stability of the products concerning liquid drop and yield are affected by phosphates**

1.3 Chemical and functional properties of phosphates

1.3.1 Phosphate additives

Phosphates comprise a group of compounds derived either from mining, refining or the combination of phosphoric acid with sodium, potassium, calcium and ammonium salts. Examples of phosphate additives are listed in Table 1.1. Under controlled temperature and pH conditions ortophosphates can polymerize to pyrophosphates. If more intense time and temperature are applied, other substances of a higher molecular weight are formed.

Considering their structure, phosphates can also be classified in monophosphates/orthophosphates, linear chained polyphoshates, and metaphosphates which bond together in a cyclic structure. Condensation determines the physical appearance of the molecule formed. The physical appearance is sometimes used as a descriptive term. The crystalline phosphates include the pyro's, tripoly's and trimeta- phosphates. The glassy phosphates comprise the hexametaphosphate and longer chained phosphates.

Table 1.1. Phosphate additives nomenclature

ORTOPHOSPHATES	SHORT NAME	FORMULA	ADDITIVE CODE
Orthophosphoric acid	(PA)	H_3PO_4	E-338
Monosodium phosphate	(MSP)	NaH_2PO_4	
Disodium phosphate	(DSP)	Na_2HPO_4	E-339
Trisodium phosphate	(TSP)	Na_3PO_4	
Monopotassium phosphate	(MKP)	KH_2PO_4	
Dipotassium phosphate	(DKP)	K_2HPO_4	E-340
Tripotassium phosphate	(TKP)	K_3PO_4	
Monocalcium phosphate	(MCP)	$Ca(H_2PO_4)_2$	
Dicalcium phosphate	(DCP)	Ca_2HPO_4	E-341
Tricalcium phosphate	(TCP)	$Ca_3(PO_4)_2$	
Monoammonium phosphate	(MAP)	$NH_4H_2PO_4$	E-342
Diammonium phosphate	(DAP)	$(NH_4)_2HPO_4$	
Monomagnesium phosphate	MMP)	$Mg(HPO_4)_2$	
Dimagnesium phosphate	(DMP)	$Mg2HPO_4$	E-343
Trimagnesium phosphate	(TMP)	$Mg_3(PO_4)_2$	

PYROPHOSPHATES	SHORT NAME	FORMULA	ADDITIVE CODE
Sodium acid pyrophosphate	(SAPP)	$Na_2H_2P_2O_7$	
Trisodium acid pyrophosphate	(TSAPP)	$Na_3H\ P_2O_7$	
Tetrasodium pyrophosphate	(TSPP)	$Na_4P_2O_7$	E-450
Tetrapotassium pyrophosphate	(TKPP)	$K_4P_2O_7$	
Calcium pyrophosphate	(CPP)	$Ca_2P_2O_7$	
TRIPHOSPHATES	**SHORT NAME**	**FORMULA**	**ADDITIVE CODE**
Sodium tripolyphosphate	(STP)	$Na_5P_3O_{10}$	E-451
Potassium tripolyphosphate	(KTP)	$K_5P_3O_{10}$	
METAPHOSPHATES	**SHORT NAME**	**FORMULA**	**ADDITIVE CODE**
Sodium trimetaphosphate	(STMP)	$(NaPO_3)_3$	
Sodium hexametaphoshate	(SHMP)	$(NaPO_3)_6$	E-452
Sodium polyphosphates	(SHMP)	$(NaPO_3)_n$	

1.3.2 Stability

Polyphosphates tend to revert back to the more stable congeners. Some have extremely long shelf life, while others revert to orthophosphate more quickly. Hexametaphosphate readily depolymerizes in aqueous solutions to form trimetaphosphate and orthophosphates. Stability is closely related to the temperature and pH of the medium. It is known that in aqueous solutions, high temperatures and

low pH favors the products hydrolysis, meanwhile low temperatures and relatively neutral or basic conditions favor stability in these compounds.
Several studies in different products stated that endogenous phosphatase enzymes in the product cause significant hydrolysis of polyphosphates even at chilled or frozen conditions (Sutton, 1973; Tenhet et *al.*, 1981; Reddy and Finne, 1986).

1.3.3 **Toxicity**

Phosphates are not considered toxic substances, with acute health effects comparable to common salt. In fact, some common medical practices consist of the intake of large amounts of phosphoric acid (20 g/day) to compensate for the lack of acidity in the stomach, without causing side effects. Phosphorus is also an essential nutrient, and an estimation of an adult needs ranges from 0.8 to 1 gram per day.

Some studies have reported that phosphates decrease the absorption of calcium, iron, magnesium and other minerals. However, the simple effect of phosphates does not appear to be important, unless they are presented combined with some vegetal compounds (as phytic acid). In animal experiments, phosphates can cause kidney problems, kidney stones, etc., but only at very high doses, much higher than those found in foods, even when they were used at levels higher than legal tolerances.

Existing data show a similar degree of toxicity among the congeners of each of the four groups of phosphates, and even among groups. However, the most important parameter is not the amount of phosphates per se, but the phosphorus/calcium ratio. This should preferably be between 1 and 1.5. In the case of diets low in calcium, phosphate intake is less recommendable than in diets rich in calcium (Weinera et *al.,* 2001).

The WHO/FAO Joint Expert Committee on Food Additives (JECFA) reviewed the safety of all food additives, addressing potential exposure to phosphorus based food additives. This group concluded that exposure to inorganic phosphate via food in the quantities used today is still far below the acceptable daily intake (70 mg / kg body weight) and therefore is not likely to cause adverse effects on consumer´s health. Surveys conducted by the International Food Additives Council (IFAC) stated that added phosphates only represent an estimated 10% of the maximum tolerable daily intake. All these data may explain why phosphates have the FDA categorization of “GRAS” (Generally Recognized As Safe) and its legislative control is not due to their toxicological effects, but to a possible consumer fraud due to elevated water binding.

1.3.4 **Utilisation of polyphosphates and their use in the seafood industry**

Phosphates are used in a wide range of products and industrial processes such as fertilizer dispersant, antifreeze, mineral supplements in feed and food, emulsifier, texture stabilizer, colour stabilizer, pH control, etc. Phosphate compounds exhibit different characteristics that are essential for their intended use. The most important features related to their application in the seafood industry are water binding capacity, buffering effect and ion chelating properties.

Product moisture is normally associated with the water-holding capacity and pH of the protein. All fish contain the protein actomyosin, which is responsible for retaining water. In living muscle, natural phosphate ATP (adenosine triphosphate) is the structure that controls actomyosin. After death, the biochemical reactions induces a fall in muscle pH, ATP levels decrease and proteins bound lose their ability to retain water, so the product becomes dry and with a fibrous texture. After harvesting, these myofibrillar proteins in fish are rapidly denatured at chilled conditions (5 °C) and may lose up to 80 % of its capacity to retain water within 5 days. Similar changes in beef take place after 45 days at temperatures above 20 °C. The poor resistance of these delicate proteins is a major drawback in seafood processing resulting in negative economic consequences for industries. Liquid lost during thawing and cooking contains vitamins and minerals negatively reducing the nutritional value and sensory quality of the product. In addition, drip loss creates protein suspensions that promote bacterial growth, and shortening product shelf life. Phosphate additives protect the protein ability to bind water, maintaining the natural humidity of the product with minimum drip loss during frozen storage, thawing or cooking.

The affinity of the fish protein varies with the phosphate type. Pyrophosphates and tripolyphosphates develop a more intense effect in protein water binding than ortophosphates and metaphosphates (Lampila, 1993), which almost develop no water retention properties not linked to their pH stabilizing ability.

The pH of the phosphate solution also depends on the selected blend and the phosphate salt used. In fresh water, pH values of diphosphate and tripolyphosphate tend to be basic (pH = 9-10). Monophosphate salts exhibit different pH values ranging from 4.5 to 10 depending on the level of hydrogen substitution for sodium, calcium or potassium. Metaphosphate solutions usually remain in neutral-basic pH conditions. The higher pH buffering capacity of monophosphates helps pyro and tripolyphosphate blends show neutral values, as well as the addition of metaphosphates, since they produce some monophosphates once in solution. Salt brine of 18-20 % usually shows a pH value around 8.5-9. A blend of pyro-tripolyphosphate (1:1) drops pH to 6.7. The addition of monophosphate to this blend increases pH to 7-7.3. Therefore the selection and design of the phosphate blend should take into account the final pH for the intended use.

Another beneficial function of phosphates include the ability to sequester and chelate metal cations such as Ca^{2+}, Mg^{2+}, Cu^{2+}, Zn^{2+}, Fe^{2+} and Fe^{3+}. Ion chelation helps to inhibit the development of oxidative rancidity and stabilizes colour. Besides, chelation of Ca^{2+}, and Mg^{2+}, also positively influences the water holding capacity (WHC). Phosphate chelation seems to be more effective against Zn^{2+} and Mg^{2+}, than Ca^{2+} and Fe^{2+}. Long-chained polyphosphates seems to show better results for lighter (Mg^{2+}- Ca^{2+}) metals meanwhile pyro and tripolyphosphates seems to have more affinity for heavier metals (Zn^{2+} and Fe^{2+}) (Kim et *al.*, 2009). Chelation may help to remove/neutralize blood iron from fish tissues and therefore preventing its oxidative effect.

Large chemical companies like Budenheim or Thermphos, among others, have developed tailored phosphate blends (BRIFISOL, CARNESAL, AFASOL, etc), which are presently available in the international market for almost every foodstuff and target application.

For fish and cephalopod processing, blends of sodium tripolyphosphate (STP), sodium acid pyrophosphate (SAPP) and / or tetrasodium pyrophosphate (TSPP), and sodium hexametaphosphate (SHMP), are frequently used since they combine good solubility, pH adjustment and selective chelation performance.

In surimi processing, the main interest of phosphates is as a cryoprotectant for proteins to develop later optimal gelification and generally short-chained polyphosphates are used. Sodium acid pyrophosphate (SAPP) is appropriate in canned cephalopods, taking advantage of its combined chelation and buffering effect. For crustaceans combinations of pyrophosphate and tripolyphosphate are frequently used to stabilize the quality of the product. Monophosphates are not commonly included unless an intense pH buffering effect is needed.

Application of phosphates varies depending on the product and desired result. The most extended application is by dipping and immersion in a bath containing phosphates at low temperatures (0-4 ^{o}C). Time of immersion and concentration are key factors and should be carefully studied to match production timing and obtaining the proposed effect in the product. Fish thickness, initial moisture contents and muscle nature are also important factors to include in the immersion time estimation. Other types of application are tumbling, glazing, spraying, and dry addition in minced products. Injection is also important and is a common practice in light-salting, where a brine containing phosphates is needle injected directly into muscle tissue, taking advantage of the osmotic diffusion to spread salt and phosphates along fillets (Gonçalves, 2008).

An excessive grade of phosphate treatment may develop sensorial defects like texture hitches, unnatural soapy surface, metallic taste, or even phosphate crystals precipitation during cooling and eventually reduced solubility. Phosphate concentrations generally oscillate between 2 and 5 % in general applications, and exposure time range from minutes to hours.

Kin et *al.* (2010) studied the effect of different blends of phosphates (BRIFISOL-BK Giulini Corp.) injected in catfish fillets in a process parallel to light-salting of cod fillets. BRIFISOL 550 (sodium tripolyphosphate, monophosphate and short chained polyphosphates blend) showed the best results in water retention probably by means of increasing muscle pH against other blends. These results were not obtained when applying this blend through vacuum-tumbling in previous trials (Kin *et al.,* 2009). All phosphate treatments increased tenderness and reduced negative colour development in fillets. BRIFISOL 550 again achieved best results in decreasing yellowness.

1.3.5 **Phosphate levels in heavy salted fish**

Thorarinsdottir et *al.,* 2001 studied the effect of phosphates in the cod salting process. Significant increase in weight yields was obtained between phosphate treated samples and control after brining, as it had been previously reported by Arnesen and Dagbjartsson (1973, 1974). No differences were found after rehydration.

Phosphorus levels increased from raw material to brined cod in phosphate treated samples, meanwhile control levels decreased. Rehydration caused the leakage of phosphates from muscle tissues resulting in no different final phosphorus levels between both samples. This final phosphorus content was below 25 % of the raw material levels. Quality evaluation showed better results in control than phosphate-treated fillets after salting, but not after rehydration or cooking.

Schröder´s (2010) studies of pacific cod salting, differ considerably from Thorarinstottir´s previous results. Injection of CARNAL 2110 (4 %) prior to brining and standard dry-salting resulted in no weight gain during the salting process compared to control samples. Schröder reported loss of phosphates during thawing of frozen cod from 4,4 g P_2O_5/kg to 3,6 g P_2O_5 /kg. After injection phosphate levels in phosphate-treated samples increased to 8,1 g P_2O_5 /kg. Meanwhile control samples decreased to 2,7 g P_2O_5/kg. Brining decreased these phosphate contents and dry-salting did not extract any of these phosphates from cod muscle. Similar to Thorarinsdottir´s studies, rehydration significantly reduced phosphorus levels even below natural contents. In this case there was still a different final level in rehydrated samples between phosphate treated samples and control (2,9 and 0,7 g P_2O_5 /kg respectively).

1.4 Present legal status.

It has been previously commented that phosphates are not considered to be toxic. However, when used improperly, excessive moisture absorption can characterized as consumer fraud. This is the main reason why these phosphates and illegal to use in most fish products.

Current EU additives legislation is under the scope of Regulation 1333/2008/EU repealing previous Directive 95/2/CE and their respective national transpositions. This directive addresses all issues concerning the application of additives to foodstuff from basic definitions and carry-over principle to procedural and labeling criteria. It also states the need of a common list of permitted additives, and also the development of an additives monitoring program. This program including timing for the re-evaluation of the different groups of additives is presented in the Regulation 257/2010/EU, and states phosphates evaluation deadline in December/2018.

Recent positive list of additives complementing Regulation 1333/2008/EU has been broadcasted in the shape of Regulation 1129/2011/EU (still in the transitional period); so until June 2013, official additive limits are those laid down in Directive 95/2/EC and in their respective national transpositions. In fishery products, international legal

background is heterogenic since; for instance, EU and Brazil establish a maximum level of additives at 5 g/kg, meanwhile FAO Codex Alimentarius Commission of WHO considers up to 10 g/kg. In contrast, USA and Canada allow free use under Good Manufacturing Practices. Concerning the use of phosphates, the new legislation does not change the previous status.

Table 1.2. Legislation concerning using of Phosphates

09	Fish and fisheries products				
09.1	Unprocessed fish and fisheries products	E-CODE	ADDITIVE NAME	Max. level (mg/kg)	Restrictions
09.1.1	Unprocessed fish	E 338-452	Phosphoric acid. Phosphates (di-tri) and Polyphosphates	5 000	Only for frozen fish fillets.
09.1.2	Unprocessed molluscs and crustaceans	E 338-452	Phosphoric acid. Phosphates (di-tri) and Polyphosphates	5 000	Only for frozen fish fillets.
09.2	Processed fish and fishery products including molluscs and crustaceans	E 338-452	Phosphoric acid. Phosphates (di-tri) and Polyphosphates	1 000	Only canned crustaceans products. Surimi and similar products
		E 338-452	Phosphoric acid. Phosphates (di-tri) and Polyphosphates	5000	Only fish and crustacean paste and in processed frozen and deep-frozen molluscs

As it is clearly shown (Tab. 1.2), the only possibility for phosphates to be legally present in salted cod is under the carry-over principle (being used during the freezing of cod raw material prior to salting). Some EU cod producers have been traditionally using phosphates based on the assumption that they are not additives but processing aids. This situation lead to some controversy, since producers from other countries claim the authorities that this unbalanced legal status among countries was behind some obtained market competitive advantages.

The Commission Standing Committee on the Food Chain and Animal Health (SCFCAH) (*SANCO – D1 (2011)D/310301)* addressed this situation in March 2011. The expert final evaluation was that ***"the use of polyphosphates during the processing and preservation of salted fish is as additives use, and not a processing aid".*** Committee experts were **not convinced** that additives are completely removed after rehydration and that they have no effect in the final product.

Common practices unveiled the need to emphasize the illegal use of phosphates during salting, and this lead to specific prohibitions in certain countries. Prohibition in Faroe Islands was enforced in November/2011. In Denmark the prohibition was effective from September/2011 and in Norway from January/2011. In Germany the prohibition is not enforced because of a court case (on whether the polyphosphates could be considered as a processing aid rather than an additive) allowing producers to continue production unchanged while awaiting the court's verdict (expected February 2012).

Icelandic prohibition is not enforced in practice yet, probably waiting for an eventual permission via an amendment to regulation 1333/2010/UE.

Considered as additives, phosphates use in food processing must be approved and included in the positive additives list of Annex II from Regulation 1333/2008/UE. In order to validate the inclusion of new additives, or new uses for current permitted additives, the request must undergo process detailed in Regulation 1331/2008/UE.

1.5 Official control compliance and analytical state of the art

The dosage of phosphate additives should respect current limits, determined by the combination of all phosphate additives, and expressed as g P_2O_5/kg. Diphosphates, triphosphates and polyphosphates may only be present due to the use as additives, while monophosphate can come from both technological and natural sources. There are some methodological limitations in the differentiation of natural phosphorus contents in foodstuff from the contents derived from the industrial use of additives. Furthermore, the effect of chemical and enzymatic degradation of polyphosphates is another drawback; and frequently develops analytical false negatives. No polyphosphates are detected after some time depending on the storage conditions, resulting in an increasing level of monophosphates due to the degradation of phosphate additives.

At present, the effective and real control of the appropriate use of phosphates by the authorities is very complex. The official method *(AOAC Official method 995.11)* is based on the determination of **total phosphorus** by the transformation and hydrolysis of phosphates and measurement of the yellow color produced by their reaction with a molibdate-vanadate solution in a UV-VIS spectrophotometer at 436 nm. Other spectroscopic methods commonly used such as FI / AAS (Flame ionization atom absorbance spectroscopy) and ICP-OES (Inductive coupled plasma - optical emission spectroscopy) are more sensitive, accurate and precise and perfectly valid.

The official method allows the identification and quantification of the total amount of phosphorus either coming from natural or from industrial processing sources.

The use of this total phosphorus method to control phosphate addition is unfair and inconsistent with the legislation criteria, that clearly addresses that the limits are just for the combination of added phosphates up to 5 g P_2O_5/kg. Sea foodstuff commonly develop natural phosphorus levels bordering or even exceeding the marked thresholds, also generating false positives. The natural variation of phosphorus in the species also creates problems to standardize the additives dosage to achieve the desired technological effect, and to comply with regulation.

Authorities are aware of this problem and, in general, do not create any trade obstacle to food products unless the dosage of phosphates is extremely high. Arguments from producers explaining the situation to inspectors usually end up in freight release, despite the positive results.

Some **analytical approaches** have been made to overcome this problem trying to separate and quantify just the phosphate additives from natural phosphorus. All the analytical methods cannot avoid the degradation of phosphate additives prior to the arrival of the sample to the laboratory. Therefore, it should be emphasized that the estimation of the appropriate use of phosphates is not accurate, and may cause false negatives. Nevertheless, the detection of polyphosphates above the legal limit would always be a real positive. Several of these methods are described below:

Method based on Nitrogen/Phosphorus permanent Natural Ratios has been applied by Italian administration *(Rapporti INTISAN 96/34)* to establish natural phosphorus content, and then calculate added phosphates by differences to total phosphorus. Basic assumption of the steadiness of this N/P relationship may work for some foodstuff (dairy products) but it is not appropriate for the evaluation in fish. Internal ANFACO-CECOPESCA studies have shown that this N/P ratio is not steady in fishery products, even within the same taxonomical group, and therefore cannot be applied for the accurate analysis of phosphates control. Nevertheless, establishing average natural phosphorus contents for each of the species (knowing and assuming its variability) can open the possibility to address the polyphosphate evaluation by simple subtraction from the total phosphorus results.

Several approaches based on ion-exchange chromatography techniques for the polyphosphates separation and quantification have been reported in the literature. Some references have evidenced its suitability for polyphosphates quantification (Kauffman *et* al., 2005; Krzynowek and Panunzio, 1995; Sekiguchi and Matsunaga, 2000; Cui et *al.*, 2000), but none of these opens the possibility for a complete phosphate additives evaluation. Besides accuracy, robustness is apparently still weak, so analytical improvements must be carried out prior to its routine use in quality control. In addition, this instrumentation is quite specific and therefore not really common in analytical laboratories. The extraction step is also very important, especially in preventing polyphosphates and methaphosphates degradation. The variety of the chemical features among the phosphate congeners makes it difficult to develop a single method for all the phosphates used by the industry, since solubility and affection by pH is specific.

Making use of the different polyphosphate degradation kinetics under controlled conditions, another method was developed known as **Thermo-differential photometry** (Kruse et *al*., 2005). In contrary to the fast monophosphate anion PO_4, condensed polyphosphates (P_2O_7 and P_3O_{10}) react much slower in the formation of the yellow molybdic vanadic acid. Differences of photometric extinction values measured at a first time after 15 minutes and finally after 90 minutes are dependent to the polyphosphate concentrations. It has also been applied in some studies carried out in ANFACO-CECOPESCA with good results in pyrophosphate and tripolyphosphate quantification. Nevertheless, results have been affected by the presence of metaphosphate congeners. Then, although this method has not undergone an intense validation, it shows good performance in those samples where cyclic phosphates are not present.

Methods based on **thin layer chromatography (TLC)** have also been applied for polyphosphates detection (Reece and Russell, 1994; Marescot et *al*., 1998), which are closely related to ISO 5553-1980 for the detection of polyphosphates in meat products by TLC. The department of Food Safety and Quality at ANFACO-CECOPESCA has adapted this method for the quantification of monophosphates, diphosphates, triphosphates and hexametaphosphate in fishery products. In this context, it has been proved that the stability of the target additives seems to be conditioned by the extraction steps, but it is still a good alternative for the evaluation of polyphosphates. Robustness is another factor to be improved, considering the general low robustness inherent to the TLC methods.

A new HPLC (High pressure liquid chromatography) method was developed in ANFACO-CECOPESCA´s laboratories, for this project, taking advantage of the performance of the CORONA CAD detector. Attending to literature Charged Aerosol Detectors seem very promising in the analysis of anions due to its capacity to detect molecules of different charge and size. Several columns and chromatographic conditions, trying to simulate ion-exchange chromatography, were used to separate these phosphate compounds. Initial extraction techniques of the analysts from fish samples were also tested.

2 MATERIALS AND METHODS

2.1 Raw materials

2.1.1 **Small scale trial**

Cod (*Gadus morhua* L.) caught in March outside the coast of Northern Norway using gillnets was used. This material (2,5-3,5 kg headed and gutted weight) was divided into two equal parts for two different sub-trials; one immediately processing the fresh fish, and another where cod was frozen (for 10 weeks) and then thawed and processed to light and heavy salted products.

2.1.2 **Large scale trial**

Cod (*Gadus morhua* L.) were caught with long line in the North Sea in early autumn (August-September). The raw material (weight class 1,0-2,5 kg - headed and gutted) was described as of lower quality than normal for long line on board frozen raw material, due to an abnormal dark colour/high content of blood. The material had been frozen for 10 weeks prior to overnight thawing and processing to heavy salted cod.

2.2 Sampling and Processing

2.2.1 **Small scale trial**

In both sub-trials with fresh and previously frozen cod, fillets without skin and bones were selected for raw material characterization. A piece from the most anterior part of the loin (200-250 gr.) was used for the determination of pyro- and triphosphates, the total phosphate level (P_2O_5) and the levels of metals. Another piece of the same weight was used for the analyses of oxidation. The last and most posterior part of the loin was used for the determination of water content, water holding capacity, and ash.

For both fresh and frozen raw material, four different heavy salting methods were used for comparison:

Method 1: Injection – Pickle salting with addition of brine (1kg brine: 5 kg fish) – Dry salting.
Method 2: Injection – Brining (bath) 24 hours at 2 - 4 ^{o}C – Drysalting.
Method 3: Pickle salting with addition of brine (1kg brine: 5 kg fish) – Drysalting.
Method 4: Pickle salting with addition of brine (1kg brine: 10 kg fish) – Drysalting.

For each of the four salting methods, Carnal 2110, a blend of sodium and potassium, pyro and triphosphate salts was used in trials in four different concentrations as

described in Table 2.1. 15 fillets were processed in each one of the groups described in Table 2.1.

Table 2.1. Small scale trial heavy salted groups.

Salting methods	Phosphate concentrations			
Injection – Pickle salting with addition of brine (1kg brine: 5 kg fish) – Drysalting.	Control (0 % P_2O_5)	0,4 % P_2O_5	0,8 % P_2O_5	1,6 % P_2O_5
Injection – Brining for 24 h – Drysalting.	Control (0 % P_2O_5)	0,4 % P_2O_5	0,8 % P_2O_5	1,6 % P_2O_5
Pickle salting with addition of brine (1kg brine: 5 kg fish) – Drysalting.	Control (0 % P_2O_5)	0,4 % P_2O_5	0,8 % P_2O_5	1,6 % P_2O_5
Pickle salting with addition of brine (1kg brine: 10 kg fish) – Drysalting.	Control (0 % P_2O_5)	0,4 % P_2O_5	0,8 % P_2O_5	1,6 % P_2O_5

Prior to the salting, weight, pH and instrumental color were determined on each fillet in all groups.

After five weeks of salting, weight, pH and instrumental color were determined. The salted cod fillets were submitted to sensorial evaluation by four scientists experienced in determining white and yellow colour, blood spots, belly blood, gaping and smell. Three fillets from each of the groups (except the group pickle salted with ratio 1:10) were taken out for determination of pyro- and triphosphates, total phosphate (P_2O_5), metals, oxidation, water content, WHC and ash in the same way as for raw material.

After additional five (fresh raw material) or seven (frozen raw material) months of storage; weight, pH and instrumental color were determined. The salted cod fillets were sensorial evaluated by four experienced scientists determining white and yellow colour, blood spots, belly blood, gaping and smell.

The same phosphate concentrations were applied in trials with light salting of cod. 10 fillets in each group were injected with 18 % NaCl brine containing phosphates, as it is described in Table 2.2. A layer of 10 % water was applied by glazing and fish were frozen stored for three months before thawing and analysis (Figure 2.1).


Figure 2.1. Glazing, packing and storage of light salted fillets.

After three months of storage, four fillets per group were thawed overnight at 2 ^{o}C and analysed as the raw material. Weight, pH and instrumental colour were determined. Samples from the muscle were taken out for determination of phosphate, metals, oxidation, water content, WHC and ash.

Table 2.2. Small scale trial light salted groups

Salting method	**Phosphate concentrations**			
Light salted by injection	Control (0% P_2O_5)	0.4 % P_2O_5	0.8 % P_2O_5	1.6 % P_2O_5

Samples from both heavy salted and light salted trials were appropriately packed and delivered by air service to ANFACO-CECOPESCA in Vigo, Spain. Salted cod fillets were kept chilled; and both raw materials and light salted cod fillets, were kept frozen. On arrival material showed neither symptoms of thawing nor sample damage. Samples were unpacked and stored in conditioned cold rooms until chemical analysis. ANFACO-CECOPESCA internal codification was applied as it can be seen in Table 2.3 and Table 2.4 for raw materials, heavy salted samples, and light salted samples.

Table 2.3. ANFACO-CECOPESCA sample codes for raw materials and heavy salted samples

FRESH RAW MATERIAL		
G.0	1105442	G.0.A
	1105443	G.0.B
	1105444	G.0.C
	1105445	G.0.D
	1105446	G.0.E

FROZEN RAW MATERIAL		
H.0	1109175	H.0.A
	1109176	H.0.B
	1109177	H.0.C
	1109178	H.0.D
	1109179	H.0.E

HEAVY SALTED

FRESH COD FILLETS

PROC	A/C internal code	0% - 1	A/C internal code	0.4% - 2	A/C internal code	0.8% - 3	A/C internal code	1.6% - 4
G.I	1105447	G.I.1.A	1105450	G.I.2.A	1105453	G.I.3.A	1105456	G.I.4.A
	1105448	G.I.1.B	1105451	G.I.2.B	1105454	G.I.3.B	1105457	G.I.4.B
	1105449	G.I.1.C	1105452	G.I.2.A	1105455	G.I.3.C	1105458	G.I.4.C
G.II	1105459	G.II.1.A	1105462	G.II.2.A	1105465	G.II.3.A	1105468	G.II.4.A
	1105460	G.II.1.B	1105463	G.II.2.B	1105466	G.II.3.B	1105469	G.II.4.B
	1105461	G.II.1.C	1105464	G.II.2.C	1105467	G.II.3.C	1105470	G.II.4.C
G.III	1105471	G.III.1.A	1105474	G.III.2.A	1105477	G.III.3.A	1105480	G.III.4.A
	1105472	G.III.1.B	1105475	G.III.2.B	1105478	G.III.3.B	1105481	G.III.4.B
	1105473	G.III.1.C	1105476	G.III.2.C	1105479	G.III.3.C	1105482	G.III.4.C

FROZEN COD FILLETS

PROC		0% - 1		0.4% - 2		0.8% - 3		1.6% - 4
H.I	1109180	H.I.1.A	1109183	H.I.2.A	1109186	H.I.3.A	1109189	H.I.4.A
	1109181	H.I.1.B	1109184	H.I.2.B	1109187	H.I.3.B	1109190	H.I.4.B
	1109182	H.I.1.C	1109185	H.I.2.A	1109188	H.I.3.C	1109191	H.I.4.C
H.II	1109192	H.II.1.A	1109195	H.II.2.A	1109198	H.II.3.A	1109201	H.II.4.A
	1109193	H.II.1.B	1109196	H.II.2.B	1109199	H.II.3.B	1109202	H.II.4.B
	1109194	H.II.1.C	1109197	H.II.2.C	1109200	H.II.3.C	1109203	H.II.4.C
H.III	1109204	H.III.1.A	1109207	H.III.2.A	1109210	H.III.3.A	1109213	H.III.4.A
	1109205	H.III.1.B	1109208	H.III.2.B	1109211	H.III.3.B	1109214	H.III.4.B
	1109206	H.III.1.C	1109209	H.III.2.C	1109212	H.III.3.C	1109215	H.III.4.C

Table 2.4. AFACO-CECOPESCA sample codes for light salted samples

LIGHT SALTED

FRESH COD FILLETS

PROC	A/C internal code	0% - 1	A/C internal code	0.4% - 2	A/C internal code	0.8% - 3	A/C internal code	1.6% - 4
L.V	1109216	L.V.1.A	1109219	L.V.2.A	1109222	L.V.3.A	1109225	L.V.4.A
	1109217	L.V.1.B	1109220	L.V.2.B	1109223	L.V.3.B	1109226	L.V.4.B
	1109218	L.V.1.C	1109221	L.V.2.A	1109224	L.V.3.C	1109227	L.V.4.C

FROZEN COD FILLETS

PROC		0% - 1		0.4% - 2		0.8% - 3		1.6% - 4
M.V	1111763	M.V.1.A	1111766	M.V.2.A	1111769	M.V.3.A	1111772	M.V.4.A
	1111764	M.V.1.B	1111767	M.V.2.B	1111770	M.V.3.B	1111773	M.V.4.B
	1111765	M.V.1.C	1111768	M.V.2.A	1111771	M.V.3.C	1111774	M.V.4.C

Three replicates were made in each of the groups except for the raw material characterization where five replicates were analysed.

2.2.2 Large scale trial

As in small scale trials, cod fillets were separated either for processing or for raw material characterization. Collection of muscle material was made by using the AOAC method “Official Method 937.07 – Fish and Marine Products sec. a”. Three pieces were taken out and skin and bones were removed (Fig. 2.2).

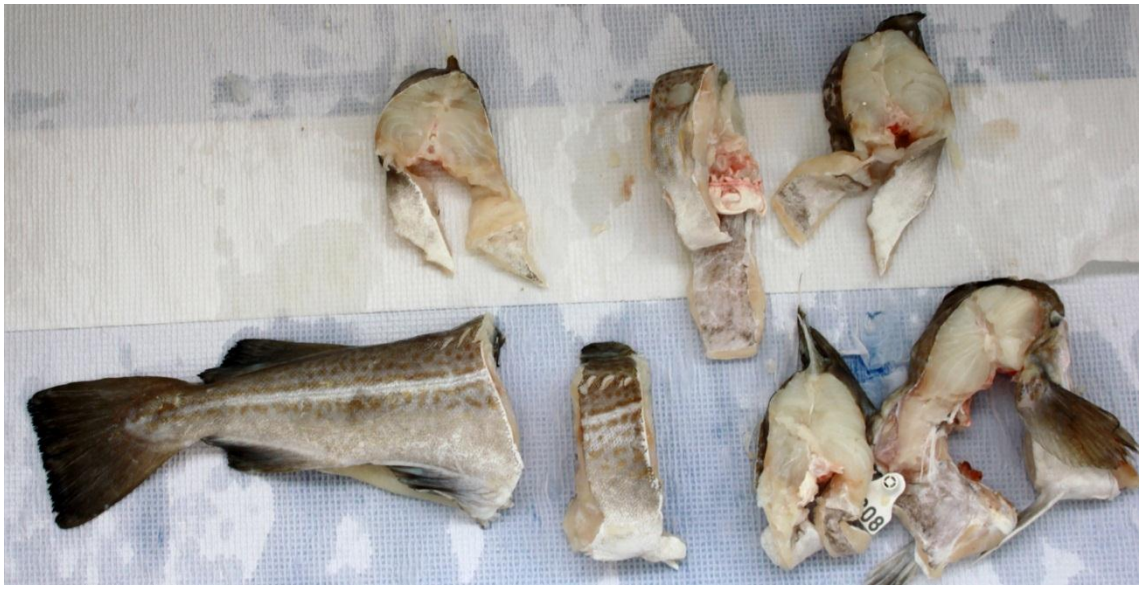


Figure 2.2. Sampling of raw materials. The three samples at the top were used in muscle analysis.

Heavy salting procedures was carried out at a processing plant during ordinary production of heavy salted, split cod in large industry scale tubs (1000 liters). Two different salting methods were tested in this trial as shown in Table 2.5. Because of sedimentation of the phosphate in the brines, the brine was continuously mixed during the trials.

Table 2.5. Large scale heavy salting trials

Salting methods	**Phosphate concentrations**		
Injection - Pickle salting (120 L of brine) - Dry salting	Control (0% P_2O_5)	1 % P_2O_5	2 % P_2O_5
Pickle salting (120 L of brine) –Dry salting	Control (0% P_2O_5)	1 % P_2O_5	2 % P_2O_5

One group was injected with brine and further pickle salted with addition of brine. After 14 days of pickle salting, the fish were dry salted for another 14 days. The other group was the same procedure without including the first injection step. Both the injected brine and the added brine contained 0 %, 1 % or 2 % Carnal 2110.

From each group 15 fish were taken out for analysis as shown in Fig. 2.3. Five fish for determination of water content and WHC. 10 fish were delivered to ANFACO-CECOPESCA facilities (five fish for phosphates and metals determination and 5 fish for TBARS and peroxide analysis).

Figure 2.3. Sampling of heavy salted cod before analysis. Samples with the muscle surface turned up were collected for analysis

After a five week storage period, ten fish were delivered to ANFACO-CECOPESCA. Five fish were sampled for the analysis of pyro- and triphosphates, quantification of metals (Ca, Na, K, P, Mg, Cu and Fe) and another five fillets were used for oxidation analysis. The same amount of fish was analyzed for the determination of water content, ash, and water holding capacity.

As the case of the small scale trial, all samples delivered showed no deterioration upon delivery, and were appropriately stored until analysis. In this trial, five replicates were analyzed for each one of the groups shown below in Table 2.6.

Table 2.6. ANFACO-CECOPESCA sample codes for large scale trial samples

FROZEN RAW MATERIAL		
J.0	17592	J.0.A
	17593	J.0.B
	17594	J.0.C
	17595	J.0.D
	17596	J.0.E

	PROC	A/C internal code	control (0%) - 1	A/C internal code	Carnal 2110 (1%) - 2	A/C internal code	Carnal 2110 (2%) - 3
FROM FROZEN COD FILLETS	G6	17597	G6.1.A	17602	G6.2.A	17607	G6.3.A
		17598	G6.1.B	17603	G6.2.B	17608	G6.3.B
		17599	G6.1.C	17604	G6.2.C	17609	G6.3.C
		17600	G6.1.D	17605	G6.2.D	17610	G6.3.D
		17601	G6.1.E	17606	G6.2.E	17611	G6.3.E
	G7	17612	G7.1.A	17617	G7.2.A	17622	G7.3.A
		17613	G7.1.B	17618	G7.2.B	17623	G7.3.B
		17614	G7.1.C	17619	G7.2.C	17624	G7.3.C
		17615	G7.1.D	17620	G7.2.D	17625	G7.3.D
		17616	G7.1.E	17621	G7.2.E	17626	G7.3.E

2.3 Analytical methods

A set of analytical determinations were applied to samples in order to extract the desired information and to be able to make comparisons among groups. Sensorial and basic analytical determinations were carried out in Norway; meanwhile special instrumental analyses took place in the certified laboratory at ANFACO-CECOPESCA.

2.3.1 **Sensorial methods**

Analysis consisted of white and yellow color determinations (Minolta Croma meter, CR-200, Japan) and the sensorial evaluation of a set of four or five scientists focusing on muscle colour, blood discoloration, belly blood, gaping and smell. Marks ranging from 0 to 3 were used for white and yellow color and gaping, where highest values determined the lowest quality. For blood spots and belly blood the marks 0 (no blood) and 1 (blood registered) were used. The smell characters were 0 (natural), 1 (deviant smell) and 2 (very deviant smell). In the large scale trial marks from 1 to 9 (highest quality) were used.

2.3.2 **Physical-chemical methods**

Water content (humidity) and ash content were determined by AOAC methods 950.46 B and 938.08 respectively; which basically consisted in gravimetric analysis of the samples before/after desiccation and combustion. Water Holding Capacity was carried out using the method of Ofstad et *al.* (1996) and pH using a pH sensor (WTW, pH 3310, Weilheim, Germany).

Oxidation methods targeted the evaluation of the primary and secondary oxidation processes. Primary oxidation begins as the long fatty acid hydrocarbon chain (L) loses one hydrogen atom, leaving a lipid radical ($L^{\cdot}$) which almost immediately reacts with the surrounding oxygen to create a peroxy-radical (LOO). Primary oxidation may be

originated by light radiation and boosted by the chemical reactivity of some ion metals as Cu^{2+}, Fe^{2+} and Zn^{2+}. Peroxy-radical (LOO) starts the propagation process extracting another hydrogen atom from the lipid chain to create a loop in the catalytic mechanism. Propagation continues unless some antioxidant molecules, as ascorbic acid or α-tocopherol, neutralize free lipid radicals to a less reactive compound. Final molecules are lipid hydroperoxides (LOOH), which can be detected by chemical methods, generally making use of their oxidation potential to oxidize iodide to iodine. A common method used in this project is the one developed by Pearson (1965), with light modifications. Basically it quantifies the idodine released by peroxides using titration with sodium thiosulfate giving the Peroxide Value (PV) as milliequivalents (mEq) peroxide per 1 kg of fat extracted from the fish. Although it gives a good estimation of an early state of oxidation, the Peroxide Value index is not directly comparable to sensory detectable defects. Besides, lipid hydroperoxides break down with time, and a low PV at a certain point during the storage of a product can indicate both, an early phase of autoxidation and a late stage of a severely oxidized product, where most of the hydroperoxides have been broken down. Provided that the PV has not been lowered through extended storage or high temperature exposure, the PV (by iodometric titration) should not be above 10-20 meq/kg fish fat (Connell, 1975).

Secondary oxidation produces smaller compounds as aldehydes, alcohols, ketones, etc. These products are behind some undesirable quality effects in fishery products as yellow tones in muscle and rancid aromas. The reaction of some of this wide range of metabolites to thiobarbituric acid can be measured, giving an estimation of the advanced oxidation state in fish muscle. TBARS is the most suitable method to estimate oxidation in highly unsaturated fats because of its simplicity. Several variations exist for TBARS, but the most extended is the one proposed by Wyncke (1970). In this project this method was used including the modification by Cervantes (1984). This method basically consists of a reaction between the lipid material and the 2-thiobarbituric acid to create a red-pink tone which is measured by UV-VIS spectrometry. TBA-results are expressed as mg malonaldehyde in 1 g of muscle tissue. Examples of guidelines for TBA-RS-values: foods with TBA-RS above 1-2 µmol MDA-equiv per g fat (Connell, 1975) or above 10, µmol MDA-equiv per 1 kg fish (Ke et al., 1976) will probably have rancid flavors.

The minerals determination of seafood samples involves a complete digestion of the samples in pressurised vessels with nitric acid and an oxidising agent (hydrogen peroxide). Samples undergo an 18 minutes heating program in a microwave-oven. After complete mineralization, samples are accurately dissolved in the desired final volume in volumetric flasks with Milli-Q water. Following this step samples are ready to be introduced in the ICP-OES instrument.

ICP-OES is an atomic emission spectrometry simultaneous technique where digested samples are pumped and nebulized in Argon plasma. All the generated spectra from electron transitions in all the present elements are captured in the detector, which is able to measure the intensities at every wavelength. Comparison to standard intensities allows quantification.

Despite its multi-elemental quantification possibilities, three methods have been developed for the analysis of seafood, due to the high differences in the amount of each of the elements present in seafood and technical hitches. At least two bands in each element where used for quantification.

Sodium and potassium. The ionic dissociation of potassium involves the use of an on-line addition ionic inhibitor (cesium chloride) and an internal standard (Yttrium). The short calibration range (1-20 mg/l) and the high sodium contents (heavy salted cod) lead to important sample dilution.

Micronutrients (Ca^{2+}, Fe^{2+}, Mg^{2+}, P, Zn^{2+}). The simultaneous analysis of these elements has been possible by means of the appropriate selection of the bands for each one of the elements. High grade acids and reagents and extreme care are required to avoid contamination (especially for iron).

Trace elements (Cu^{2+}, Sn^{2+}) Interfering bands and very low levels involve the development of a separate method for these elements. Sensitivity for some of these elements is reduced and does not allow quantification.

The low copper contents made the use of the graphite furnace atomic absorption spectrometry technique necessary. This technique is similar to the ICP-OES. It is a mono-elemental technique but the sensitivity is higher. It shares the preliminary digestion steps with the previous method but the digested sample is introduced in a small graphite furnace and then a thermal atomization takes place and the Cu^{2+} is detected at an accurate wavelength. Zeeman Effect and the detector allow the quantification of the absorption intensity of the element by comparison to the standard signal. The use of this method made possible the quantification of copper levels below 1 mg/kg.

Polyphosphates analysis by high performance thin layer chromatography (HPTLC) consists of a separation of trichloroacetic (TCA) fish extracts on a cellulose layer. After elution and developing, quantitative results are produced by a densitometric scanner at 586 nm. Method consisted on the steps detailed below:
Extraction: Homogenization of samples in ultraturrax is carried out with diluted TCA to dissolve the target compounds. Mixture is left to stand at 4 °C for 30 min before being vacuum filtered to a volumetric flask.
Injection: Injection is done soon after filtration to avoid polyphosphates degradation in acid medium. The injection is automatically performed by the LINOMAT 5 system, coupled to WINCATS software.
Elution in ADC-2: Mobile phase is prepared and poured in ADC-2 reservoir. Plate is also allocated inside and automatic elution is carried out up to a final distance of 8 cm.
Chromogenic reaction and Detection: After solvent residues evaporation, spraying with two different reagents ((1) tartaric acid in a solution of nitric acid and ammonium molybdate tetrahydrate, (2) 4-amino-3-hydroxy-1-naphthalenesulfonic acid in sodium metabisulphite and sodium sulphite solution mixture) is carried out creating blue spots. The detection and quantification by densitometry at 586 nm is performed by the system TLC SCANNER from CAMAG assisted by the WINCATS software.

Calculations and result expression**:** A calibration curve with tailored standards is previously constructed, to obtain quantization; the results are expressed as grams of P_2O_5 /100g.

3 RESULTS

3.1 Small scale trials with fresh and frozen raw material

3.1.1 Salting trials with fresh raw material

Trials with light salted fillets

Fillets were injected and stored on plastic covered trays before freezing. After one day in frozen storage (-30 ^{o}C) the fillets were glazed before further frozen storage in cartons. Light salted fillets were thawed and analyzed after 3 months of frozen storage. Results of yield, pH and color measurements are shown in Figures 3.1-3.3.

Weight yields after injection were similar for all groups (122-124 %). After frozen storage the yield was lowest for fish in the control group where no phoshpate was added (133 %) and highest for fish injected with 1.6 % phosphate (136 %). After thawing the same trend was observed.

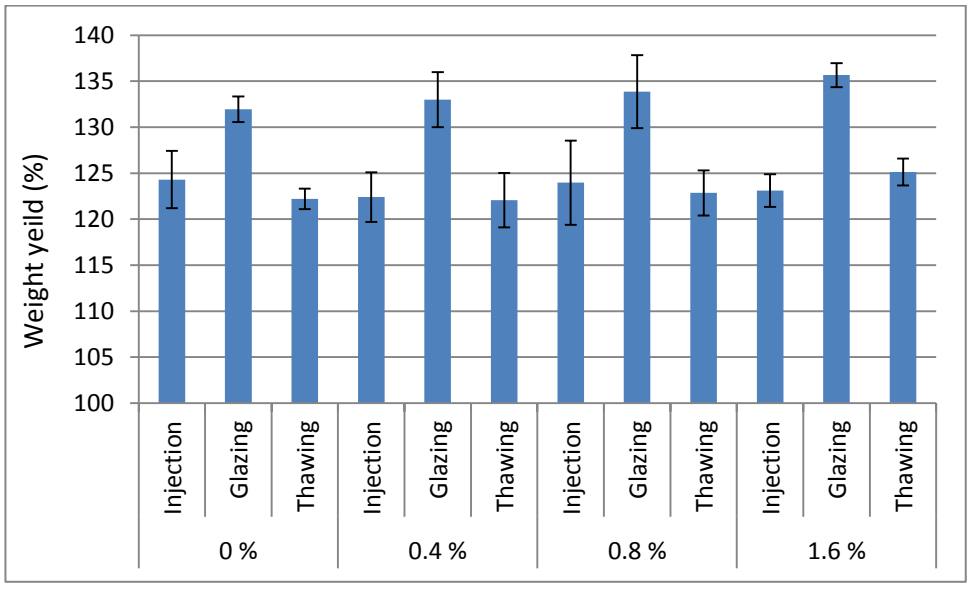


Figure 3.1. Weight yields of light salted fillets from fresh raw material. The weight yields were based on raw fillet weights and calculated after injection of 0, 0.4, 0.8 or 1.6 phosphate (N=10), glazing (before thawing) (N=4) and after thawing (N=4). Means and standard deviation of each group are shown.

Color determinations showed that L-values of raw material were 56-57 (Fig. 3.2). For thawed samples the L- value seemed to increase from 54 to 59 with higher phosphate levels.

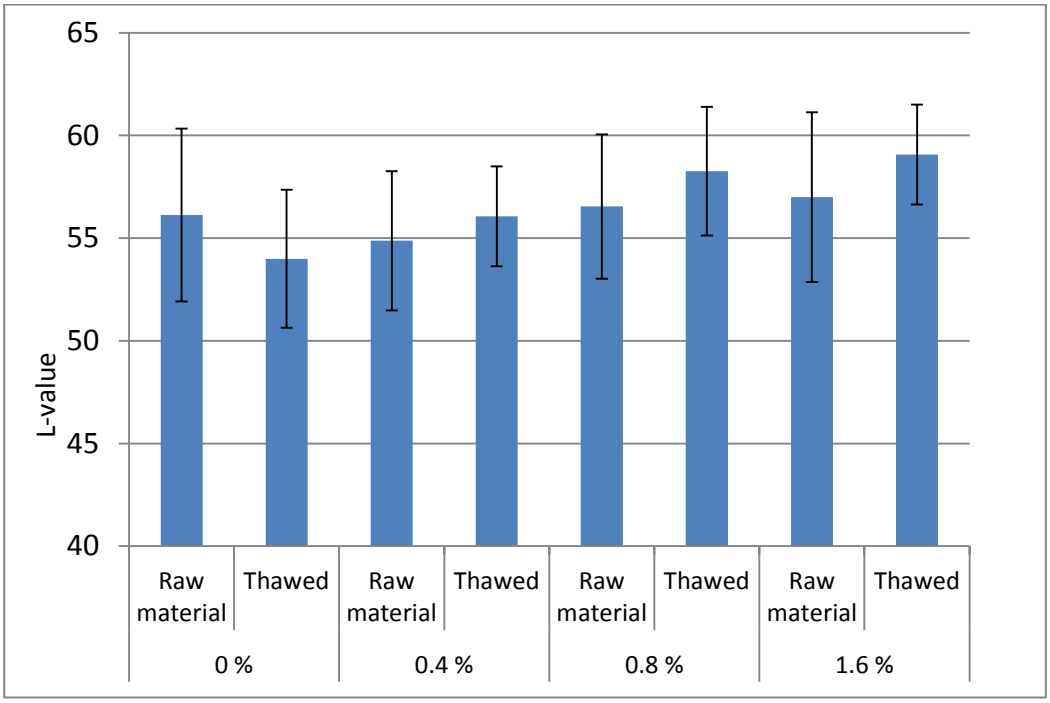


Figure 3.2. Instrumental measurement of lightness (L-value) on raw material (N=10) and after thawing (N=4) of light salted fillets injected with 0, 0.4, 0.8 or 1.6 % phosphate. Means and standard deviations are shown for each group.

The group treated with the highest phosphate concentration (1.6 %) had the highest score in the sensorial evaluation, with a whiter surface, less blood, less yellow color and less gaping. The three other groups were difficult to separate (Fig. 3.3). However, the variation in raw material quality was evident also after treatment for all groups.

Figure 3.3. Light salted fillets before (image above) and after thawing (below). Control (G51), 0,4 % P (G52), 0,8 % P (G53) and 1,6 % P (G54).

There was a decrease in pH from raw material pH (7.0-7.1) to the light salted groups after storage and thawing (pH 6,6) as shown in Fig. 3.4. No differences were found in pH between the light salted samples.

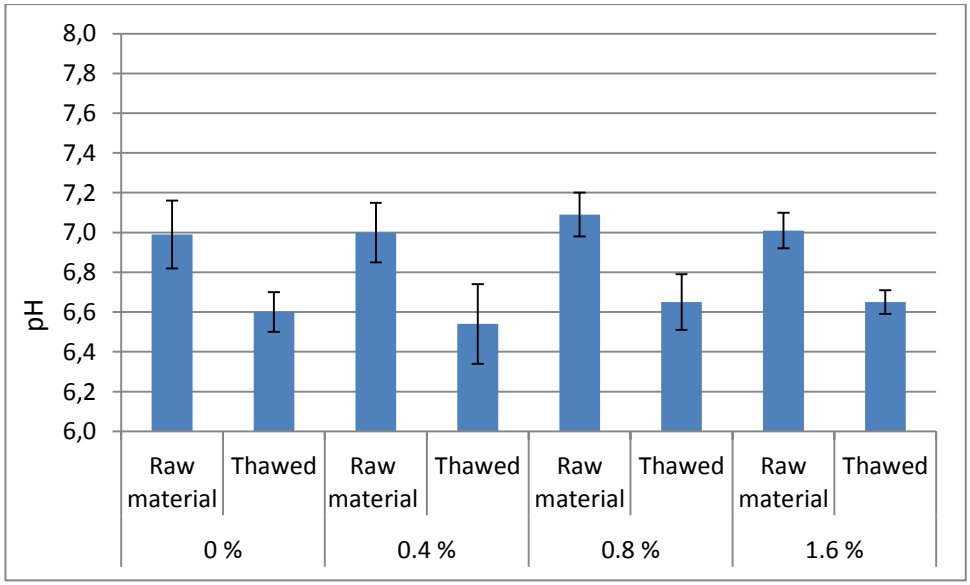


Figure 3.4. pH of loins in raw material and light salted fillet after thawing. Fillets injected with salt brine containing 0, 0.4, 0.8 or 1.6 % phosphate. Means and standard deviations of four fish are shown.

As predicted, the water content (mass balances) increased from control to the highest phosphate concentration (Fig. 3.5). This balance leads to an increase in tissue NaCl from 0.23 g/100g in raw material to 4.5 g/100g in final light salted cod.

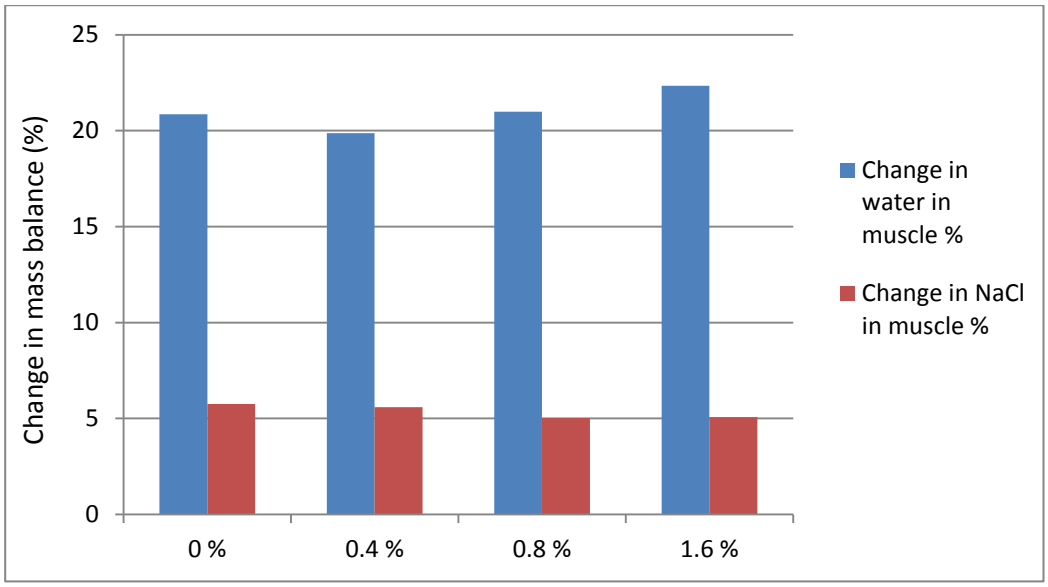


Figure 3.5. Mass balances for change in water and NaCl in light salted fillets injected with salt brine containing 0, 0.4, 0.8 or 1.6 % phosphate (3 fillets average shown).

Final potassium and phosphate levels are corresponding to phosphate addition (Fig. 3.6). This was expected as CARNAL 2110 is composed of sodium and potassium phosphate salts. Levels drop from raw material to control because of the water and salt uptake, but recover as more additive is included in the injected brine. Even with a 1.6 % P_2O_5 addition, final levels comply with legislation.

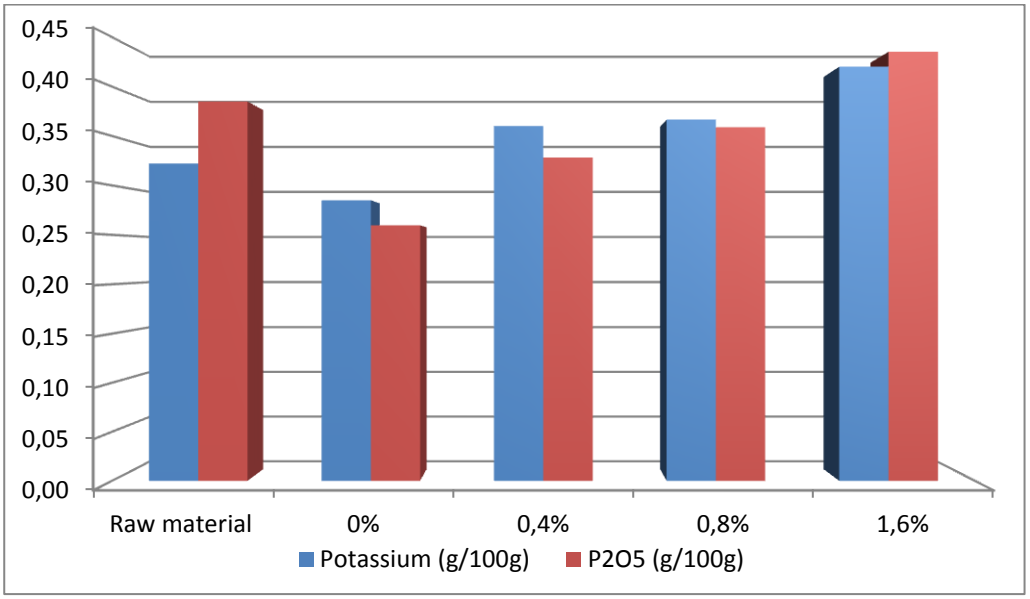


Figure 3.6. Average values of phosphate and potassium in light salted fillets injected with, 0, 0.4, 0.8 or 1.6 % phosphate (n=3).

Minor triphosphate residues (below quantification threshold) were only detected at phosphate concentrations of0.8 % and 1.6 %. No pyrophosphate was detected in any of the studied groups. Apart from the phosphate degradation effect, it seems that sensitivity of HPTLC method only allows detection where important phosphate additions have been applied.

The results from primary oxidation were difficult to interpret. Results of peroxides and TBARS are presented in Table 3.1. Raw material shows minimal primary oxidation which increases considerably when processed, displaying a large variability in all samples, even within replicates in the same group for PV. Secondary oxidation (TBA index) also seems not to be related to the amount of phosphates used in the injected brine, however these values are very low (<1mg TBARS/kg muskel).

Table 3.1. Oxidation values of light salted fillets (n=3).

	SMP	*PEROXIDES INDEX* (meq.O2/Kg.fat)	*TBA INDEX* (mg/Kg muscle tissue)
RAW MATERIAL	Raw material-A	<2,00	0.5
	Raw material-B	<2,00	0.3
	Raw material-C	<2,00	0.6
	Raw material-D	<2,00	0.4
	Raw material-E	<2,00	0.4
FRESH RAW MATERIAL TRIAL	Light salted - 0% .A	216,22	0,7
	Light salted - 0% .B	205,88	0,5
	Light salted - 0% .C	74,07	0,6
	Light salted - 0,4% .A	115,38	0,6
	Light salted - 0,4% .B	94,59	0,7
	Light salted - 0,4% .C	406,25	0,8
	Light salted - 0,8% .A	62,50	0,3
	Light salted - 0,8% .B	104,17	0,4
	Light salted - 0,8% .C	43,48	0,8
	Light salted - 1,6% .A	62,50	0,3
	Light salted - 1,6% .B	125,00	0,5
	Light salted - 1,6% .C	152,78	0,6

Trial with heavy salted fillets after 5 weeks of chilled storage

Calculated yields for each group are shown in Fig. 3.7. Injected groups have significant ($p<0.05$) higher yields than pickle salted groups. Treatment with higher phosphate concentration gave higher yields for all groups and this effect was more pronounced for injected groups.

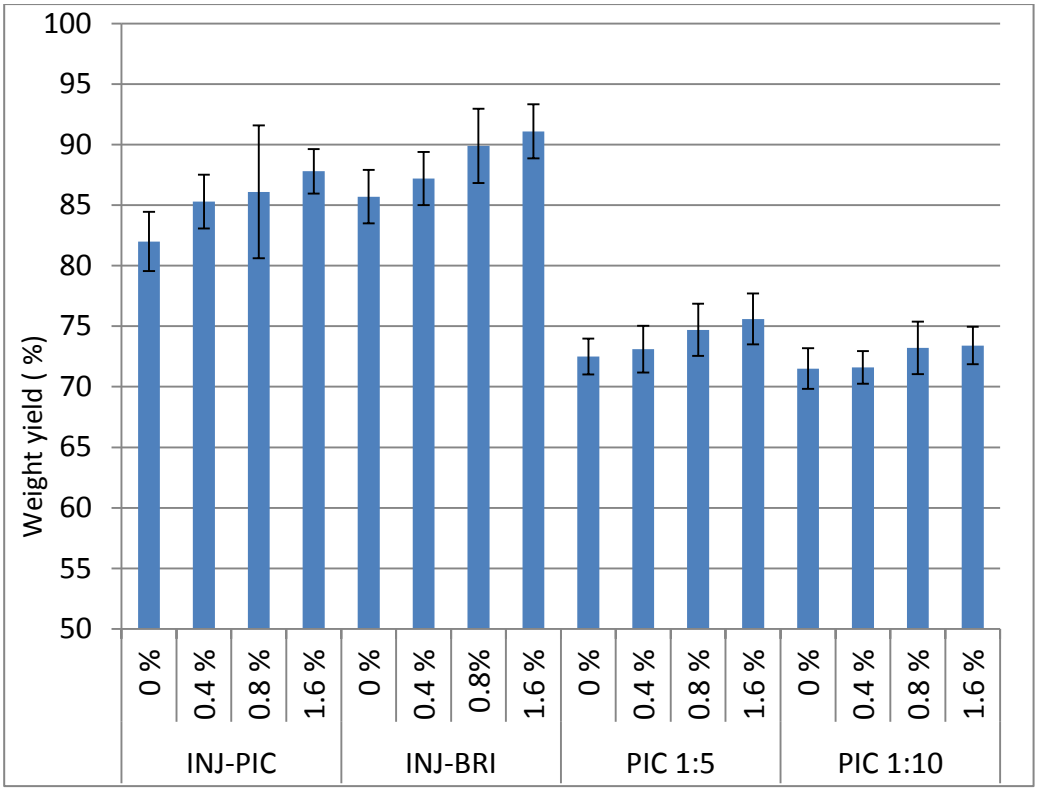


Figure 3.7. Weight yields after five weeks of salting in % of raw material weight (N=15). For the four salting methods, 0, 0.4, 0.8 or 1.6 % phosphates were used. Means and standard deviations are shown.

Results of the instrumental determination of white and yellow color are shown in Fig. 3.8 and 3.9, respectively. Samples injected followed by pickling seemed to have a declining trend in L-value with an increasing phosphate concentration, while injection followed by brining seemed to increase the L-value. No clear trend could be observed in fish that were pickle salted.

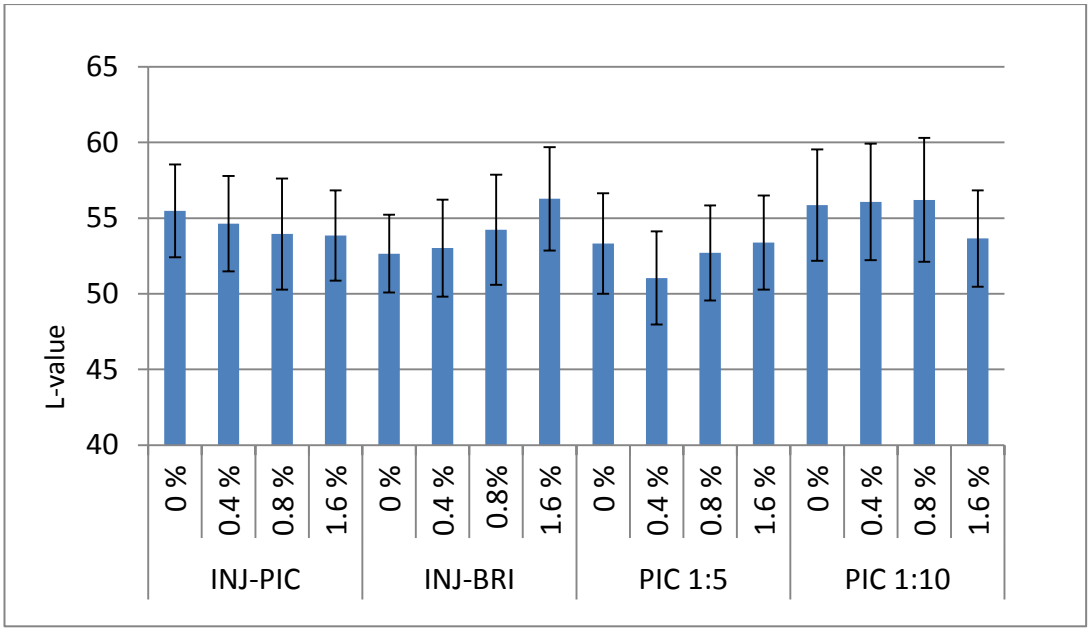


Figure 3.8. Instrumental determination of whiteness (L-value) in heavy salted fillets. For the four salting methods tested, 0, 0.4, 0.8 or 1.6 % phosphates were used. Higher values mean whiter fillet color (N=15). Means and standard deviations of fish are shown.

No significant effect of phosphate on yellow colour was found (Fig.3.9). No clear common trend has been found in groups regarding salting method or phosphate concentration.

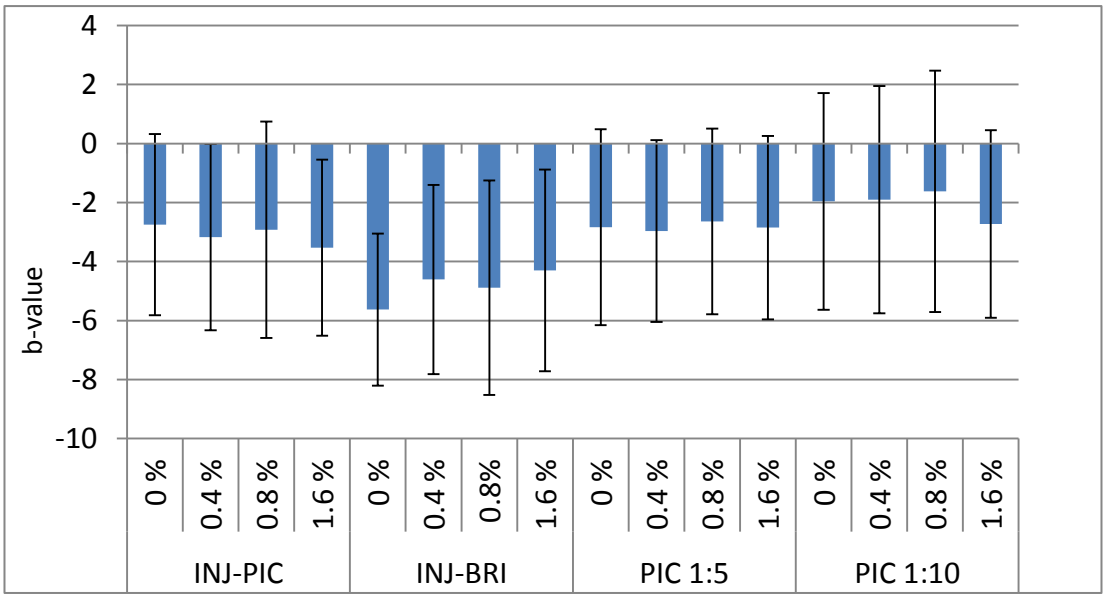


Figure 3.9. Instrumental determination of yellow color (b-value) in heavy salted fillets. For the four salting methods tested, 0, 0.4, 0.8 or 1.6 % phosphates were used. Lower values determine less yellow fillet than higher values (N=15). Means and standard deviations are shown.

In the sensorial evaluation, the fish that were injected and brined were ranked as the superior group, due to the whitest surface and smallest amount of blood in the belly flaps. Also, it was observed that blood in the belly flaps was reduced with an increase in phosphate concentration. No significant trends were registered within the fish which were injected and pickled. A lower intensity of whiteness and a higher level of yellow color was found in fish that were only pickle salted. A trend observed within these fish was that the whiteness increased and yellow color intensity and blood in belly flaps decreased with higher phosphate levels. The general quality of the group pickle salted with 1:10 ration between brine and fish was similar to the group salted with the ratio 1:5, but no clear trends were registered except for gaping which was reduced with higher phosphate levels.
In the sensorial evaluation, no significant trends were registered within the group injected and pickle salted. A lower intensity of whiteness and a higher level of yellow color were found in the group pickle salted groups compared to the injected groups. A trend observed within the pickle salted group (1:5 ratio) was that the whiteness increased and yellow color intensity and blood in belly flaps decreased with higher phosphate levels.

When the groups were visually evaluated by judges in an expert panel laying out as shown in Fig. 3.10 it was registered that injected fish were found visually whiter than fish only pickled. It was also noted, with the exception of pickled 1:5, that a higher level of phosphate gave a whiter muscle surface.

The pH of the raw material was between 6.9 and 7.1. After salting and curing the pH shifted to 6.1-6.2 for fish injected and pickled, 6.2 – 6.3 for fish injected and brined, 6.1-6.2 for fish pickled 1:10 and 6.1 for fish pickled 1:5.

Figure 3.10. Salt fillets after 40 days of salting (N=5). Fish in group G11-G14 are injected and pickled, G21-24 are injected and brined, G31-34 are pickled 1:5 (brine:fish) and G41-44 are pickled 1:10 (brine:fish). Fish from left to right are treated with 0 %, 0.4 %, 0.8 % and 1.6 % phosphate.

Since fish pickled with 1:5 showed similar effects of phosphate treatment as fish pickled with 1:10, the latter group was excluded from the further analysis. The change in salt and water content from raw material to heavy salted cod is shown in Fig. 3.11. As in light salted samples, there was an increased retention of water with higher phosphate concentration.

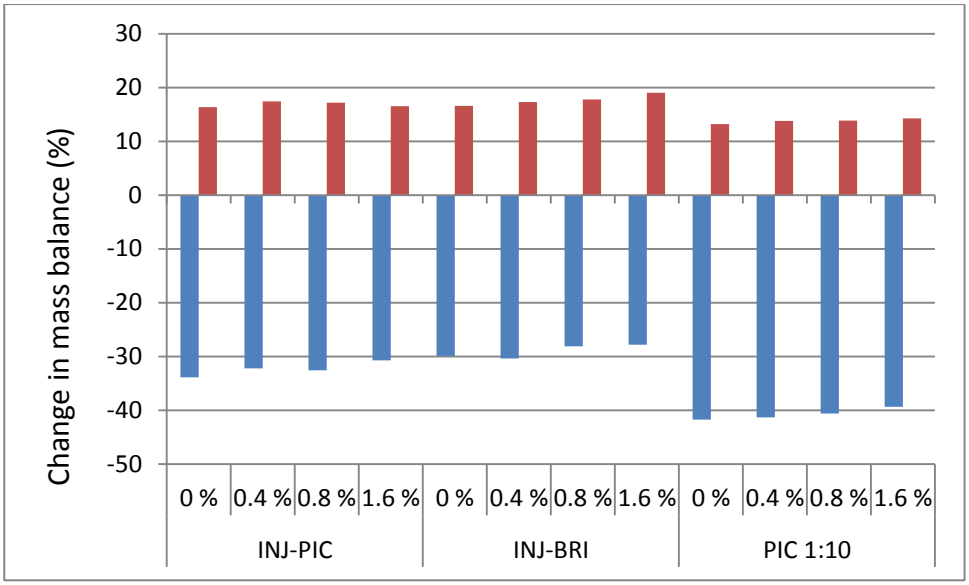


Figure 3.11. Mass balances for water and NaCl in heavy salted fillets. For the three salting methods tested, 0, 0.4, 0.8 or 1.6 % phosphates were used. Means of three fish are shown.

This salt and water balance leads to an average (all groups) final salt content of 19.7 %. Statistical data treatment (ANOVA) found significant differences between heavy salting procedures; where both injection groups gave similar results, while the pickle salted treatment resulted in heavy salted cod with a significantly reduced water content (results not shown). Between the heavy salted methods, the pickle salted fish had a significant lower NaCl content compared to the fish that were injected (Tab 3.2).

Table 3.2. ANOVA Post-Hoc (SNK) from salt contents. Different subsets indicate significant differences. (p<0.05).

Student-Newman-Keuls - NaCl

Salting	N	Subset for alpha = .05			
		1	2	3	4
G0 (Raw material)	5	0,2260			
G5 (Light salted)	12		4,5417		
G3 (Pickelsalting 20 % Brine Drysalting)	12			18,9250	
G1 (Injection - Pickelsalting - Drysalting)	12				20,0330
G2 (injection - Brining - Drysalting)	12				20,1750
Sig.		1.000	1.000	1.000	.602

The level of potassium and phosphates (initial level in raw material; 0.32g K/100g and 0.38g P_2O_5/100g) was reduced during heavy-salting (Fig. 3.12) This may indicate that part of the natural soluble phosphates and potassium are released from the tissues within the outgoing liquid during salting, as it has been also documented in literature (Thorarinsdottir et *al*. 2006).

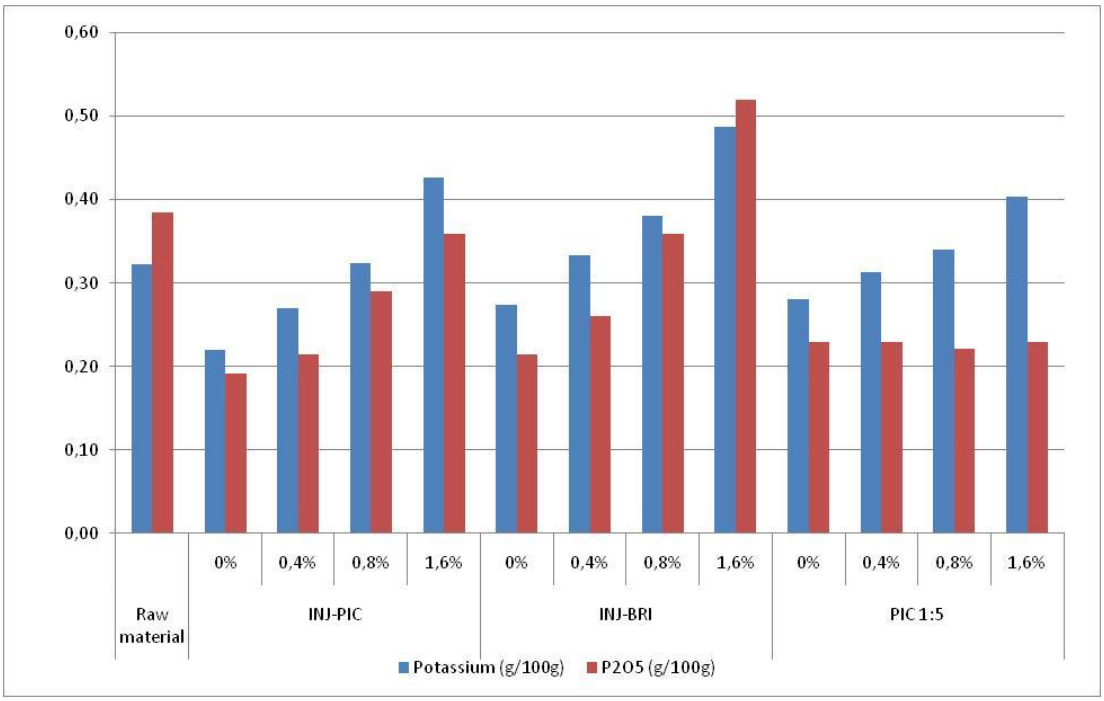


Figure 3.12. Average value of phosphate and potassium in raw material and heavy salted fillets (n=3) after 5 weeks of storage. For the three salting methods tested, 0, 0.4, 0.8 or 1.6 % phosphates were used.

Samples treated with greater levels of phosphate had an increased concentration of potassium and phosphates after 5 weeks of storage. All but one group (INJ-BRI-1.6 %) was within the legal tolerance level (0.5 P_2O_5/100g). In fact phosphate additives help samples to recover their natural phosphorus content, and only the most intense treatment was sufficient to reach the raw material levels. It seems that the salting procedure with injection and brining is the most effective method for the absorption of phosphates by the cod muscle tissues.

The pickling procedure shows no increase in phosphates. This is not consistent with the previously commented potassium pattern which seemed to show some additives absorption.

It seems that degradation of phosphate has been important. Only the most intense treatments results in trace levels of triphosphate residues. No pyrophosphate was detected in any of the analyzed samples.

As in the case of light salted samples, primary oxidation displays no systematic results (Fig 3.13) regarding phosphate treatment and no trend can be associated to previous sensorial results. Within each group large variations in oxidation were registered.

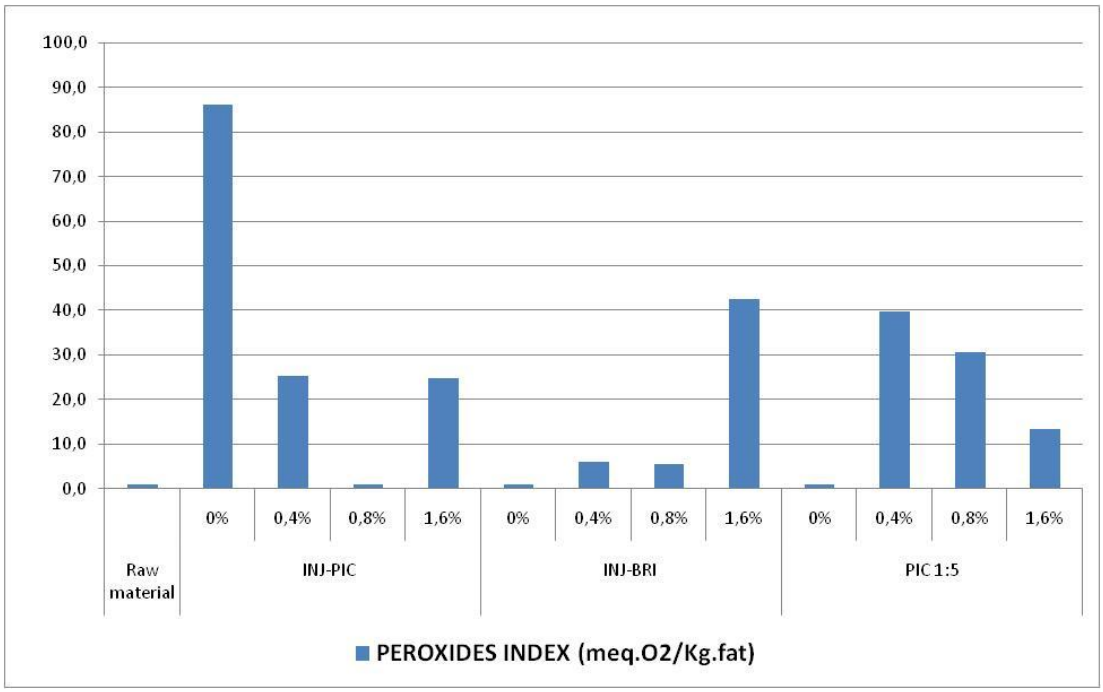


Figure 3.13. Primary oxidation results in raw material and heavy salted groups. Means of three fish are shown. For the three salting methods tested, 0, 0.4, 0.8 or 1.6 % phosphates were used.

As the case of primary oxidation, TBARS values do not show any systematic effect of phosphate treatment. The TBARS values are low while the internal deviation is high (Fig 3.14). Group injected and brine treated seems to show a reducing oxidation trend with the intensity of the phosphates addition, in accordance with previous sensorial data, but this was not significant in an Anova test (p=0.05) due to large variations within each group.

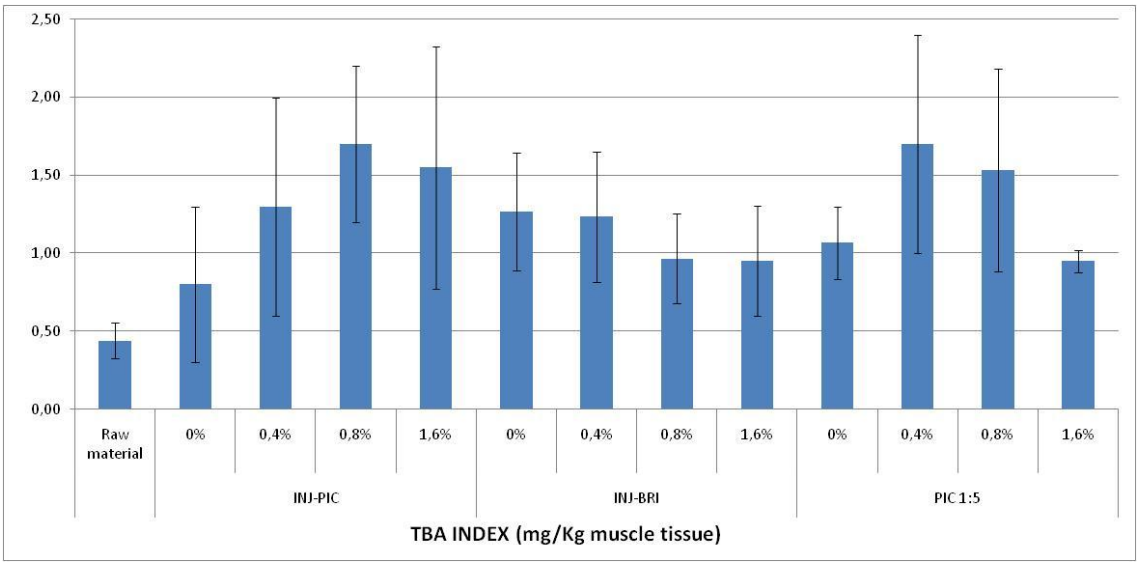


Figure 3.14. Secondary oxidation (TBARS) results in raw material and heavy salted groups. Mean and standard deviation of three fish are shown. For the three salting methods tested, 0, 0.4, 0.8 or 1.6 % phosphates were used.

Analysis after 7 months of storage of salted fillets

After seven months of chilled storage, the remaining nine fillets were analyzed in the same way as they were after five weeks. Fish pickle salted with ratio 1:10 were excluded from these trials due to low uptake of phosphates, and no chemical analysis were carried.

Determination of yields showed the similar trends as those seen for five weeks storage (Fig. 3.15). The weight loss had been approximately the same for all groups, between 5 and 7 %. There was a trend that the highest phosphate levels lost less weight than the control groups.

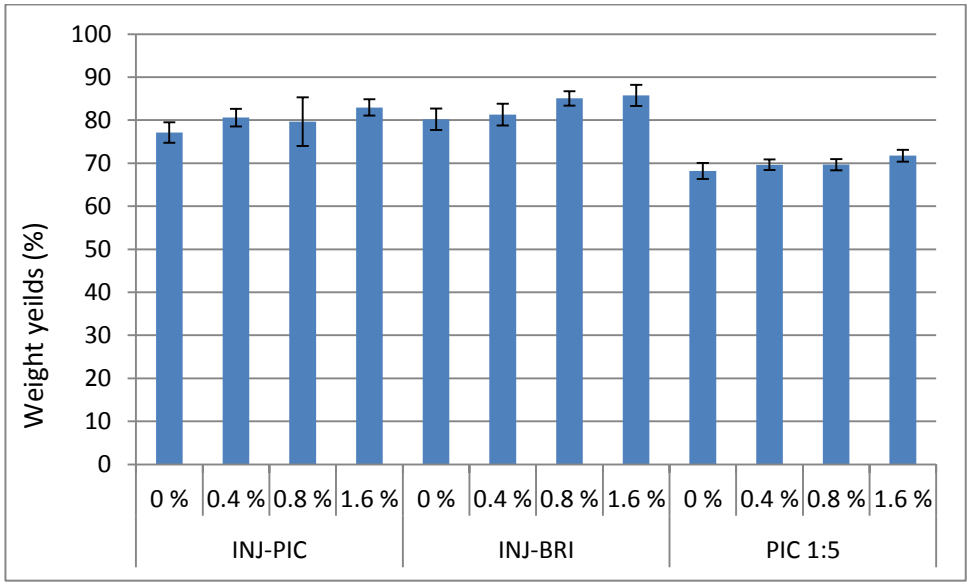


Figure 3.15. Salt cured fillets yields after 7 months of storage, shown in % for raw material weight. Means and standard deviations of nine fillets are shown. For the three salting methods tested, 0, 0.4, 0.8 or 1.6 % phosphates were used.

The pH in muscle was stable at 7.0 ± 0.1 for all groups.

There was an increase in colour values between 5 and 7 weeks salting but the increase was similar across groups. L-values increased with increasing addition of phosphates only for fish that were injected and brine salted (Fig 3.16) but large standard deviation gave seemed to give no differences.

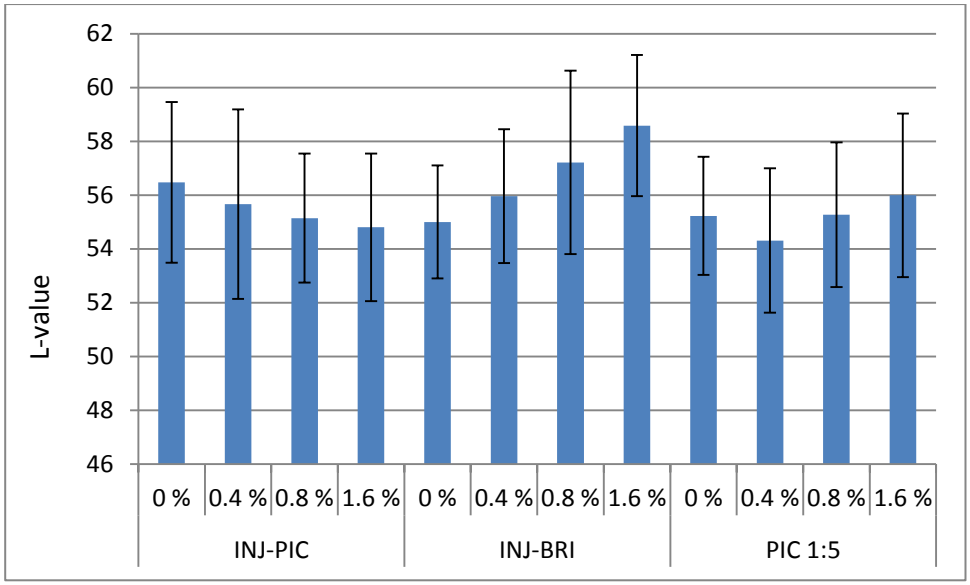


Figure 3.16. Instrumental determination of whiteness (L-value) after seven months of storage. Three measurements were carried out on each of nine fillets per group. For the three salting methods tested, 0, 0.4, 0.8 or 1.6 % phosphates were used. Means and standard deviations of nine fillets are shown.

There was a decreasing trend in b-values (yellow colour) in fish injected and pickle salted with higher phosphate levels, while the opposite was registered for fish injected followed by brining. These fish seemed overall to be the least yellow while pickled fish were the most yellow (Fig. 3.17).

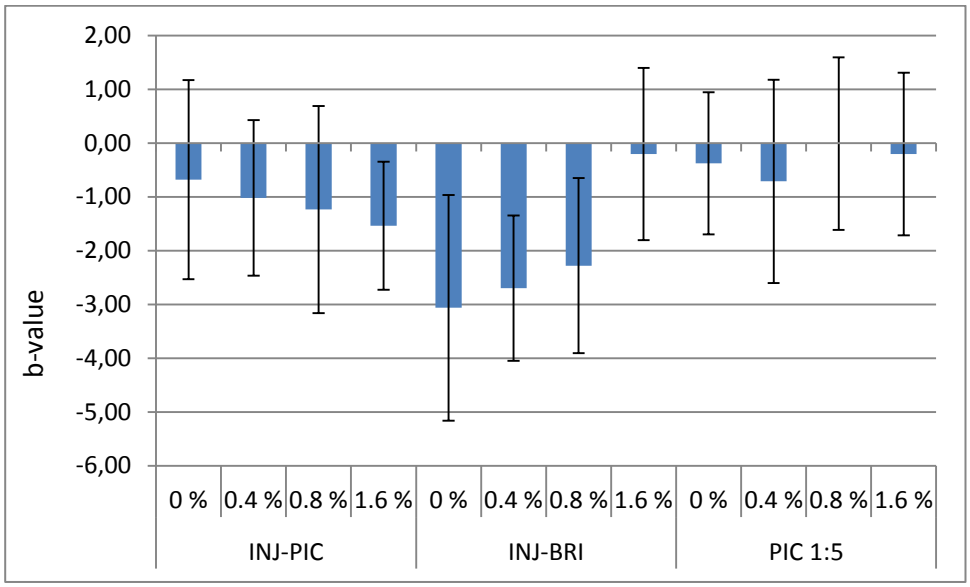


Figure 3.17. Instrumental determination of yellow color (b-value) after seven months of storage. Three measurements on each of nine fillets per group. For the three salting methods tested, 0, 0.4, 0.8 or 1.6 % phosphates were used. Means and standard deviations of nine fillets are shown.

All groups were ranked when simultaneously lying on tables as shown in Fig. 3.18. Injected and pickled group had lower overall quality with higher phosphates level. In injected and brined group, 0.4 % phosphate treatment was ranked highest, followed by 1.6 %, 0.8 % and control due to yellow color. For the pickle salted group, 0.4 % was ranked highest, followed by control, 0.8 % and 1.6 % phosphate treatment. Overall injected and pickled group was slightly better than injected and brined group, while the pickle salted group had the lowest quality.

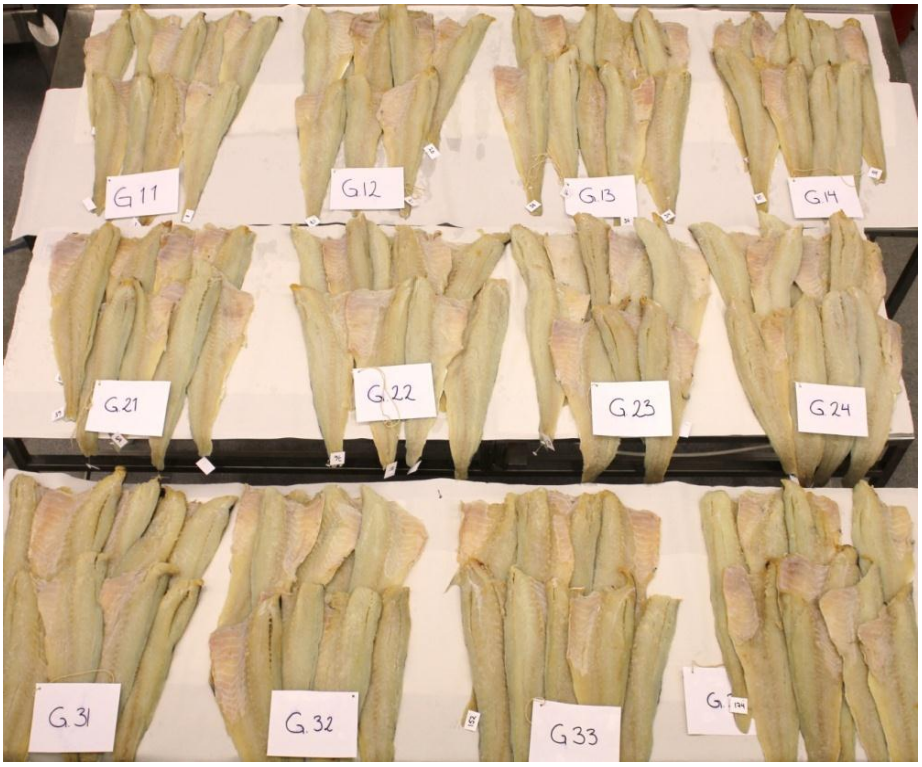


Figure 3.18. Heavy salted fillets after seven months storage (N=9). Fish in group G11-G14 are injected and pickled, G21-24 are injected and brined and G31-34 are pickled 1:5 (brine:fish). Fish from left to right are treated with 0 %, 0.4 %, 0.8 % and 1.6 % phosphate.

There were no significant trends in the sensorial evaluations for different levels of phosphate concentrations in the different salting methods.

3.1.2 **Salting trials with frozen raw material**

The same raw material was used in these trials as in the trials with fresh raw material. The frozen raw material had been stored for three months at -30 °C.

Light salting of fillets

After three months of frozen storage at – 30 °C, fillets were thawed for 24 hours. The weight gain after injection was 34.5 % ±1.0 % (N = 40). Before thawing (including glazing) the yields of all phosphate concentrations were similar and between 144-146 % regardless of phosphate concentration. After thawing the yields were between 132-133 % with no trends concerning phosphate concentration (Fig. 3.19).

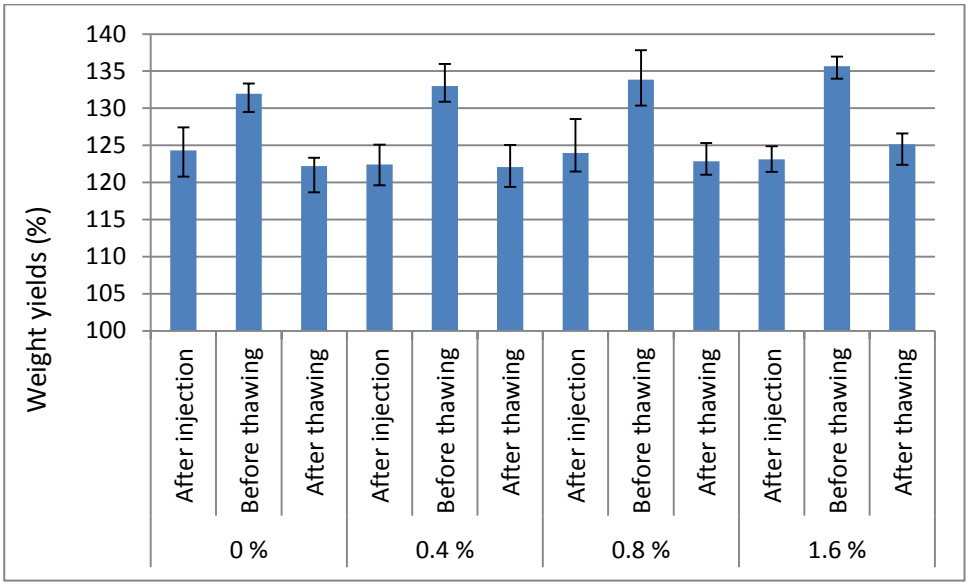


Figure 3.19. Light salted fillet yields based on raw material weight for light salted cod fillets (N=10 after injection) (N=4 before thawing) (N=4 after thawing) injected with 0, 0.4, 0.8 or 1.6 % phosphate. Means and standard deviations are shown.

Results for whiteness (L-value) are shown in Figure 3.20. The L-values for raw material was 63-65 while L-values of thawed light salted fillets increased with higher phosphate concentration from 47.5 to 54.4.

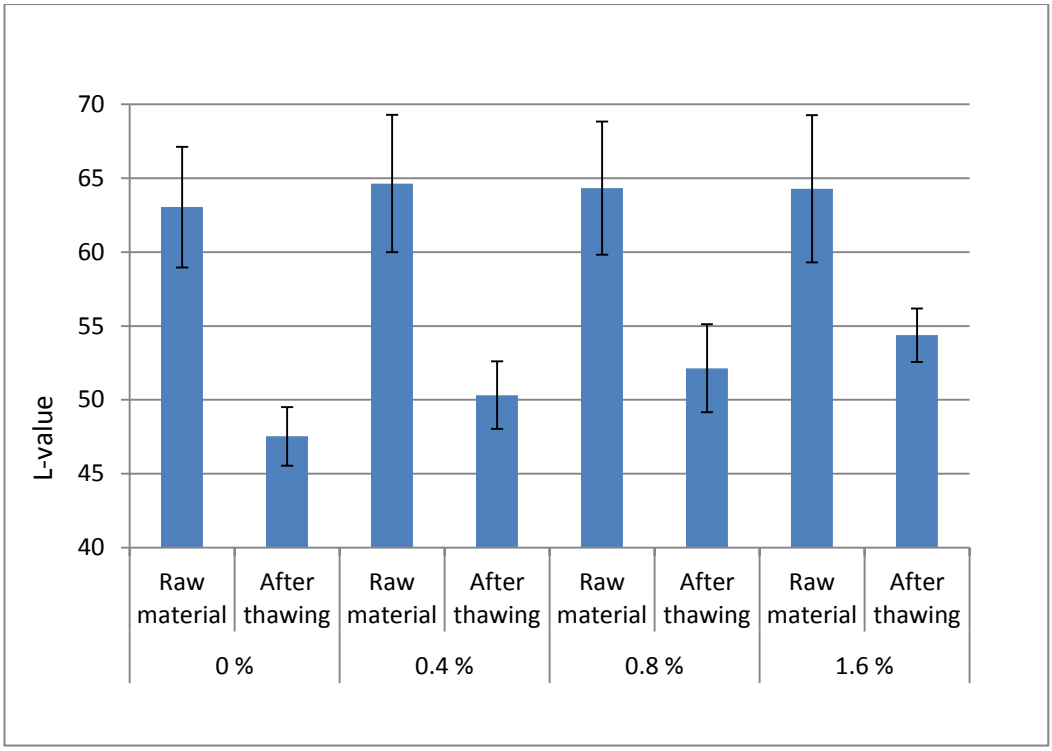


Figure 3.20. Measurement of whiteness (L-value) on raw material (N=10) and after thawing (N=4) of light salted fillets injected with 0, 0.4, 0.8 or 1.6 % phosphate. Means and standard deviations are shown.

The b-values were between -7 and -9 for all groups and the values are shown in Figure 3.21. There was a slight trend of lower yellowness with increased phosphate concentration.

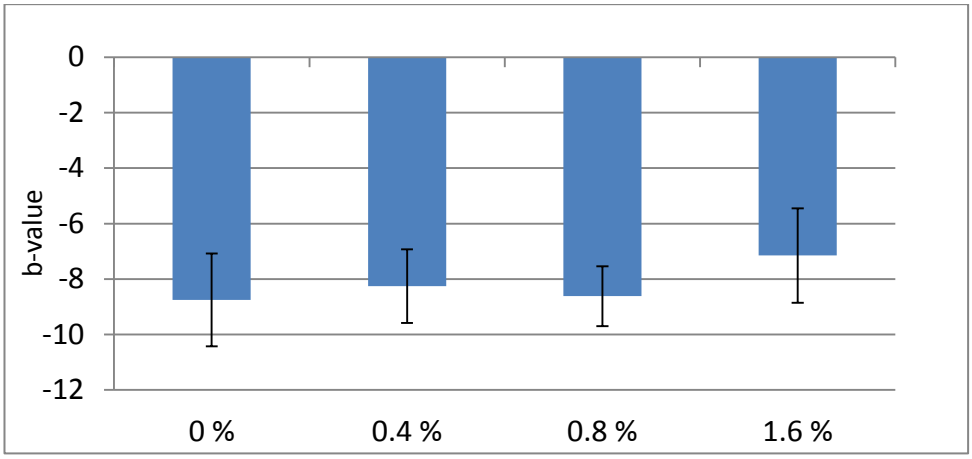


Figure 3.21. Instrumental measurement of yellow colour (b-value) on light salted fillet after thawing (N=10) of light salted fillets injected with 0, 0.4, 0.8 or 1.6 % phosphate. Means and standard deviations are shown.

In the sensorial ranking the fish treated with the highest concentration of phosphate scored highest on whiteness (G54), had less yellow tone and less blood followed by 0.4 %, 0.8 % and 0 % phosphate treatment. The raw material influenced largely on the quality of the light salted fillets. The exception was fish treated with 1.6 % phosphate where no red belly flaps were registered (Fig. 3.22).

Figure 3.22. Light salted fillets before (above) and after thawing (below). G51 = treated with 0 % phosphate, G52 = 0.4 % phosphate, G53 = 0.8 % phosphate and G54 = 1.6 % phosphate.

pH ranged from 6.8-6.9 for raw material in all groups (Fig. 3.23). In thawed fillets the pH seemed to increase from 6.5 (fish treated without phosphate) to 6.9 (fish treated with 0.8 % phosphate) while fish treated with the highest concentration of phosphate had a pH of 6.6.

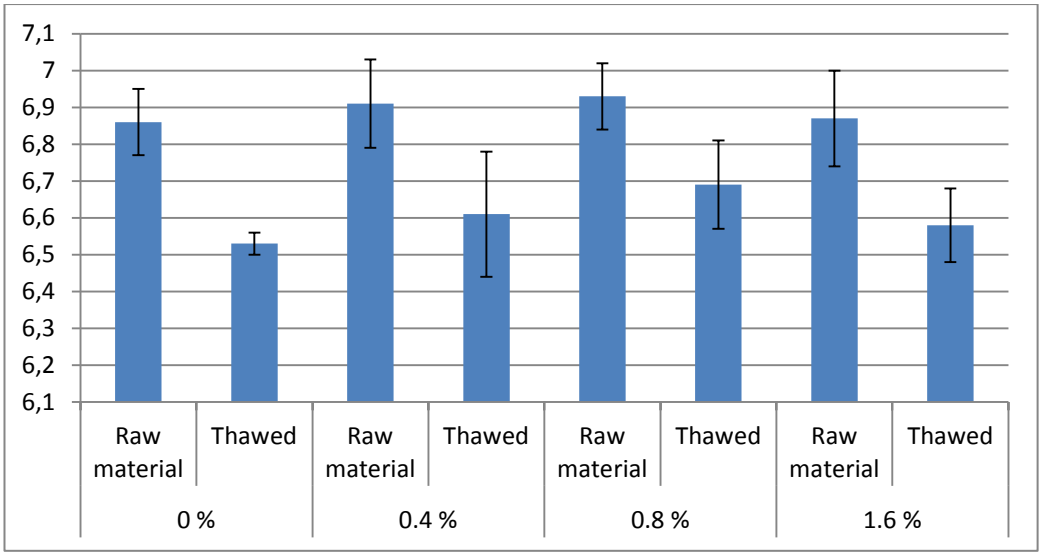


Figure 3.23. pH in loins for raw material (N=10) and light salted fillets after thawing (N=4) of light salted fillets injected with 0, 0.4, 0.8 or 1.6 % phosphate. Means and standard deviations are shown.

No trends were found concerning water or NaCl mass balances (Fig. 3.24). The final salt content was 6.2 % NaCl, which is significantly higher than the salt content in light salted fillets from fresh raw material (4.5 % NaCl).

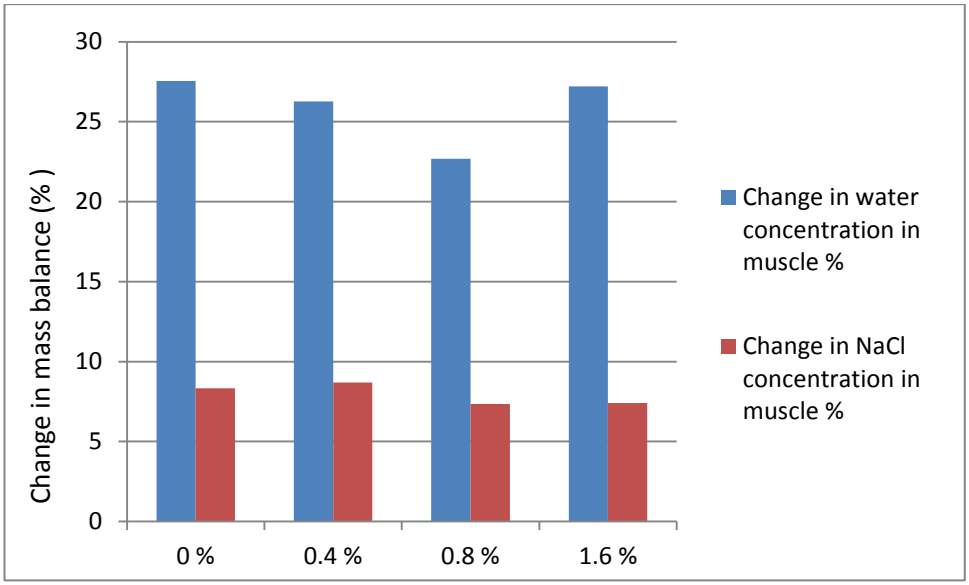


Figure 3.24. Mass balances for water and NaCl in light salted fillets (frozen raw material) injected with 0, 0.4, 0.8 or 1.6 % phosphate. Mean of 3 fish is displayed.

Potassium and total phosphorus levels did not follow the same pattern as in fresh raw material. Unexplainable results were obtained, neither potassium nor phosphates increased with the higher concentration of the CARNAL addition (Fig.3.25).

HPTLC results detected pyro- and triphosphate trace levels (below quantification limit) in fish treated with 0.4% and 1.6 % phosphate.

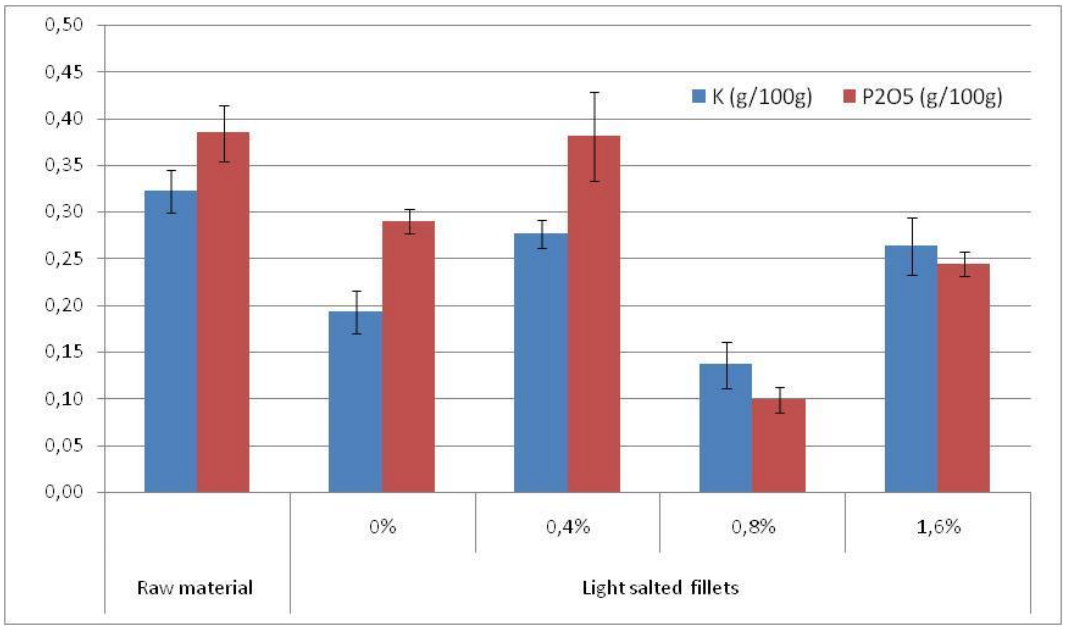


Figure 3.25. Potassium and phosphorus contents in raw material and light salted samples injected with 0, 0.4, 0.8 or 1.6 % phosphate. Means and standard deviations of three fish are shown.

Oxidation in frozen raw material, as in fresh raw material tests, displays an important internal variation which minimizes the possibilities of extracting information (Fig. 3.26). Primary oxidation in frozen raw material samples was very high compared to fresh raw material, where almost no oxidation levels were detected. The three month storage could have caused some important oxidation in these samples, but control and light salted material shows a reduced level.

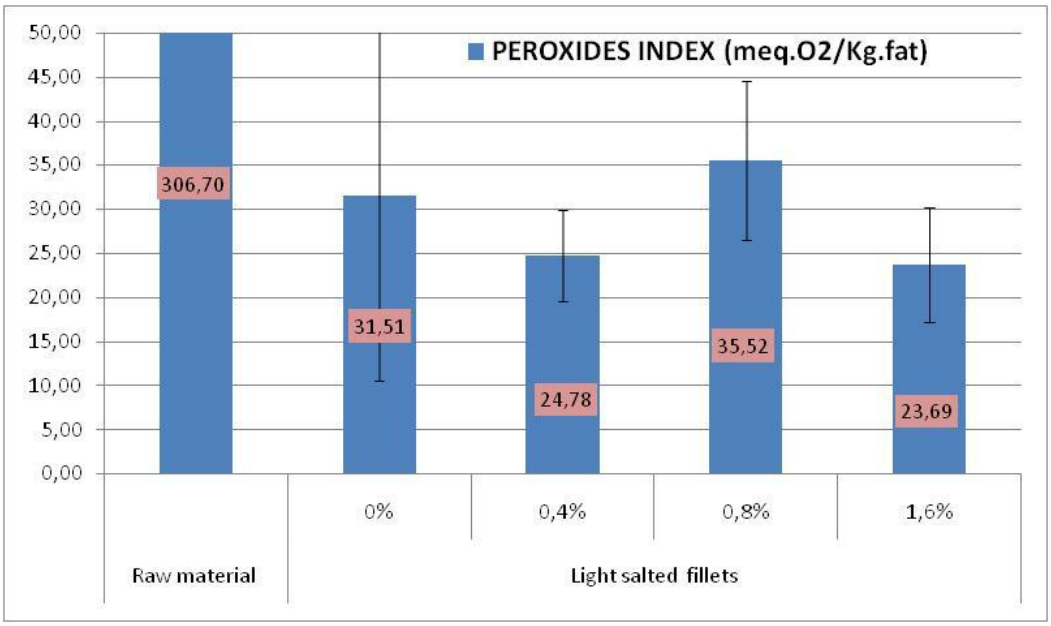


Figure 3.26. Peroxide index in light salted fillets injected with 0, 0.4, 0.8 or 1.6 % phosphate . Means and standard deviation of three fish are shown.

The secondary oxidation results seem to reflect that there is an antioxidant protection of phosphates in the samples. The TBAR levels are reduced as more phosphate is included in the injected brine (Fig.3.27).

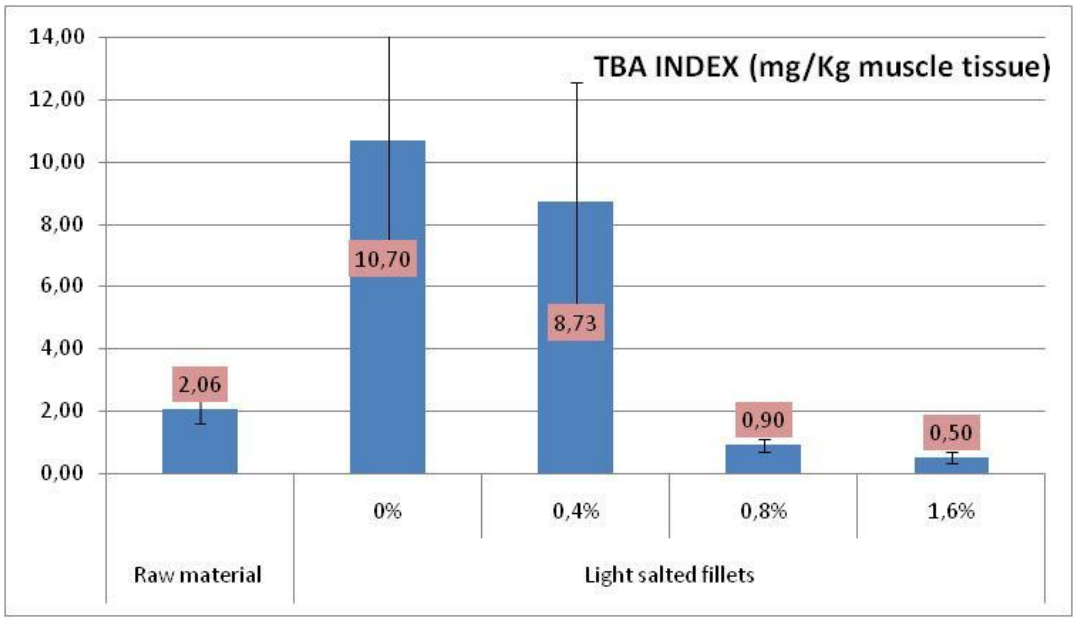


Figure 3.27. TBA index in light salted fillets injected with 0, 0.4, 0.8 or 1.6 % phosphate . Means and standard deviations of three fish are shown.

Trial with heavy salted fillets after 5 weeks of chilled storage

There were significant differences in the yield between groups and yields increased with higher phosphate concentration for fish that were injected, but not for those that were only pickled (Fig 3.28).

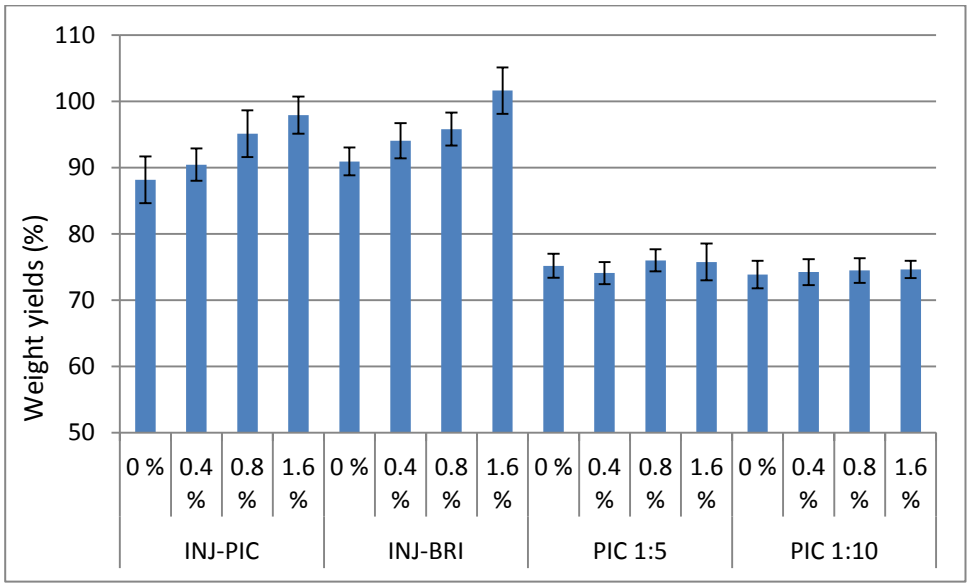


Figure 3.28. Heavy salted fillet yields in % of raw material weight after five weeks of storage. For the four salting methods fillets were injected with 0, 0.4, 0.8 or 1.6 % phosphate. Means and standard deviations of 15 fish are shown.

The results of pH measurements showed a very stable pH on raw material of 6.8-7.0. pH in salt-cured fillet ranged from5.9 to 6.3 for all groups. For fish injected, there was a small decrease in pH with increased phosphate concentration, while the pH in those only pickled were stable around pH 6.0.

In the instrumental analysis of muscle surface lightness (L-value), fish that were injected and pickle salted scored higher than fish salted in the other ways (Fig. 3.29). There were no clear relation between whiteness and phosphate concentration.

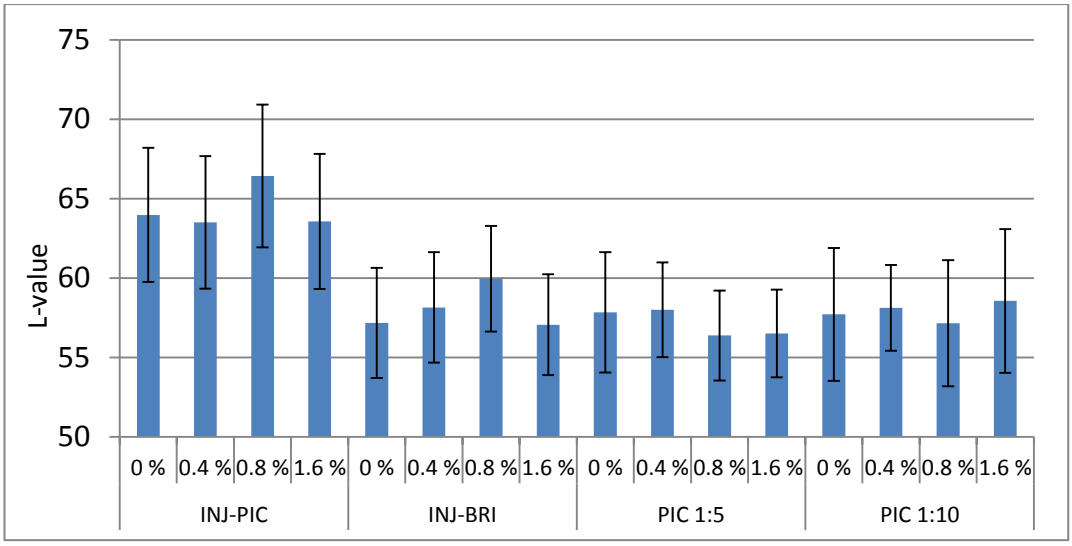


Figure 3.29. Instrumental measurements of muscle lightness (L-value) for heavy salted fillets. For the four salting methods fillets were injected with 0, 0.4, 0.8 or 1.6 % phosphate. For each of the 15 fillets in each group, three measurements were taken. Means and standard deviations of 15 fish are shown.

The was an insignificant trend that fish injected and brined had lower scores (less yellow) than those which were only pickle salted (Fig. 3.30), but there were no relation between whiteness and phosphate concentration.

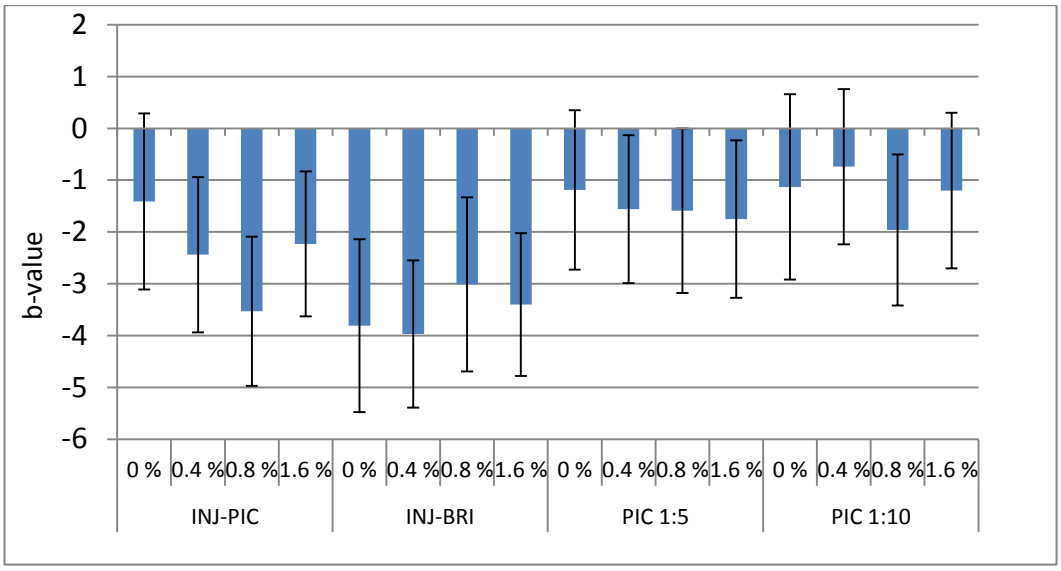


Figure 3.30. Instrumental measurement of muscle yellow color (b-value). For the four salting methods fillets were injected with 0, 0.4, 0.8 or 1.6 % phosphate. For each of the 15 fillets in each group, three measurement were taken (N=15). Mean and standard deviation are shown.

In the sensory evaluations, all groups were described as fully salt-cured and with normal salt fillet features. However, some of the groups had a lower intensity of salt cured smell.

Fillets injected followed by pickle salting were described as the whitest and least yellow of all fillets, but they were not affected by phosphate concentration. However, the degree of gaping seemed to be reduced with increasing phosphate concentration. The quality of fillets injected followed by brining was not affected by phosphate concentration. For fillets pickle salted 1 kg brine to 5 kg fish, grey and yellow color increased with higher phosphate content. Fillets which were pickle salted with 1 kg brine to 10 kg fish was evaluated as the group with lowest quality due to a yellow and grey muscle surface. There was no increase in quality with higher phosphate content and the raw material was of very variable quality due to blood (Fig. 3.31).

Figure 3.31. Salted fillets after 40 days of salting (N=5). G11-G14 injected and pickle salted, G21-G24 injected and brined, G31-G34 pickle salted 1:5 and G41-G44 pickle salted 1:10.

Since the group pickle salted with 1 kg brine to 10 kg fish showed similar or lower effects of phosphate treatment than the same salting method with 1 kg brine to 5 kg fish, the first group was excluded from further analysis.

The water loss was higher for pickle salted fillets than the injected fillets. A reduction in water loss was registered with increased phosphate concentration (Fig.3.32).

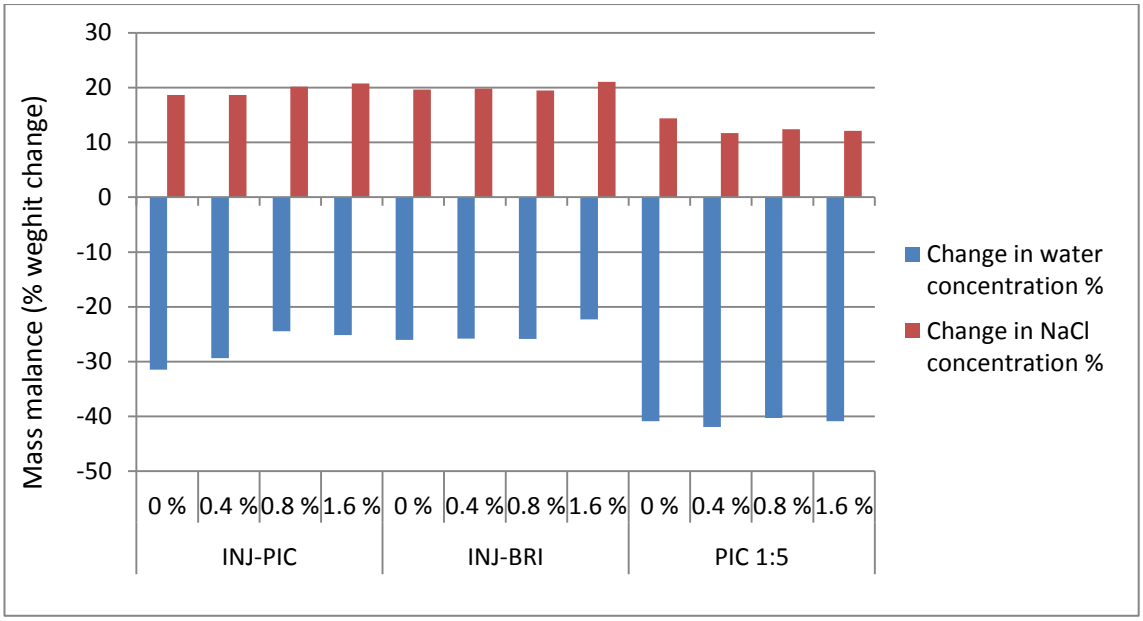


Figure 3.32. Mass balances for water and NaCl in heavy salted fillets. For the three salting methods fillets were injected with 0, 0.4, 0.8 or 1.6 % phosphate. Mean of three fish are shown.

Despite the fact that less water leaks out from fish tissue when more phosphate is added, it seems that this is compensated with more salt income, because final salt levels are similar to initial levels. Nevertheless, as the case of fresh cod testing, there are some differences in final salt concentration in the different types of processing. Heavy-salted injected samples show a slightly higher level of salt when compared to pickle salted group (Tab. 3.3).

Table 3.3 ANOVA reflecting differences in final salt levels based on processing. Different subsets indicate significant differences (p<0.05).

Student-Newman-Keuls - Salt contents.					
		Subgroups: alfa = .05			
Salting methods	N	2	3	4	1
Raw material	5	0,172			
Light salting	6		6,5333		
Pickelsalting with brine addition (1:5) - Dry-salting	12			17,0333	
Injection - Brining (bath) 24 h. – Dry-salting	12				21,0833
Injection - Pickelsalting with brine addition (1:10) - Dry-salting.	12				21,0917
Sig.		1	1	1	0,984

Total phosphorus and potassium contents are similar to previous tests. Initial natural phosphorus contents are reduced during the salt/water diffusion; meanwhile potassium levels seem unaffected by this process. The addition of phosphates increased both mineral levels in the final product proportionally to the intensity of the additives use. This helps the flesh to regain natural levels, but a 2 % P_2O_5 addition makes samples go slightly beyond the legislation limit in the injected and brined group. Again, the pickelsalting – dry-salting does not seem to give any phosphate uptake in

cod tissues, even though potassium levels (coming from additives) increase with higher CARNAL 2110 addition (Fig. 3.33).

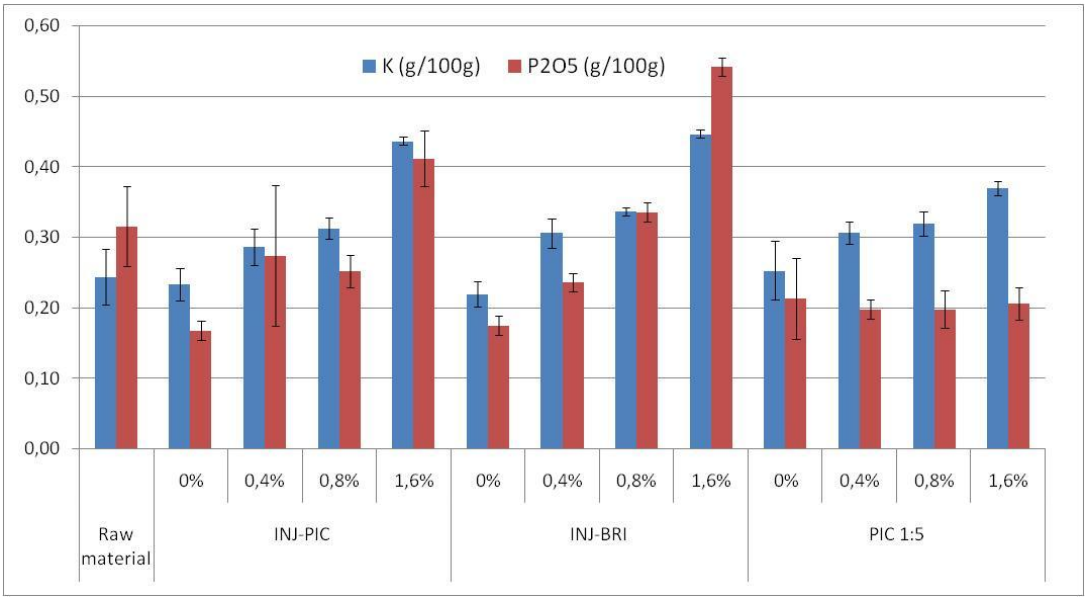


Figure 3.33. Potassium and phosphorus content in heavy salted samples. For the three salting methods fillets were injected with 0, 0.4, 0.8 or 1.6 % phosphate. Means and standard deviations of three fillets are shown.

Pyro- and triphosphates could only be detected in injected groups with the highest phosphate additions. This is consistent to previous results giving evidence that the HPTLC method sensitivity is limited, only detecting phosphates when a relative high level of phosphorus is present in the final product. This might be caused, as detailed in introduction, by phosphate degradation.

After three months of frozen storage, oxidation values in frozen raw material are significantly higher than in fresh raw material samples (Fig. 3.34). Average peroxides value in frozen raw material is much higher than heavy salted samples. Differences among control samples are high as well, ranging from 18.5 to 251.8 meq. O2/kg fat. Both injected groups show a decreasing oxidation trend with the amount of phosphates used. Besides, the pickle salted group did not follow up this trend, possibly because of the non-absorption of phosphates.

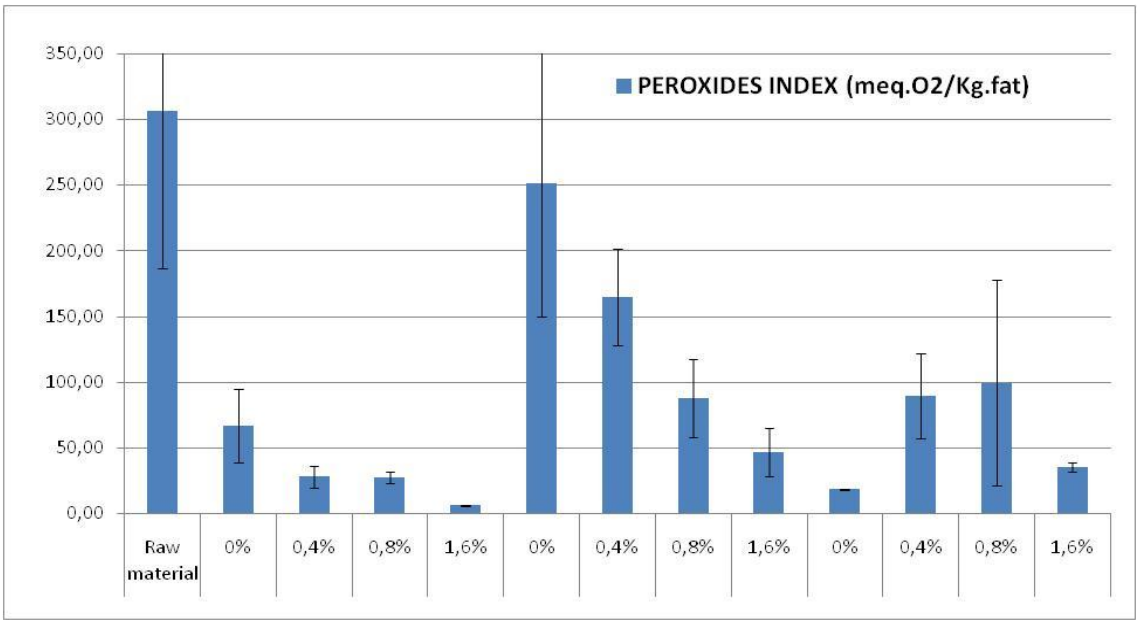


Figure 3.34. Peroxides index in heavy salted samples. For the three salting methods fillets were injected with 0, 0.4, 0.8 or 1.6 % phosphate. Means and standard deviations of three fillets are shown.

Although the total amount of samples is reduced (n=3), there was a significant difference in oxidation levels between the treated and untreated samples for both injected groups (ANOVA-Post Hoc (SNK)) (Table 3.4).

Table 3.4. ANOVA Post-Hoc (SNK) for peroxides values between phosphate treatments for injected and pickle salted group (left) injected and brine salted group (right). Different subgroup indicates significant differences.

POLYPHOSPHATES TREATMENT	N	Subgroup for para α = .05	
		2	1
1.6%	3	6,2667	
0.8%	3	27,7867	
0.4%	3	28,09	
0%	3		66,7533
Sig.		0,23	1

POLYPHOSPHATES TREATMENT	N	Subgroup for para α = .05	
		2	1
1.6%	3	46,8533	
0.8%	3	88,1967	
0.4%	3	164,73	164,73
0%	3		251,2867
Sig.		0,078	0,098

Secondary oxidation was very low and did not show any systematic trend (Fig.3.35). As for primary oxidation, initial levels in raw material were higher than in heavy-salted samples.

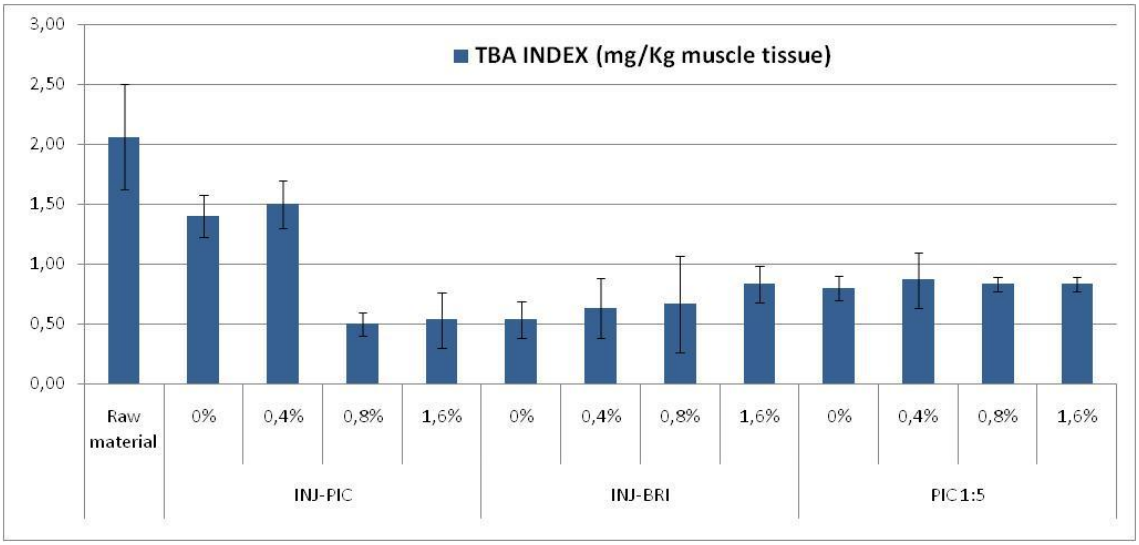
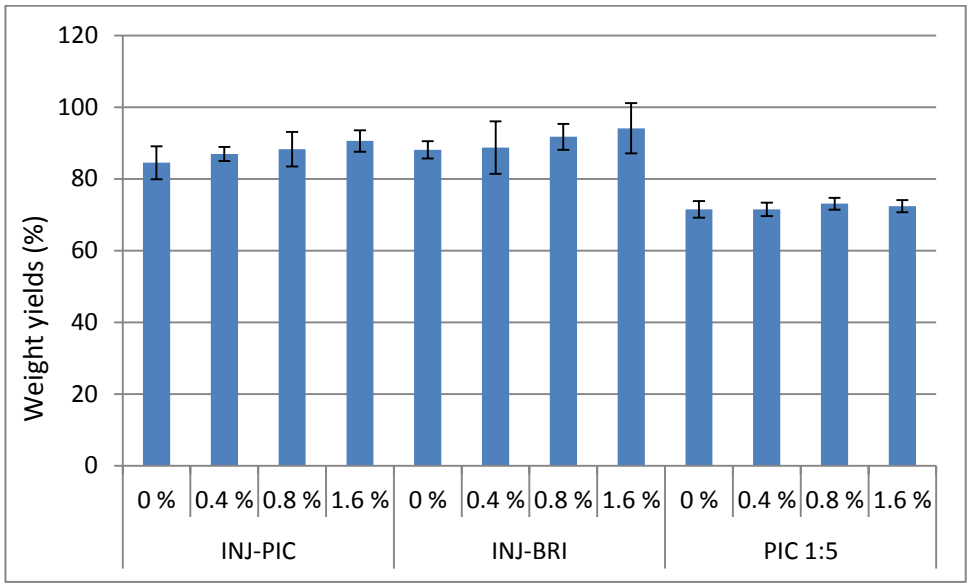


Figure 3.35. TBARS content in heavy salted samples. For the three salting methods fillets were injected with 0, 0.4, 0.8 or 1.6 % phosphate. Mean and standard deviation for tree fish is shown.

Analysis after 5 months storage of salted fillets

Since the pickle salted group with the lowest level of brine added (1:10 brine:fish) showed similar or less effects of phosphate treatment than the other pickle salted group (1:5 brine:fish), the group treated with the prior group was excluded from the further analysis.

Results of yields showed a similar trend to that seen after 5 weeks, with a yield of 84-91 % for injected and pickle salted group, 88-94 % for injected and brine group and 72-73 % for pickled group. In all three groups the yields showed a slight increasing trend with increased levels of phosphate (Fig. 3.36).



Figur 3.36. Salted fillet yields after five months of storage. For the three salting methods fillets were injected with 0, 0.4, 0.8 or 1.6 % phosphate. Mean and standard deviations of nine fish are shown.

Average muscle pH was 6.9 ± 0.1 for all groups.

The L-value was in the range 57 to 61 for all groups (Fig. 3.37). For injected and pickle salted group, the whiteness was stable around 60-61 scoring the highest of all groups. For the injected and brine group there was a slight positive correlation with phosphate concentration. The pickle salted group showed an opposite trend and had the lowest L-values.

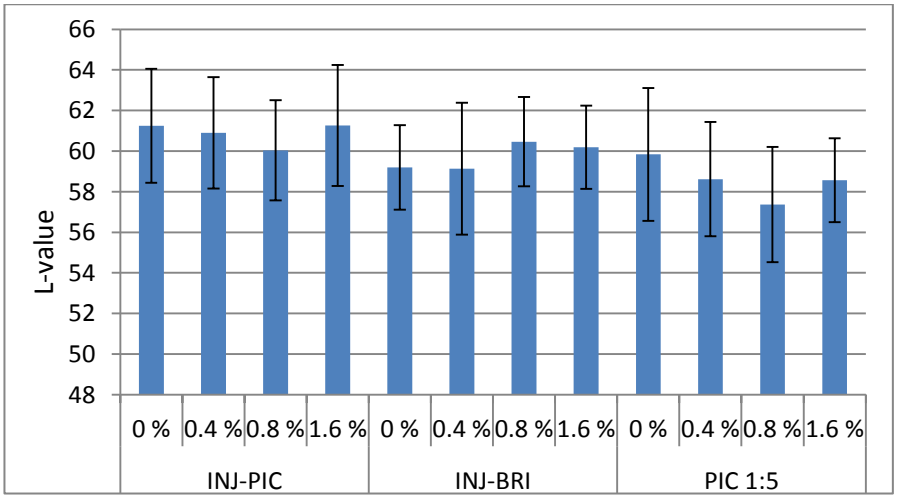


Figure 3.37. Instrumental measurements of whiteness (L-value) on salted fillets. Means and standard deviations of nine fish are shown.

The b-value for injected and pickle salted group had an increasing trend (less yellow) with higher phosphate concentration except for the highest concentration of 1.6 % (Fig. 3.38). For injected and brined group, increased phosphate concentration resulted in increased b-values, while the pickled group showed no trends and was most yellow of all groups. Large standard deviation resulted however in no significant differences between groups or treatments.

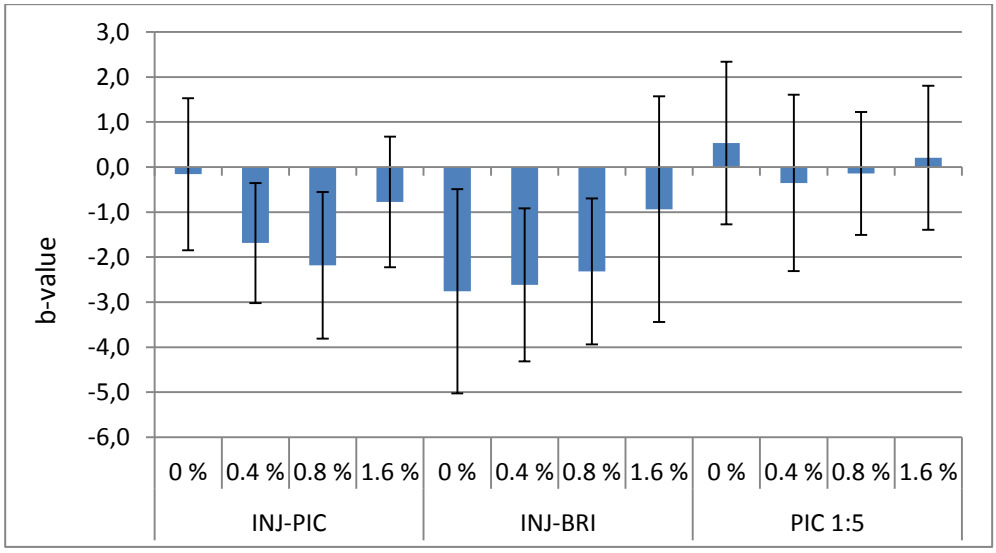


Figure 3.38. Instrumental measurement of yellow color (b-value) of salted fillets. For the three salting methods fillets were injected with 0, 0.4, 0.8 or 1.6 % phosphate. Means and standard deviations of nine fillets are shown.

Figure 3.39 shows fillets from all of the salting groups. The sensorial analysis of injected and pickle salted group showed small differences between subgroups with different phosphate concentration. There was an insignificant, increasing off-odor described as raw meat with increased phosphate concentration. Fillets in injected and brined group increased in white and yellow muscle colour, red colour in belly flaps and lower gaping with increased phosphate concentration from 0 % to 0.4 % phosphate addition, while the respective quality categories showed a decreasing trend for group 0,8 % and 1,6 %. There were no significant off-odors for this group. For pickle salted group there were small differences for the various quality parameters with increased phosphate concentration.

Figure 3.39. Heavy salted fillets after five months of chilled storage (N=9). G11-G14 injected and pickle salted, G21-G24 injected and brined, G31-G34 pickle salted 1:5. Increasing phosphate levels moving to the right.

All groups were visually characterized and ranked by all judges when fillets were gathered as shown in Figure 3.39. For injected and pickled group, subgroups 0.4 % and 0.8 % were ranked as having highest quality followed by 1.6 % and control due to dark/yellow surface colour. In injected and brined group, 0.4 % had highest quality, followed by control, 0.8 % and 1.6 % phosphate due to yellow discoloration. In the pickle salted group, subgroup control was ranked highest, followed by 0.4 %, 0.8 % and 1.6 % due to increased yellow, red and darker colour. Injected and pickled group had slightly higher overall quality than injected and brined group while the pickle salted group was clearly of lowest overall quality due to yellow and red discoloration.

3.2 Large scale trials

3.2.1 Analysis of heavy salted cod after 4 weeks of chilled storage

The yields for salted cod groups were from 76-80 % for injected and pickle salted group where increased phosphate concentration resulted in increased yields (Fig. 3.40). For the pickle salted group the yield was 73-74 % for all groups

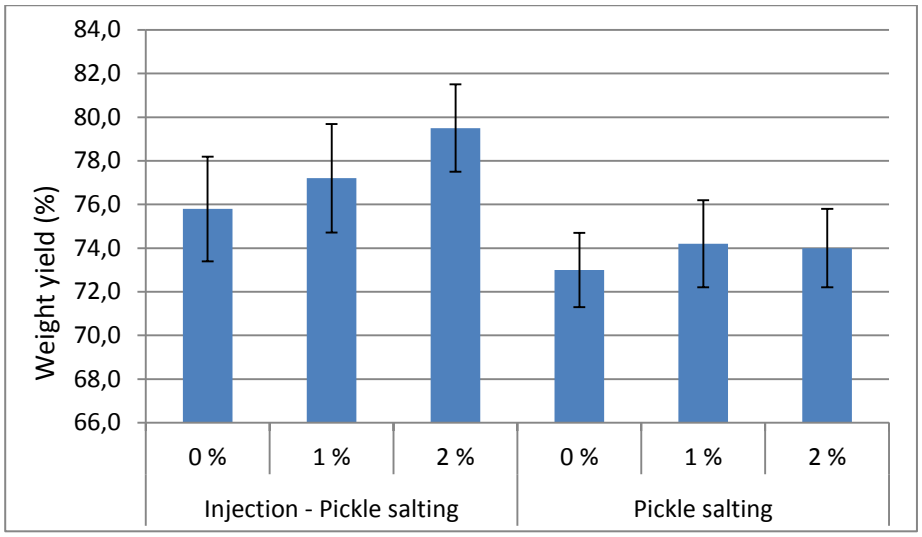


Figure 3.40. Weight yields from raw material weights (split fish) for heavy salted cod after four weeks of chilled storage. For the two salting methods 0, 1 or 2 % phosphate were tested. Means and standard deviations of 30 fish are shown.

In Fig. 3.41 instrumental measurements of muscle surface lightness (L-value) are shown for heavy salted fish. Group injected and pickled had levels of 55-57 and pickle salted group levels of 52-53.

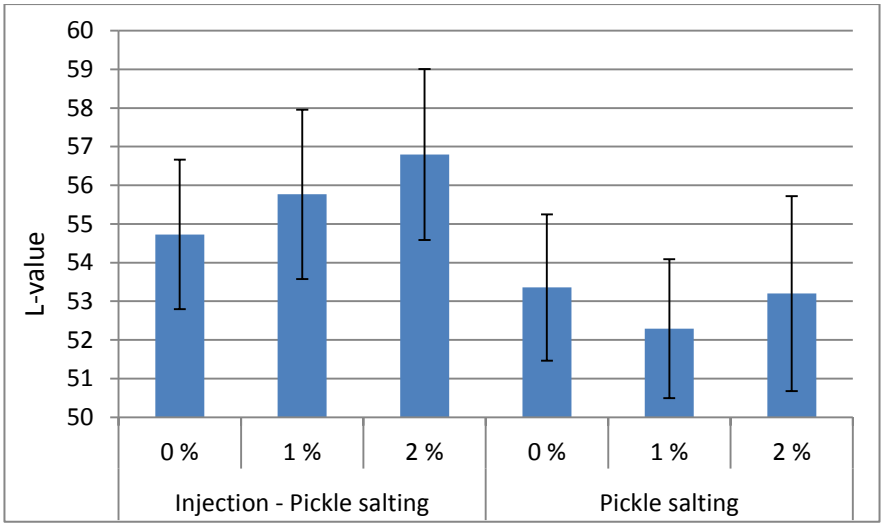


Figure 3.41. Measurements of whiteness (L-value) on muscle surface of heavy salted fish. For the two salting methods 0, 1 or 2 % phosphate were tested. Four measurements were conducted on each split fillet. Means and standard deviations of 30 fish are shown.

The b-values of the raw material were in the range -2.9 to -3.3 for injected and pickled group and -3.5 to -3.9 for pickled group. For salted fish, the b-value was found to be between -2 and -3 for all groups. For injected groups, yellow colour increased with increased phosphate concentration while the opposite as registered for pickle salted groups (Fig. 3.42).

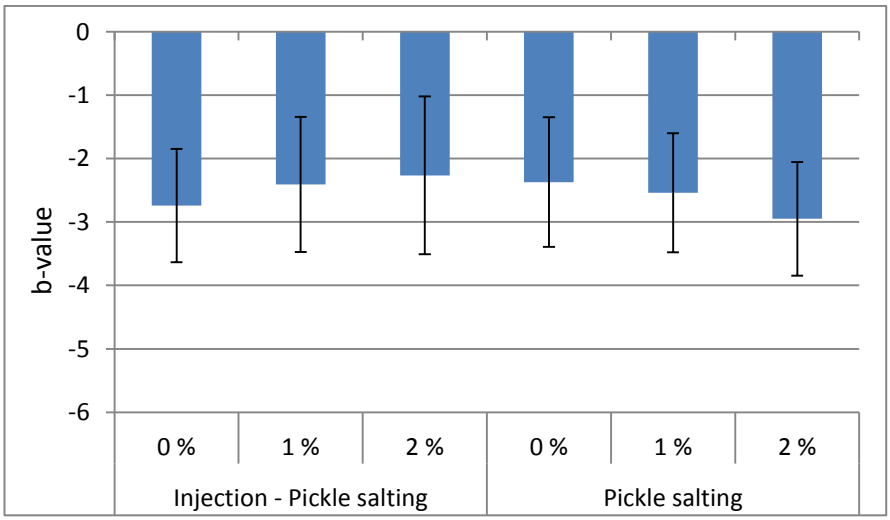


Figure 3.42. Instrumental measurements of yellow colour (b-value) on muscle surface of heavy salted fish after four weeks storage. For the two salting methods 0, 1 or 2 % phosphate were tested. Four measurements were conducted on each split fish. Means and standard deviations of 30 fish are shown.

The sensorial evaluation showed a weak increase in muscle base colour (increased whiteness) and a reduced intensity of yellow colour with increased phosphate concentration for injected groups, while the opposite was registered for pickle salted

groups. Gaping was reduced slightly with higher phosphate concentration for injected group but not for pickle salted group. The level of characteristic heavy salted cod smell was slightly reduced with higher phosphate concentration for injected and pickled group, while the smell was stable at a slightly higher level for the pickled group (Fig. 3.43). None of the differences were statistically significant. A comparison of all salted groups (Fig. 3.44) showed the same trend as found in the sensorial evaluation (Fig. 3.43). pH in muscle loin was measured to be 6,17 ± 0,06 for all groups in the trial.

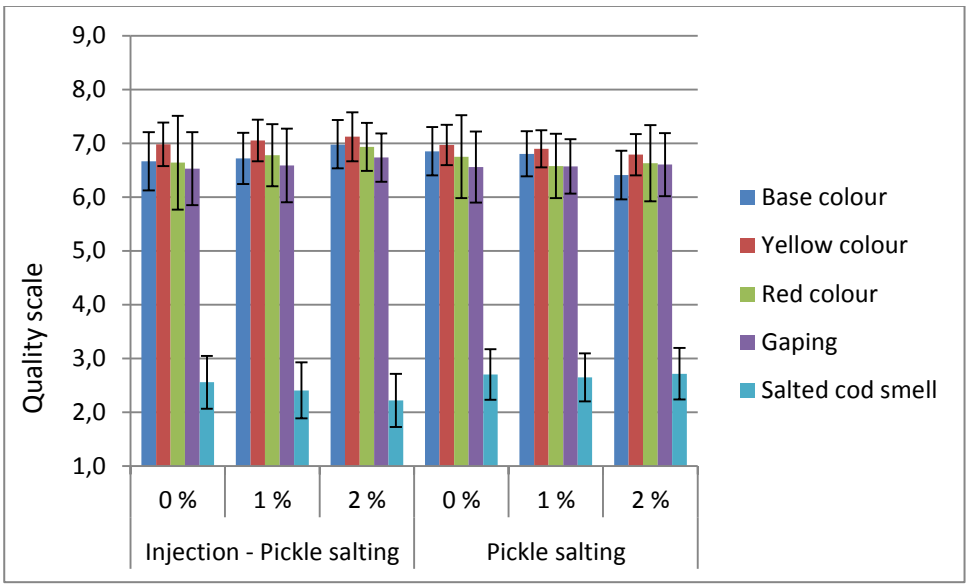


Figure 3.43. Sensorial evaluation of heavy salted cod on a scale from 1 to 9 (best quality). For the two salting methods 0, 1 or 2 % phosphate were tested. Main values and standard deviations for four judges shown. For each of the 6 groups, 30 fish were analysed

Figure 3.44. Pickle salted groups above with increasing phosphate concentration going to the right (0 % to the left, 1 % in the middle and 2 % to the right). Injected and pickle salted groups are shown below.

The water loss was in the range -38 to -39 % for the pickle salted group while the water loss was reduced with higher phosphate concentration in the injected and pickled group (from – 37 to – 35 %) shown in Fig. 3.45.

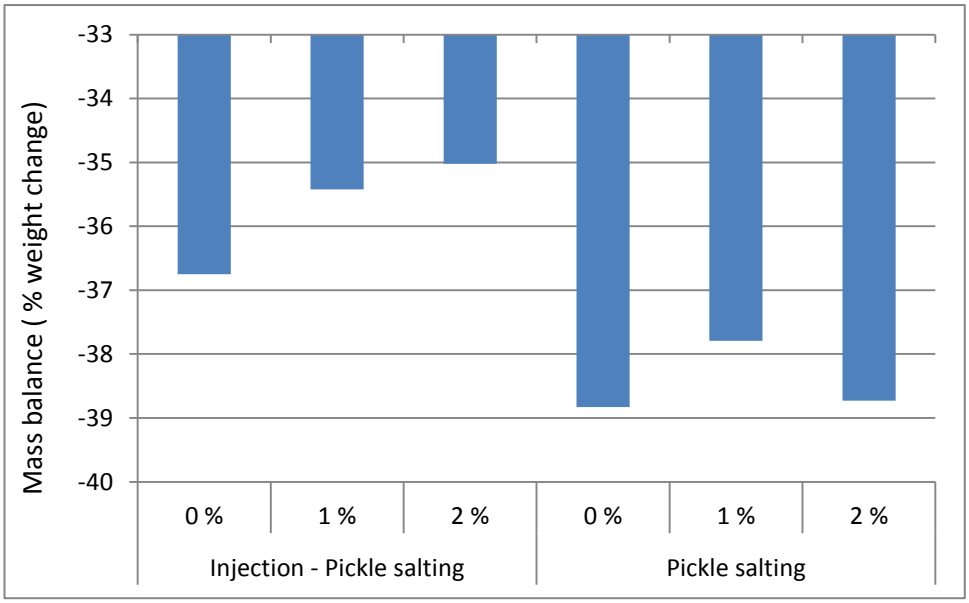


Figure 3.45. Mass balances for water content in heavy salted split fish. For the two salting methods 0, 1 or 2 % phosphate were tested. Means of three fish are shown.

The mass balance for NaCl showed a weight gain ranging from 12-15 % for both groups and no systematic trends were found (Fig. 3.46).

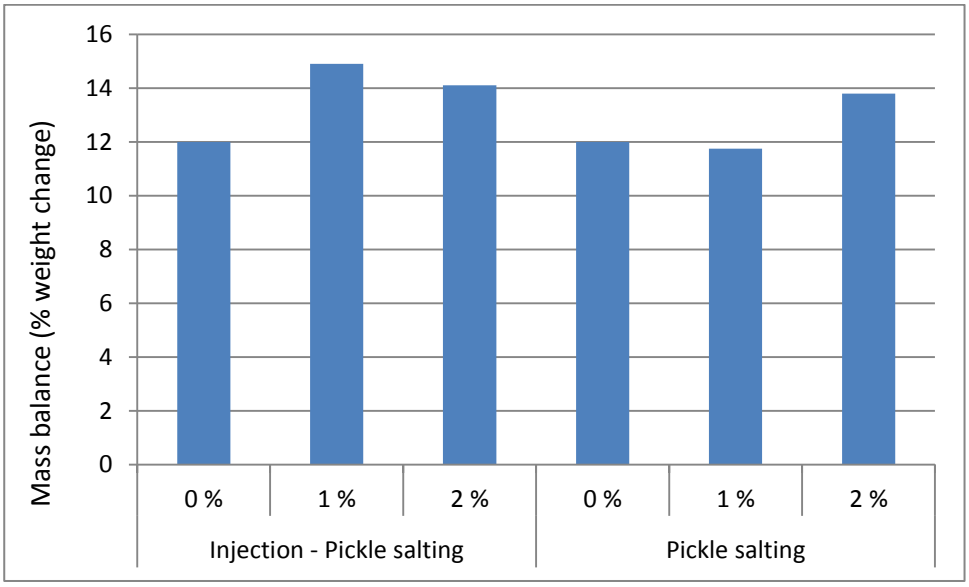


Figure 3.46. Mass balances for NaCl content in heavy salted split fish. For the two salting methods 0, 1 or 2 % phosphate were tested. Means of three fish are shown.

Average NaCl content in large scale trial was 18.1 %. Higher water retention in the injected and pickle salted group seems to be followed by an increase in the salt uptake from muscle tissue.

The phosphate and potassium content shows similar trends in both groups. The P_2O_5-level is reduced from 0.34 g/100 g in the raw material to 0.10 g/100 g in the salt cured fish. Increased phosphate addition leads to slightly elevated levels of muscle P_2O_5 in the injected and pickle salted group, but not in the pickle salted group (Fig. 3.47).

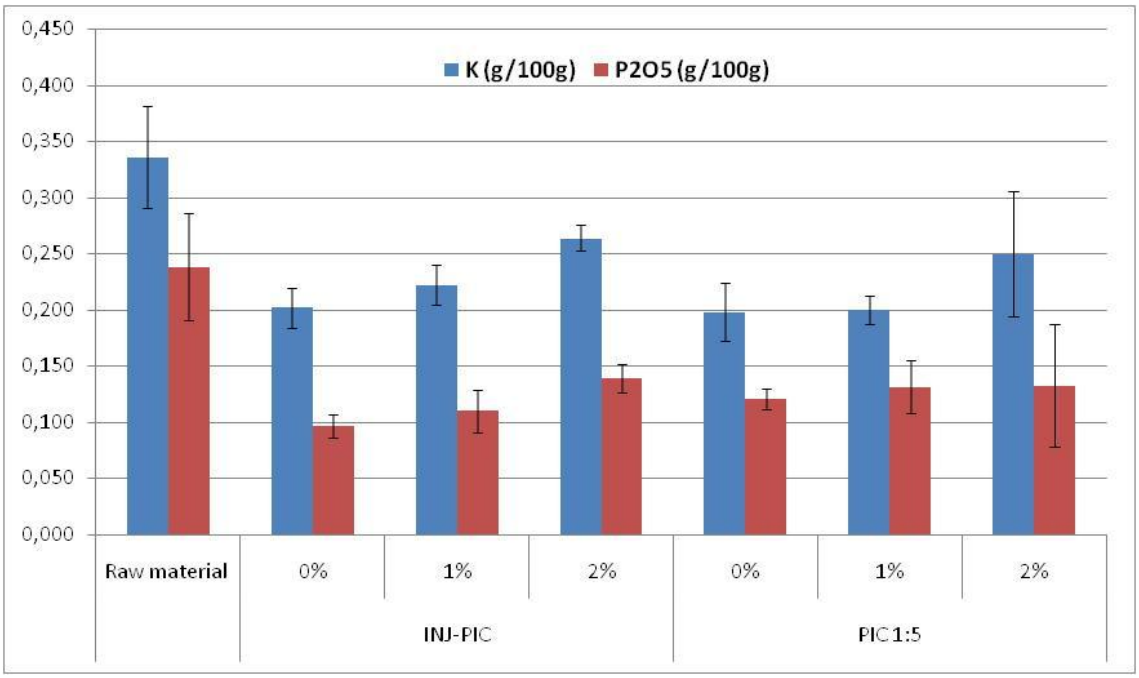


Figure 3.47. Potassium and phosphate content in raw material and heavy salted cod. For the two salting methods 0, 1 or 2 % phosphate were tested. Means and standard deviations of five fish are shown.

The treatment injection and pickle salting increases the phosphorus content, since significant differences (Sig<0.05) were detected by Post-Hoc test. However, the treatment pickle salting shows almost no increase in phosphates level, leading to no statistically significant differences (Sig>0.05), as shown below (Tab. 3.5).

Table 3.5. Post-Hoc SNK test for phosphate contents (P_2O_5) for injected and pickle salted group (left) and pickle salted group (right). Different subsets indicate significant differences.

POLYPHOSPHATES TREATMENT	N	Subset for alpha = .05	
		Group 1	Group 2
Control	5	.0940	
1%	5	.1080	
2%	5		.1420
Sig.		.164	1.000

POLYPHOSPHATES TREATMENT	N	Subset for alpha = .05
		Group 1
Control	5	.1200
1%	5	.1320
2%	5	.1320
Sig.		.839

Enhanced yields were obtained only when absorption of phosphates were registered. Low final phosphate values and additives degradation did not make it possible to detect either pyrophosphate or triphosphate in any of the studied samples.

Regarding to primary oxidation, variability of peroxide values was high with no evident trends in the data (no statistical differences were detected in ANOVA tests).

Concerning to TBARS values, the pattern was different. A decrease in secondary oxidation was detected with increasing phosphates concentration. This trend is statistically significant (ANOVA p<0.05.)(Table 3.6, Figure 3.48). Fillets treated with 2 % phosphate have significantly lower TBARS values than fillets treated with 0 % or 1 % phosphate.

Table 3.6. Post-Hoc SNK test for oxidation (TBARS) for injected and pickle salted group. Different subsets indicate significant differences.

POLYPHOSPHATES TREATMENT	N	Subset for alpha = .05	
		1	2
2%	5	.340	
1%	5		.740
Control	5		.780
Sig.		1.000	.670

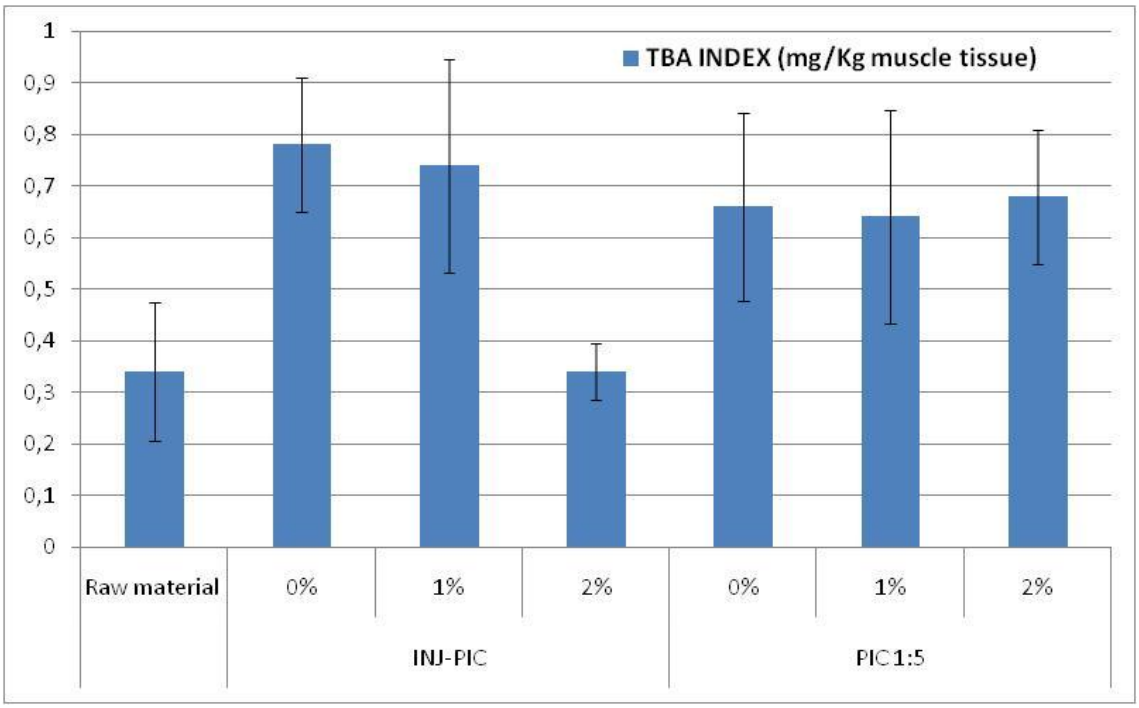


Figure 3.48. TBARS levels in heavy salted fish. For the two salting methods 0, 1 or 2 % phosphate were tested. Means and standard deviations of five fish are shown.

3.3 Fatty acid profiling

The long line raw material used in the large scale trial was of poorer quality than expected due to more blood and darker surface colour than normal long line caught raw material. Various hypothesis have been made to this; a higher fat content and enzyme activity, higher processing temperatures, a significant change in the fatty acids profile, remaining blood boosting oxidation, etc. Possibly, the final explanation is a combination of these and other unknown factors. Several determinations have been carried out in order to detect an increase in the fat levels and a change in the fatty acid profile. Five samples from the raw materials and five samples from processed cod in both trials were analyzed.

The muscle water content decreased slightly from fish caught in spring to early autumn (Fig. 3.49). This was not associated with an increase in fat content as there was no concurrent decrease in fat content. As it has been documented in literature, seasonal variation of muscle fat contents is not significant *(Ingolsfdottir* et *al., 1998)* and this is consistent with our obtained data where no fat content differences were detected (all samples 0.1%). It should be noted that fat determination at such low values might not possess the required sensitivity to detect any differences.

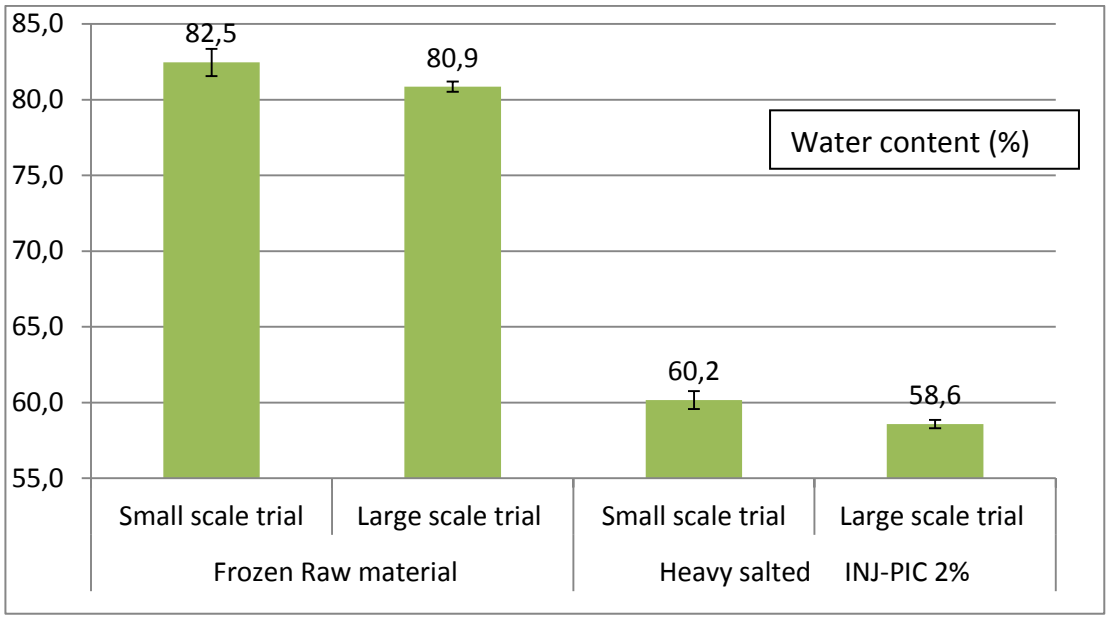


Figure 3.49. Water content (%) in cod raw material and heavy salted cod. Both small and large scale heavy salted fish was injected and pickle salted with 1.6 % and 2 % phosphate, respectively. Means and standard deviations of five fish are shown.

Although internal variability is high, Figure 3.50 shows an increase in the poly-unsaturated fatty acids in early autumn caught raw material as a result of a decrease in mono-unsaturated fatty acids (especially oleic acid). Omega-3 congeners (mainly EPA and DHA) are responsible for this change meanwhile omega-6 remains almost constant. It should be discussed whether such a reduced increase is sufficient to develop the significant sensorial differences. Possibly, this elevated poly-unsaturated

fatty acids level contribute to this sensorial differences, but other factors (maybe residual blood) might be playing a more important role.

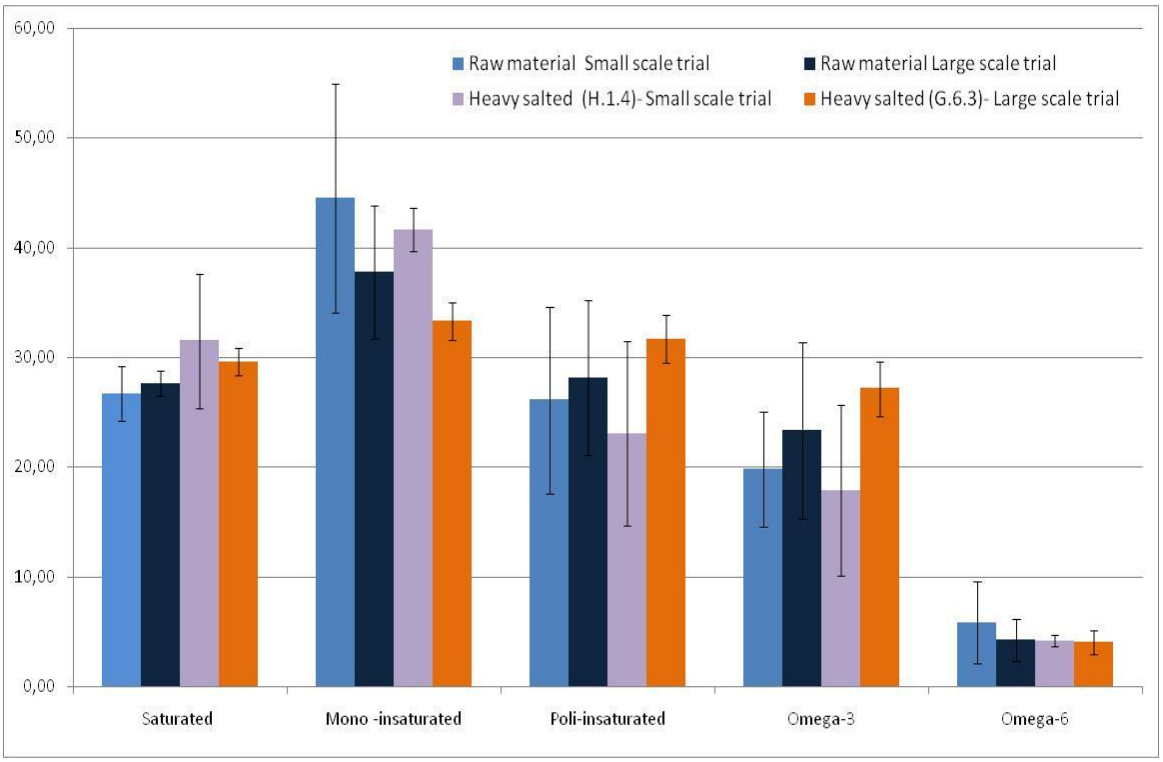


Figure 3.50. General fatty acid profile (%) in the studied samples. Means and standard deviations of five fish are shown.

There was also a difference in the natural trans fatty acid contents in fish harvested in spring compared to cod caught in August-September.

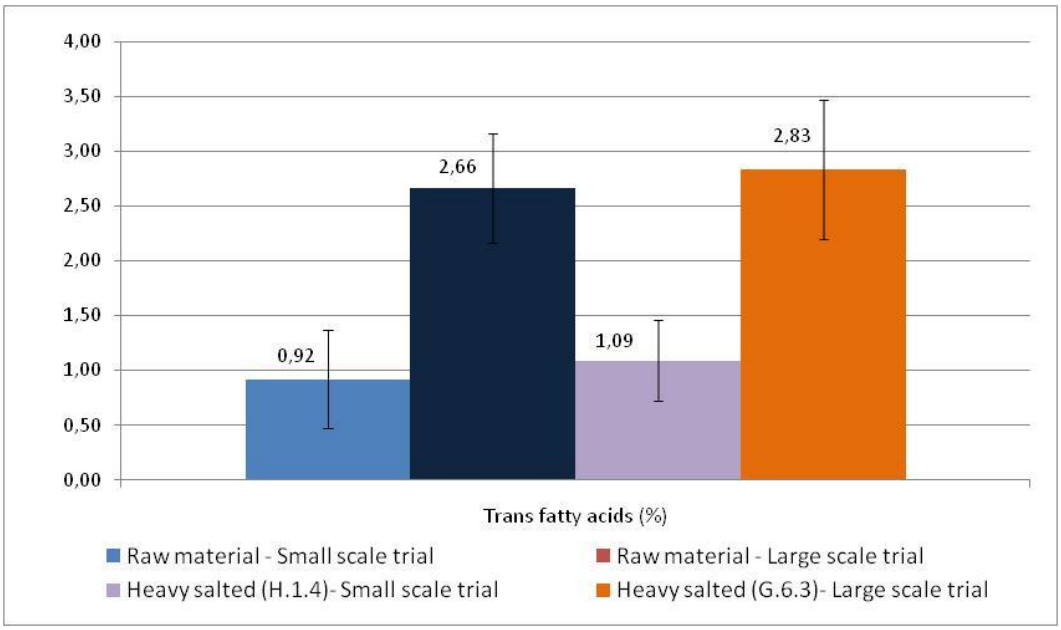


Figure 3.51 Trans fatty acids (%) in the study samples. Mean and standard deviation for five fish are shown.

4 DISCUSSION

Results from both small and large scale trial show that **injection seems to be the most effective method for addition of phosphates** in the muscle tissue. Pure pickle salting without including the pre-injection step ended in almost no absorption of the additive. When introducing a brine step after the injection the phosphate levels were further elevated. This suggests that some phosphate absorption takes place during the brining process although the majority of phosphate is introduced by the physical injection of brine. Minor phosphate uptake is taking place during the pickle salting stage. This is as expected due to the large out flux of moisture at this stage in the salting process.

In both small and large scale trials the natural phosphate content (P_2O_5) was approximately reduced by half from raw material to heavy salted product. In the small scale trials, the threshold level of 0.5 g P_2O_5/100 g (maximum allowable level in frozen fillets) was achieved only when the highest phosphate concentration was used (1.6 %). When using 2 % phosphate in the large scale trials however, only 0.14 g P_2O_5/100 g was achieved in the final products. This is probably due to the lower yields obtained in the large scale trials, but another factor could be that other injection parameters were used in large scale than in small scale trials.

Chelation studies aimed to investigate if phosphates chelate metals in the fish muscle and contribute to remove metals from tissue during salt-water diffusion. The low values and the high variability in measured metal levels did not allow any conclusive statement, but **it seems that a phosphate chelation effect is not present**. Copper is present in trace levels below 1 mg/Kg in almost all the samples. Zinc levels are also not affected by phosphates and remain relatively steady around 4.1 mg/kg in salted samples and 3.2 mg/kg in raw material in the small scale trial. Iron values randomly vary and might possibly be related to the quality of the fillet (bleeding). In fact, the second trial samples were of a poorer quality and presented more blood spotting. Both iron and zinc in these samples vary largely from one sample to another with no specific trend, reinforcing the residual blood theory.

The **Peroxides and Thiobarbituric Indexes**, used for the evaluation of the chemical oxidation in the samples, **gave no systematic results which enabled any conclusions to be made concerning phosphates and oxidation**. Fresh raw material was almost not oxidized and therefore no differences were measured. Oxidation results in unprocessed frozen raw materials in the small scale trial were very high, showing that poor net caught raw material is not suitable for frozen storage. The oxidation reactions are not inhibited by frozen storage at – 30 ^{o}C. Pre-injection groups from frozen raw material (where absorption of phosphates effectively took place), show a Peroxides Value diminishing trend with the intensity of the phosphates use, especially when 1.6% P_2O_5 is used. This is not reflected in neither sensorial nor colour results, but, it should be reminded that significant primary oxidation is not usually linked to sensorial defects.

The relatively poor quality of large scale trial material is not detected in the chemical oxidation analysis, with low peroxides and TBA levels detected. Compared to the frozen raw material in the small scale trials which was stored for several days before being frozen, this raw material was onboard frozen. Nevertheless, and despite the small amount of samples analyzed, a certain reduction in secondary oxidation was obtained with the 2 % P_2O_5 addition in the injected group. However, this is corresponding neither with previous total phosphorus data nor sensorial test ranking. Therefore, it would be too risky to assign phosphates the capacity of preventing oxidation and improving sensorial appearance.

The sensorial evaluation of the products shows no clear influence of phosphates on improving heavy salted cod quality. **Phosphates seem however to enhance quality for light salted cod**. Although conditioned by varying quality in raw material, both fresh and frozen raw fillets developed higher lightness (L-value) and less intense yellow color (b-value) with higher phosphate treatment of light salted cod. In general, in sensorial quality testing, the phosphate treated light salted samples were best ranked.

As earlier reported (Bjørkevoll et *al*., 2011; Thorarinsdottir et *al*., 2001), **the positive effect of di- and triphosphates on yield gain for heavy salted products has been demonstrated** in this study. Meanwhile, light salted samples reflected very small yield differences after thawing. The five weeks stored salted fillets showed a relatively high weight increase between control and phosphate treated samples. The yields increased with elevated phosphate levels. This effect seems to be more diffuse after five and seven months storage due to moisture loss. Both small and large scale trials, and either fresh or frozen raw material, followed this pattern. Part of this weight gain is because phosphates reduce the amount of muscle water leaking out during salting without a decrease in the levels of the incoming salt. Again, the **processing methods which included pre-injection contributed to a slightly higher salt content** compared to common pickle salting.

Calcium and magnesium doubled their contents from raw material to salted fillets as a result of the weight effect, which gives some evidence that these metals **are not washed out during the salting process,** but remaining within the tissue. The contribution of the incoming salt to raise the tissue calcium content has also been observed; since in light salting the calcium contents increase even though there is a considerable yield gain because of the salt and water injection. Magnesium however, slightly reduces its concentration. There is no correlation between phosphate treatments and calcium or magnesium, so CARNAL 2110 does not affect these elements in significant amounts.

Despite some changes in absolute data, **heavy-salted fillets** showed the same trends for five weeks as for five or seven month of storage. In the **case of prolonged storage, results were sometimes rather diffuse or even contradictory (colour)**. Nevertheless, the highest sensorial scores were obtained in samples with highest phosphates absorption. In fact, salting methods including pre-injection had, in general, higher quality than the rest of the processing methods studied. As the light salted samples, increased L-value and reduced b-value seemed in most cases to be correlated to the

intensity of the use of these additives. Sensorial ranking (including gaping, smell, color, and blood spotting) seemed not to present any trend in small scale trials, but in large scale trials reduced “heavy salted cod smell” and reduced gaping were correlated with phosphates concentration.

In summary, the **analytical data concerning oxidation and muscle colour are not conclusive**. Although some trends have been detected, it becomes necessary to study them more in detail and with a higher amount of samples.

5 CONCLUSIONS

- Injection is a successful method for introduction of phosphates to fish muscle. Adding phosphates blended in brine during pickle salting does not lead to effective phosphate uptake.

- It seems that phosphates are working better, concerning quality parameters, when raw materials of optimal rather than poor quality are used

- No consistent effects of phosphate on colour were detected in small scale. In large scale, a slight improvement in colour was noted. Phosphate does not seem to remove or camouflage raw material of poor quality, but more data is needed on documenting if phosphates affect the surface colour and blood level in fish muscle.

- No chelation effect from phosphates in the selected oxidizing minerals was detected based on the final levels of these metals in processed fillets. However, large variations in raw material quality could have overshadowed the chelation effect.

- Minor differences between fresh and raw materials were detected on the oxidation effects of phosphates in heavy salted products. However, frozen net caught raw materials were more oxidized both prior to and after salting. Analytical data concerning oxidation is not conclusive yet. Some trends have been detected, but more data are needed on the effects of phosphates in large scale production with raw materials of various quality.

- The phosphate Carnal 2110 seems to be effective in water retention, and therefore increased yields, when added in 1.6 % level in small scale and 2 % in large scale.

- The levels of phosphates added were below the legislation levels in almost all the tested samples. Natural phosphates are lost during heavy-salting. Di- and triphosphate addition partially compensates this loss.

- Retaining of white muscle surface, reducing oxidation and yield improvements was registered on light salted cod.

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7 APPENDIX.

7.1 Phosphate results.

		1st trial							
				DIPHOSPHATE		TRIPHOSPHATE		HEXAMETAPHOSPHATE		COMMENTS
		SMP	ANFACO CODE	(gP/100g)	(gP2O5/100g)	(gP/100g)	(gP2O5/100g)	(gP/100g)	(gP2O5/100g)	
HEAVY SALTED - FRESH RAW MATERIAL	G.O	G.0.A	1105442	< 0,03	< 0,06	<0,03	<0,07	< 0,04	< 0,09	NO ADDED PHOSPHATE RESIDUES DETECTED
		G.0.B	1105443	< 0,03	< 0,06	<0,03	<0,07	< 0,04	< 0,09	NO ADDED PHOSPHATE RESIDUES DETECTED
		G.0.C	1105444	< 0,03	< 0,06	<0,03	<0,07	< 0,04	< 0,09	NO ADDED PHOSPHATE RESIDUES DETECTED
		G.0.D	1105445	< 0,03	< 0,06	<0,03	<0,07	< 0,04	< 0,09	NO ADDED PHOSPHATE RESIDUES DETECTED
		G.0.E	1105446	< 0,03	< 0,06	<0,03	<0,07	< 0,04	< 0,09	NO ADDED PHOSPHATE RESIDUES DETECTED
	G.1	G.I.1.A	1105447	< 0,03	< 0,06	<0,03	<0,07	< 0,04	< 0,09	NO ADDED PHOSPHATE RESIDUES DETECTED
		G.I.1.B	1105448	< 0,03	< 0,06	<0,03	<0,07	< 0,04	< 0,09	NO ADDED PHOSPHATE RESIDUES DETECTED
		G.I.1.C	1105449	< 0,03	< 0,06	<0,03	<0,07	< 0,04	< 0,09	NO ADDED PHOSPHATE RESIDUES DETECTED
		G.I.2.A	1105450	< 0,03	< 0,06	<0,03	<0,07	< 0,04	< 0,09	NO ADDED PHOSPHATE RESIDUES DETECTED
		G.I.2.B	1105451	< 0,03	< 0,06	<0,03	<0,07	< 0,04	< 0,09	NO ADDED PHOSPHATE RESIDUES DETECTED
		G.I.2.C	1105452	< 0,03	< 0,06	<0,03	<0,07	< 0,04	< 0,09	NO ADDED PHOSPHATE RESIDUES DETECTED
		G.I.3.A	1105453	< 0,03	< 0,06	<0,03	<0,07	< 0,04	< 0,09	NO ADDED PHOSPHATE RESIDUES DETECTED
		G.I.3.B	1105454	< 0,03	< 0,06	<0,03	<0,07	< 0,04	< 0,09	NO ADDED PHOSPHATE RESIDUES DETECTED
		G.I.3.C	1105455	< 0,03	< 0,06	<0,03	<0,07	< 0,04	< 0,09	NO ADDED PHOSPHATE RESIDUES DETECTED
		G.I.4.A	1105456	< 0,03	< 0,06	<0,03	<0,07	< 0,04	< 0,09	NO ADDED PHOSPHATE RESIDUES DETECTED
		G.I.4.B	1105457	< 0,03	< 0,06	<0,03	<0,07	< 0,04	< 0,09	NO ADDED PHOSPHATE RESIDUES DETECTED
		G.I.4.C	1105458	< 0,03	< 0,06	<0,03	<0,07	< 0,04	< 0,09	NO ADDED PHOSPHATE RESIDUES DETECTED
	G.2	G.II.1.A	1105459	< 0,03	< 0,06	<0,03	<0,07	< 0,04	< 0,09	NO ADDED PHOSPHATE RESIDUES DETECTED
		G.II.1.B	1105460	< 0,03	< 0,06	<0,03	<0,07	< 0,04	< 0,09	NO ADDED PHOSPHATE RESIDUES DETECTED
		G.II.1.C	1105461	< 0,03	< 0,06	<0,03	<0,07	< 0,04	< 0,09	NO ADDED PHOSPHATE RESIDUES DETECTED
		G.II.2.A	1105462	< 0,03	< 0,06	<0,03	<0,07	< 0,04	< 0,09	NO ADDED PHOSPHATE RESIDUES DETECTED
		G.II.2.B	1105463	< 0,03	< 0,06	<0,03	<0,07	< 0,04	< 0,09	NO ADDED PHOSPHATE RESIDUES DETECTED
		G.II.2.C	1105464	< 0,03	< 0,06	<0,03	<0,07	< 0,04	< 0,09	NO ADDED PHOSPHATE RESIDUES DETECTED
		G.II.3.A	1105465	< 0,03	< 0,06	<0,03	<0,07	< 0,04	< 0,09	MINOR TRIPHOSPHATE RESIDUES DETECTED
		G.II.3.B	1105466	< 0,03	< 0,06	<0,03	<0,07	< 0,04	< 0,09	MINOR TRIPHOSPHATE RESIDUES DETECTED
		G.II.3.C	1105467	< 0,03	< 0,06	<0,03	<0,07	< 0,04	< 0,09	MINOR TRIPHOSPHATE RESIDUES DETECTED
		G.II.4.A	1105468	< 0,03	< 0,06	<0,03	<0,07	< 0,04	< 0,09	NO ADDED PHOSPHATE RESIDUES DETECTED
		G.II.4.B	1105469	< 0,03	< 0,06	<0,03	<0,07	< 0,04	< 0,09	MINOR TRIPHOSPHATE RESIDUES DETECTED
		G.II.4.C	1105470	< 0,03	< 0,06	<0,03	<0,07	< 0,04	< 0,09	MINOR TRIPHOSPHATE RESIDUES DETECTED
	G.3	G.III.1.A	1105471	< 0,03	< 0,06	<0,03	<0,07	< 0,04	< 0,09	NO ADDED PHOSPHATE RESIDUES DETECTED
		G.III.1.B	1105472	< 0,03	< 0,06	<0,03	<0,07	< 0,04	< 0,09	NO ADDED PHOSPHATE RESIDUES DETECTED
		G.III.1.C	1105473	< 0,03	< 0,06	<0,03	<0,07	< 0,04	< 0,09	NO ADDED PHOSPHATE RESIDUES DETECTED
		G.III.2.A	1105474	< 0,03	< 0,06	<0,03	<0,07	< 0,04	< 0,09	MINOR TRIPHOSPHATE RESIDUES DETECTED
		G.III.2.B	1105475	< 0,03	< 0,06	<0,03	<0,07	< 0,04	< 0,09	MINOR TRIPHOSPHATE RESIDUES DETECTED
		G.III.2.C	1105476	< 0,03	< 0,06	<0,03	<0,07	< 0,04	< 0,09	MINOR TRIPHOSPHATE RESIDUES DETECTED
		G.III.3.A	1105477	< 0,03	< 0,06	0,07	0,15	< 0,04	< 0,09	TRIPHOSPHATE RESIDUES QUANTIFIED
		G.III.3.B	1105478	< 0,03	< 0,06	0,05	0,11	< 0,04	< 0,09	TRIPHOSPHATE RESIDUES QUANTIFIED
		G.III.3.C	1105479	< 0,03	< 0,06	0,1	0,23	< 0,04	< 0,09	TRIPHOSPHATE RESIDUES QUANTIFIED
		G.III.4.A	1105480	< 0,03	< 0,06	<0,03	<0,07	< 0,04	< 0,09	NO ADDED PHOSPHATE RESIDUES DETECTED
		G.III.4.B	1105481	< 0,03	< 0,06	<0,03	<0,07	< 0,04	< 0,09	NO ADDED PHOSPHATE RESIDUES DETECTED
		G.III.4.C	1105482	< 0,03	< 0,06	<0,03	<0,07	< 0,04	< 0,09	NO ADDED PHOSPHATE RESIDUES DETECTED

Raw material
Injection . Pickelsalting with brine addition(1:10).Dy-salting.
Injection . Brining (bath) 24 h. - Drysalting.
Pickelsalting with brine addition(1:5).Dry-salting.

		SMP	ANFACO CODE	DIPHOSPHATE		TRIPHOSPHATE		HEXAMETAPHOSPHATE		COMMENTS
				(gP/100g)	(gP2O5/100g)	(gP/100g)	(gP2O5/100g)	(gP/100g)	(gP2O5/100g)	
HEAVY SALTED-FROZEN RAW MATERIAL	H.O	H.0.1	1109175	< 0,03	< 0,06	< 0,03	<0,07	< 0,04	< 0,09	NO ADDED PHOSPHATE RESIDUES DETECTED
		H.0.2	1109176	< 0,03	< 0,06	< 0,03	<0,07	< 0,04	< 0,09	NO ADDED PHOSPHATE RESIDUES DETECTED
		H.0.3	1109177	< 0,03	< 0,06	< 0,03	<0,07	< 0,04	< 0,09	NO ADDED PHOSPHATE RESIDUES DETECTED
		H.0.4	1109178	< 0,03	< 0,06	< 0,03	<0,07	< 0,04	< 0,09	NO ADDED PHOSPHATE RESIDUES DETECTED
		H.0.5	1109179	< 0,03	< 0,06	< 0,03	<0,07	< 0,04	< 0,09	NO ADDED PHOSPHATE RESIDUES DETECTED
	H.1	H.I.1.A	1109180	< 0,03	< 0,06	< 0,03	<0,07	< 0,04	< 0,09	NO ADDED PHOSPHATE RESIDUES DETECTED
		H.I.1.B	1109181	< 0,03	< 0,06	< 0,03	<0,07	< 0,04	< 0,09	NO ADDED PHOSPHATE RESIDUES DETECTED
		H.I.1.C	1109182	< 0,03	< 0,06	< 0,03	<0,07	< 0,04	< 0,09	NO ADDED PHOSPHATE RESIDUES DETECTED
		H.I.2.A	1109183	< 0,03	< 0,06	< 0,03	<0,07	< 0,04	< 0,09	NO ADDED PHOSPHATE RESIDUES DETECTED
		H.I.2.B	1109184	< 0,03	< 0,06	< 0,03	<0,07	< 0,04	< 0,09	NO ADDED PHOSPHATE RESIDUES DETECTED
		H.I.2.C	1109185	< 0,03	< 0,06	< 0,03	<0,07	< 0,04	< 0,09	NO ADDED PHOSPHATE RESIDUES DETECTED
		H.I.3.A	1109186	< 0,03	< 0,06	< 0,03	<0,07	< 0,04	< 0,09	NO ADDED PHOSPHATE RESIDUES DETECTED
		H.I.3.B	1109187	< 0,03	< 0,06	< 0,03	<0,07	< 0,04	< 0,09	NO ADDED PHOSPHATE RESIDUES DETECTED
		H.I.3.C	1109188	< 0,03	< 0,06	< 0,03	<0,07	< 0,04	< 0,09	MINOR TRIPHOSPHATE RESIDUES DETECTED
		H.I.4.A	1109189	< 0,03	< 0,06	< 0,03	<0,07	< 0,04	< 0,09	MINOR TRIPHOSPHATE RESIDUES DETECTED
		H.I.4.B	1109190	< 0,03	< 0,06	< 0,03	<0,07	< 0,04	< 0,09	MINOR TRIPHOSPHATE RESIDUES DETECTED
		H.I.4.C	1109191	< 0,03	< 0,06	< 0,03	<0,07	< 0,04	< 0,09	MINOR TRIPHOSPHATE RESIDUES DETECTED
	H.2	H.II.1.A	1109192	< 0,03	< 0,06	< 0,03	<0,07	< 0,04	< 0,09	NO ADDED PHOSPHATE RESIDUES DETECTED
		H.II.1.B	1109193	< 0,03	< 0,06	< 0,03	<0,07	< 0,04	< 0,09	NO ADDED PHOSPHATE RESIDUES DETECTED
		H.II.1.C	1109194	< 0,03	< 0,06	< 0,03	<0,07	< 0,04	< 0,09	NO ADDED PHOSPHATE RESIDUES DETECTED
		H.II.2.A	1109195	< 0,03	< 0,06	< 0,03	<0,07	< 0,04	< 0,09	NO ADDED PHOSPHATE RESIDUES DETECTED
		H.II.2.B	1109196	< 0,03	< 0,06	< 0,03	<0,07	< 0,04	< 0,09	NO ADDED PHOSPHATE RESIDUES DETECTED
		H.II.2.C	1109197	< 0,03	< 0,06	< 0,03	<0,07	< 0,04	< 0,09	NO ADDED PHOSPHATE RESIDUES DETECTED
		H.II.3.A	1109198	< 0,03	< 0,06	< 0,03	<0,07	< 0,04	< 0,09	MINOR TRIPHOSPHATE RESIDUES DETECTED
		H.II.3.B	1109199	< 0,03	< 0,06	< 0,03	<0,07	< 0,04	< 0,09	MINOR TRIPHOSPHATE RESIDUES DETECTED
		H.II.3.C	1109200	< 0,03	< 0,06	< 0,03	<0,07	< 0,04	< 0,09	MINOR TRIPHOSPHATE RESIDUES DETECTED
		H.II.4.A	1109201	< 0,03	< 0,06	< 0,03	<0,07	< 0,04	< 0,09	MINOR TRIPHOSPHATE RESIDUES DETECTED
		H.II.4.B	1109202	< 0,03	< 0,06	< 0,03	<0,07	< 0,04	< 0,09	MINOR TRIPHOSPHATE RESIDUES DETECTED
		H.II.4.C	1109203	< 0,03	< 0,06	< 0,03	<0,07	< 0,04	< 0,09	MINOR TRIPHOSPHATE RESIDUES DETECTED
	H.3	H.III.1.A	1109204	< 0,03	< 0,06	< 0,03	<0,07	< 0,04	< 0,09	NO ADDED PHOSPHATE RESIDUES DETECTED
		H.III.1.B	1109205	< 0,03	< 0,06	< 0,03	<0,07	< 0,04	< 0,09	NO ADDED PHOSPHATE RESIDUES DETECTED
		H.III.1.C	1109206	< 0,03	< 0,06	< 0,03	<0,07	< 0,04	< 0,09	NO ADDED PHOSPHATE RESIDUES DETECTED
		H.III.2.A	1109207	< 0,03	< 0,06	< 0,03	<0,07	< 0,04	< 0,09	NO ADDED PHOSPHATE RESIDUES DETECTED
		H.III.2.B	1109208	< 0,03	< 0,06	< 0,03	<0,07	< 0,04	< 0,09	NO ADDED PHOSPHATE RESIDUES DETECTED
		H.III.2.C	1109209	< 0,03	< 0,06	< 0,03	<0,07	< 0,04	< 0,09	NO ADDED PHOSPHATE RESIDUES DETECTED
		H.III.3.A	1109210	< 0,03	< 0,06	< 0,03	<0,07	< 0,04	< 0,09	NO ADDED PHOSPHATE RESIDUES DETECTED
		H.III.3.B	1109211	< 0,03	< 0,06	< 0,03	<0,07	< 0,04	< 0,09	NO ADDED PHOSPHATE RESIDUES DETECTED
		H.III.3.C	1109212	< 0,03	< 0,06	< 0,03	<0,07	< 0,04	< 0,09	NO ADDED PHOSPHATE RESIDUES DETECTED
		H.III.4.A	1109213	< 0,03	< 0,06	< 0,03	<0,07	< 0,04	< 0,09	NO ADDED PHOSPHATE RESIDUES DETECTED
		H.III.4.B	1109214	< 0,03	< 0,06	< 0,03	<0,07	< 0,04	< 0,09	NO ADDED PHOSPHATE RESIDUES DETECTED
		H.III.4.C	1109215	< 0,03	< 0,06	< 0,03	<0,07	< 0,04	< 0,09	NO ADDED PHOSPHATE RESIDUES DETECTED

LIGHT SALTED

FRESH RAW MATERIAL

SMP	ANFACO CODE	DIPHOSPHATE		TRIPHOSPHATE		HEXAMETAPHOSPHATE		COMMENTS
		(gP/100g)	(gP2O5/100g)	(gP/100g)	(gP2O5/100g)	(gP/100g)	(gP2O5/100g)	
L.V.1.A	1109216	< 0,03	< 0,06	< 0,03	<0,07	< 0,04	< 0,09	NO ADDED PHOSPHATE RESIDUES DETECTED
L.V.1.B	1109217	< 0,03	< 0,06	< 0,03	<0,07	< 0,04	< 0,09	NO ADDED PHOSPHATE RESIDUES DETECTED
L.V.1.C	1109218	< 0,03	< 0,06	< 0,03	<0,07	< 0,04	< 0,09	NO ADDED PHOSPHATE RESIDUES DETECTED
L.V.2.A	1109219	< 0,03	< 0,06	< 0,03	<0,07	< 0,04	< 0,09	NO ADDED PHOSPHATE RESIDUES DETECTED
L.V.2.B	1109220	< 0,03	< 0,06	< 0,03	<0,07	< 0,04	< 0,09	NO ADDED PHOSPHATE RESIDUES DETECTED
L.V.2.C	1109221	< 0,03	< 0,06	< 0,03	<0,07	< 0,04	< 0,09	NO ADDED PHOSPHATE RESIDUES DETECTED
L.V.3.A	1109222	< 0,03	< 0,06	< 0,03	<0,07	< 0,04	< 0,09	NO ADDED PHOSPHATE RESIDUES DETECTED
L.V.3.B	1109223	< 0,03	< 0,06	< 0,03	<0,07	< 0,04	< 0,09	MINOR TRIPHOSPHATE RESIDUES DETECTED
L.V.3.C	1109224	< 0,03	< 0,06	< 0,03	<0,07	< 0,04	< 0,09	MINOR TRIPHOSPHATE RESIDUES DETECTED
L.V.4.A	1109225	< 0,03	< 0,06	< 0,03	<0,07	< 0,04	< 0,09	MINOR TRIPHOSPHATE RESIDUES DETECTED
L.V.4.B	1109226	< 0,03	< 0,06	< 0,03	<0,07	< 0,04	< 0,09	MINOR TRIPHOSPHATE RESIDUES DETECTED
L.V.4.C	1109227	< 0,03	< 0,06	< 0,03	<0,07	< 0,04	< 0,09	MINOR TRIPHOSPHATE RESIDUES DETECTED

FROZEN RAW MATERIAL

SMP	ANFACO CODE	DIPHOSPHATE		TRIPHOSPHATE		HEXAMETAPHOSPHATE		COMMENTS
		(gP/100g)	(gP2O5/100g)	(gP/100g)	(gP2O5/100g)	(gP/100g)	(gP2O5/100g)	
M.V.1.A	1111763	< 0,03	< 0,06	< 0,03	<0,07	< 0,04	< 0,09	NO ADDED PHOSPHATE RESIDUES DETECTED
M.V.1.B	1111764	< 0,03	< 0,06	< 0,03	<0,07	< 0,04	< 0,09	NO ADDED PHOSPHATE RESIDUES DETECTED
M.V.1.C	1111765	< 0,03	< 0,06	< 0,03	<0,07	< 0,04	< 0,09	NO ADDED PHOSPHATE RESIDUES DETECTED
M.V.2.A	1111766	< 0,03	< 0,06	< 0,03	<0,07	< 0,04	< 0,09	MINOR TRIPHOSPHATE RESIDUES DETECTED
M.V.2.B	1111767	< 0,03	< 0,06	< 0,03	<0,07	< 0,04	< 0,09	MINOR TRIPHOSPHATE RESIDUES DETECTED
M.V.2.C	1111768	< 0,03	< 0,06	< 0,03	<0,07	< 0,04	< 0,09	MINOR TRIPHOSPHATE RESIDUES DETECTED
M.V.3.A	1111769	< 0,03	< 0,06	< 0,03	<0,07	< 0,04	< 0,09	NO ADDED PHOSPHATE RESIDUES DETECTED
M.V.3.B	1111770	< 0,03	< 0,06	< 0,03	<0,07	< 0,04	< 0,09	NO ADDED PHOSPHATE RESIDUES DETECTED
M.V.3.C	1111771	< 0,03	< 0,06	< 0,03	<0,07	< 0,04	< 0,09	NO ADDED PHOSPHATE RESIDUES DETECTED
M.V.4.A	1111772	< 0,03	< 0,06	< 0,03	<0,07	< 0,04	< 0,09	MINOR TRIPHOSPHATE RESIDUES DETECTED
M.V.4.B	1111773	< 0,03	< 0,06	< 0,03	<0,07	< 0,04	< 0,09	MINOR TRIPHOSPHATE RESIDUES DETECTED
M.V.4.C	1111774	< 0,03	< 0,06	< 0,03	<0,07	< 0,04	< 0,09	MINOR TRIPHOSPHATE RESIDUES DETECTED

2nd trial

		SMP	ANFACO CODE	DIPHOSPHATE (gP/100g)	DIPHOSPHATE (gP2O5/100g)	TRIPHOSPHATE (gP/100g)	TRIPHOSPHATE (gP2O5/100g)	HEXAMETAPHOSPHATE (gP/100g)	HEXAMETAPHOSPHATE (gP2O5/100g)	COMMENTS
HEAVY SALTED-FROZEN RAW MATERIAL	J.0	J.0.A	17592	< 0,03	< 0,06	<0,03	<0,07	< 0,04	< 0,09	NO ADDED PHOSPHATE RESIDUES DETECTED
		J.0.B	17593	< 0,03	< 0,06	<0,03	<0,07	< 0,04	< 0,09	NO ADDED PHOSPHATE RESIDUES DETECTED
		J.0.C	17594	< 0,03	< 0,06	<0,03	<0,07	< 0,04	< 0,09	NO ADDED PHOSPHATE RESIDUES DETECTED
		J.0.D	17595	< 0,03	< 0,06	<0,03	<0,07	< 0,04	< 0,09	NO ADDED PHOSPHATE RESIDUES DETECTED
		J.0.E	17596	< 0,03	< 0,06	<0,03	<0,07	< 0,04	< 0,09	NO ADDED PHOSPHATE RESIDUES DETECTED
	G.6	G6.1.A	17597	< 0,03	< 0,06	<0,03	<0,07	< 0,04	< 0,09	NO ADDED PHOSPHATE RESIDUES DETECTED
		G6.1.B	17598	< 0,03	< 0,06	<0,03	<0,07	< 0,04	< 0,09	NO ADDED PHOSPHATE RESIDUES DETECTED
		G6.1.C	17599	< 0,03	< 0,06	<0,03	<0,07	< 0,04	< 0,09	NO ADDED PHOSPHATE RESIDUES DETECTED
		G6.1.D	17600	< 0,03	< 0,06	<0,03	<0,07	< 0,04	< 0,09	NO ADDED PHOSPHATE RESIDUES DETECTED
		G6.1.E	17601	< 0,03	< 0,06	<0,03	<0,07	< 0,04	< 0,09	NO ADDED PHOSPHATE RESIDUES DETECTED
		G6.2.A	17602	< 0,03	< 0,06	<0,03	<0,07	< 0,04	< 0,09	NO ADDED PHOSPHATE RESIDUES DETECTED
		G6.2.B	17603	< 0,03	< 0,06	<0,03	<0,07	< 0,04	< 0,09	NO ADDED PHOSPHATE RESIDUES DETECTED
		G6.2.C	17604	< 0,03	< 0,06	<0,03	<0,07	< 0,04	< 0,09	NO ADDED PHOSPHATE RESIDUES DETECTED
		G6.2.D	17605	< 0,03	< 0,06	<0,03	<0,07	< 0,04	< 0,09	NO ADDED PHOSPHATE RESIDUES DETECTED
		G6.2.E	17606	< 0,03	< 0,06	<0,03	<0,07	< 0,04	< 0,09	NO ADDED PHOSPHATE RESIDUES DETECTED
		G6.3.A	17607	< 0,03	< 0,06	<0,03	<0,07	< 0,04	< 0,09	NO ADDED PHOSPHATE RESIDUES DETECTED
		G6.3.B	17608	< 0,03	< 0,06	<0,03	<0,07	< 0,04	< 0,09	NO ADDED PHOSPHATE RESIDUES DETECTED
		G6.3.C	17609	< 0,03	< 0,06	<0,03	<0,07	< 0,04	< 0,09	NO ADDED PHOSPHATE RESIDUES DETECTED
		G6.3.D	17610	< 0,03	< 0,06	<0,03	<0,07	< 0,04	< 0,09	NO ADDED PHOSPHATE RESIDUES DETECTED
		G6.3.E	17611	< 0,03	< 0,06	<0,03	<0,07	< 0,04	< 0,09	NO ADDED PHOSPHATE RESIDUES DETECTED
	G.7	G7.1.A	17612	< 0,03	< 0,06	<0,03	<0,07	< 0,04	< 0,09	NO ADDED PHOSPHATE RESIDUES DETECTED
		G7.1.B	17613	< 0,03	< 0,06	<0,03	<0,07	< 0,04	< 0,09	NO ADDED PHOSPHATE RESIDUES DETECTED
		G7.1.C	17614	< 0,03	< 0,06	<0,03	<0,07	< 0,04	< 0,09	NO ADDED PHOSPHATE RESIDUES DETECTED
		G7.1.D	17615	< 0,03	< 0,06	<0,03	<0,07	< 0,04	< 0,09	NO ADDED PHOSPHATE RESIDUES DETECTED
		G7.1.E	17616	< 0,03	< 0,06	<0,03	<0,07	< 0,04	< 0,09	NO ADDED PHOSPHATE RESIDUES DETECTED
		G7.2.A	17617	< 0,03	< 0,06	<0,03	<0,07	< 0,04	< 0,09	NO ADDED PHOSPHATE RESIDUES DETECTED
		G7.2.B	17618	< 0,03	< 0,06	<0,03	<0,07	< 0,04	< 0,09	NO ADDED PHOSPHATE RESIDUES DETECTED
		G7.2.C	17619	< 0,03	< 0,06	<0,03	<0,07	< 0,04	< 0,09	NO ADDED PHOSPHATE RESIDUES DETECTED
		G7.2.D	17620	< 0,03	< 0,06	<0,03	<0,07	< 0,04	< 0,09	NO ADDED PHOSPHATE RESIDUES DETECTED
		G7.2.E	17621	< 0,03	< 0,06	<0,03	<0,07	< 0,04	< 0,09	NO ADDED PHOSPHATE RESIDUES DETECTED
		G7.3.A	17622	< 0,03	< 0,06	<0,03	<0,07	< 0,04	< 0,09	NO ADDED PHOSPHATE RESIDUES DETECTED
		G7.3.B	17623	< 0,03	< 0,06	<0,03	<0,07	< 0,04	< 0,09	NO ADDED PHOSPHATE RESIDUES DETECTED
		G7.3.C	17624	< 0,03	< 0,06	<0,03	<0,07	< 0,04	< 0,09	NO ADDED PHOSPHATE RESIDUES DETECTED
		G7.3.D	17625	< 0,03	< 0,06	<0,03	<0,07	< 0,04	< 0,09	NO ADDED PHOSPHATE RESIDUES DETECTED
		G7.3.E	17626	< 0,03	< 0,06	<0,03	<0,07	< 0,04	< 0,09	NO ADDED PHOSPHATE RESIDUES DETECTED

7.2 Mineral results

			1st trial												
		SMP	ANFACO CODE	Na (g/100g)	ClNa (g/100g)	K (g/100g)	Ca (mg/Kg)	Mg (mg/Kg)	P (g/100g)	P2O5 (g/100g)	Fe (mg/Kg)	Cu (mg/Kg)	Zn (mg/Kg)	Cd (mg/Kg)	As (mg/Kg)
HEAVY SALTED - FRESH RAW MATERIAL	G.O	G.0.A	1105442	0,10	0,25	0,32	87	223	0,17	0,39	5,8	0,12	3,5	<0,25	14,5
		G.0.B	1105443	0,09	0,23	0,35	95	246	0,18	0,41	5,2	< 0,10	3,4	<0,25	31,6
		G.0.C	1105444	0,08	0,20	0,34	84	242	0,18	0,41	24,6	0,14	3,8	<0,25	6,1
		G.0.D	1105445	0,08	0,20	0,30	91	239	0,15	0,34	10,4	0,1	3,1	<0,25	10,9
		G.0.E	1105446	0,10	0,25	0,30	107	248	0,16	0,37	5,8	< 0,10	3,1	<0,25	17,6
	G.1	G.I.1.A	1105447	8,08	20,5	0,23	366	622	0,08	0,18	<1,0	<0,10	3,6	< 0,25	1,6
		G.I.1.B	1105448	7,84	19,9	0,21	506	705	0,09	0,21	2,5	<0,10	3,9	< 0,25	1,9
		G.I.1.C	1105449	7,91	20,1	0,22	408	685	0,08	0,18	2,5	0,1	5,1	< 0,25	1,5
		G.I.2.A	1105450	8,28	21,0	0,29	242	444	0,11	0,25	2,4	0,14	3,9	< 0,25	2,96
		G.I.2.B	1105451	8,21	20,9	0,27	240	458	0,09	0,21	2	<0,10	4	< 0,25	1,92
		G.I.2.C	1105452	7,90	20,1	0,25	344	630	0,08	0,18	2	<0,10	4	< 0,25	2,18
		G.I.3.A	1105453	7,87	20,0	0,32	241	550	0,12	0,27	2,4	0,17	6	< 0,25	1,44
		G.I.3.B	1105454	8,17	20,8	0,33	290	594	0,13	0,30	2,1	0,12	4,3	< 0,25	1,4
		G.I.3.C	1105455	7,83	19,9	0,32	309	537	0,13	0,30	1,9	0,17	4,6	< 0,25	1,27
		G.I.4.A	1105456	7,41	18,8	0,42	248	436	0,16	0,37	<1,0	0,10	4,2	< 0,25	1,93
		G.I.4.B	1105457	7,70	19,6	0,45	210	466	0,17	0,39	10,5	<0,10	3,7	< 0,25	1,47
		G.I.4.C	1105458	7,40	18,8	0,41	221	518	0,14	0,32	<1,0	0,26	4,7	< 0,25	3,87
	G.2	G.II.1.A	1105459	7,56	19,2	0,27	208	291	0,1	0,23	<1,0	0,10	2,8	< 0,25	1,73
		G.II.1.B	1105460	7,86	20,0	0,28	197	311	0,09	0,21	2	<0,10	3,1	< 0,25	5,05
		G.II.1.C	1105461	7,68	19,5	0,27	227	298	0,09	0,21	<1,0	0,11	4	< 0,25	3,32
		G.II.2.A	1105462	7,86	20,0	0,33	246	414	0,11	0,25	<1,0	0,16	3,6	< 0,25	2
		G.II.2.B	1105463	7,97	20,3	0,32	274	495	0,11	0,25	1,9	<0,10	4,1	< 0,25	1,4
		G.II.2.C	1105464	7,87	20,0	0,35	224	522	0,12	0,27	2,4	<0,10	4,1	< 0,25	5,8
		G.II.3.A	1105465	7,90	20,1	0,37	267	587	0,16	0,37	<1,0	0,86	3,6	< 0,25	2,5
		G.II.3.B	1105466	7,78	19,8	0,40	215	391	0,16	0,37	2,40	0,27	4,00	< 0,25	2,40
		G.II.3.C	1105467	7,81	19,9	0,37	236	438	0,15	0,34	<1,0	0,22	4	< 0,25	2,6
		G.II.4.A	1105468	8,69	22,1	0,49	368	673	0,23	0,53	2	0,19	3,3	< 0,25	2
		G.II.4.B	1105469	8,09	20,6	0,47	265	546	0,22	0,50	2,1	0,22	3,6	< 0,25	5
		G.II.4.C	1105470	8,10	20,6	0,50	350	586	0,23	0,53	25,30	0,34	4,50	< 0,25	2,70
	G.3	G.III.1.A	1105471	7,52	19,1	0,31	278	479	0,11	0,25	2,1	<0,10	4,3	< 0,25	6,3
		G.III.1.B	1105472	7,35	18,7	0,29	260	573	0,1	0,23	< 1,0	<0,10	3,8	< 0,25	3,5
		G.III.1.C	1105473	7,00	17,8	0,24	387	628	0,09	0,21	< 1,0	<0,10	4,6	< 0,25	5,4
		G.III.2.A	1105474	7,68	19,5	0,33	236	476	0,1	0,23	2,1	0,16	4,8	< 0,25	4,2
		G.III.2.B	1105475	7,75	19,7	0,33	202	425	0,11	0,25	2	0,23	3,6	< 0,25	2,5
		G.III.2.C	1105476	7,18	18,3	0,28	366	685	0,09	0,21	< 1,0	0,19	4,4	< 0,25	3,6
		G.III.3.A	1105477	7,38	18,8	0,36	201	506	0,12	0,27	1,9	<0,10	4,5	< 0,25	4,3
		G.III.3.B	1105478	7,40	18,8	0,33	276	633	0,09	0,21	<1,0	<0,10	3,9	< 0,25	4
		G.III.3.C	1105479	7,48	19,0	0,33	342	724	0,08	0,18	2,2	<0,10	4,4	< 0,25	5,2
		G.III.4.A	1105480	7,48	19,0	0,40	297	596	0,11	0,25	6,40	0,10	5,40	< 0,25	2,50
		G.III.4.B	1105481	7,58	19,3	0,39	359	629	0,1	0,23	5,6	<0,10	6,4	< 0,25	2
		G.III.4.C	1105482	7,53	19,1	0,42	386	615	0,09	0,21	14,9	0,22	5,4	< 0,25	3,2

		SMP	ANFACO CODE	Na (g/100g)	ClNa (g/100g)	K (g/100g)	Ca (mg/Kg)	Mg (mg/Kg)	P (g/100g)	P2O5 (g/100g)	Fe (mg/Kg)	Cu (mg/Kg)	Zn (mg/Kg)	Cd (mg/Kg)	As (mg/Kg)
HEAVY SALTED-FROZEN RAW MATERIAL	H.O	H.0.A	1109175	0,08	0,20	0,31	93	240	0,18	0,41	3,5	<0,5	4,1	< 0,25	1,0
		H.0.B	1109176	0,07	0,18	0,22	79	204	0,12	0,27	2,9	<0,5	2,8	< 0,25	7,4
		H.0.C	1109177	0,07	0,18	0,24	101	220	0,13	0,30	5,0	<0,5	2,6	< 0,25	1,3
		H.0.D	1109178	0,06	0,15	0,21	96	203	0,12	0,27	2,7	<0,5	3,0	< 0,25	1,8
		H.0.E	1109179	0,06	0,15	0,24	90	217	0,14	0,32	4,6	<0,5	3,0	< 0,25	16,6
	H.1	H.I.1.A	1109180	8,51	21,6	0,26	432	575	0,08	0,18	2,53	<0,5	4,34	< 0,25	1,31
		H.I.1.B	1109181	8,16	20,7	0,22	554	598	0,07	0,16	2,66	<0,5	3,6	< 0,25	2,27
		H.I.1.C	1109182	8,34	21,2	0,22	625	689	0,07	0,16	3,14	<0,5	3,71	< 0,25	1,82
		H.I.2.A	1109183	8,42	21,4	0,31	435	897	0,1	0,23	2,14	<0,5	3,99	< 0,25	1,5
		H.I.2.B	1109184	8,16	20,7	0,29	1653	779	0,17	0,39	2,11	<0,5	3,57	< 0,25	1,24
		H.I.2.C	1109185	7,99	20,3	0,26	458	665	0,09	0,21	2,4	<0,5	4,47	< 0,25	1,33
		H.I.3.A	1109186	8,03	20,4	0,33	404	623	0,12	0,27	<1,0	<0,5	3,24	< 0,25	2,1
		H.I.3.B	1109187	7,93	20,2	0,3	540	739	0,11	0,25	2	<0,5	3,55	< 0,25	1,4
		H.I.3.C	1109188	8,58	21,8	0,31	484	701	0,1	0,23	5,84	<0,5	4,33	< 0,25	1,23
		H.I.4.A	1109189	8,46	21,5	0,43	338	559	0,19	0,44	2,08	<0,5	2,98	< 0,25	4,4
		H.I.4.B	1109190	8,49	21,6	0,44	254	509	0,16	0,37	3,9	<0,5	3,35	< 0,25	1,85
		H.I.4.C	1109191	8,55	21,7	0,44	335	669	0,19	0,44	3,43	<0,5	2,97	< 0,25	2,43
	H.2	H.II.1.A	1109192	8,50	21,6	0,23	283	296	0,08	0,18	4,58	<0,5	2,8	< 0,25	1,84
		H.II.1.B	1109193	8,30	21,1	0,23	306	337	0,08	0,18	3,11	<0,5	3,43	< 0,25	1,85
		H.II.1.C	1109194	8,67	22,0	0,2	376	412	0,07	0,16	3,85	<0,5	3,11	< 0,25	< 1,0
		H.II.2.A	1109195	8,66	22,0	0,33	258	270	0,11	0,25	2,87	<0,5	3,86	< 0,25	1,98
		H.II.2.B	1109196	8,33	21,2	0,3	351	503	0,1	0,23	3,1	<0,5	2,77	< 0,25	5,64
		H.II.2.C	1109197	8,14	20,7	0,29	402	518	0,1	0,23	4,3	<0,5	3,61	< 0,25	4,29
		H.II.3.A	1109198	8,16	20,7	0,34	369	379	0,14	0,32	<1,0	<0,5	4,46	< 0,25	1,45
		H.II.3.B	1109199	7,91	20,1	0,34	255	279	0,15	0,34	<1,0	<0,5	3,92	< 0,25	< 1,0
		H.II.3.C	1109200	8,24	20,9	0,33	483	542	0,15	0,34	<1,0	<0,5	4,14	< 0,25	1,09
		H.II.4.A	1109201	8,37	21,3	0,45	298	393	0,24	0,55	<1,0	<0,5	4,03	< 0,25	2,26
		H.II.4.B	1109202	8,26	21,0	0,45	347	460	0,24	0,55	<1,0	<0,5	4,06	< 0,25	< 1,0
		H.II.4.C	1109203	8,01	20,4	0,44	324	324	0,23	0,53	<1,0	<0,5	4,47	< 0,25	1,61
	H.3	H.III.1.A	1109204	7,59	19,3	0,22	649	812	0,12	0,27	2,57	<0,5	4,17	< 0,25	2,61
		H.III.1.B	1109205	7,61	19,3	0,24	557	764	0,07	0,16	2,25	<0,5	4,75	< 0,25	2,08
		H.III.1.C	1109206	7,58	19,3	0,3	465	753	0,09	0,21	2,93	<0,5	5,57	< 0,25	2,38
		H.III.2.A	1109207	6,27	15,9	0,31	368	645	0,09	0,21	<1,0	<0,5	4,3	< 0,25	3
		H.III.2.B	1109208	6,30	16,0	0,29	413	522	0,09	0,21	<1,0	<0,5	3,9	< 0,25	4,9
		H.III.2.C	1109209	6,37	16,2	0,32	457	741	0,08	0,18	<1,0	<0,5	5,5	< 0,25	7
		H.III.3.A	1109210	6,57	16,7	0,31	430	620	0,08	0,18	<1,0	<0,5	4,2	< 0,25	5,6
		H.III.3.B	1109211	6,48	16,5	0,31	391	562	0,08	0,18	2,4	<0,5	6	< 0,25	3,9
		H.III.3.C	1109212	6,50	16,5	0,34	574	795	0,1	0,23	<1,0	<0,5	5,2	< 0,25	5
		H.III.4.A	1109213	6,44	16,4	0,38	430	733	0,08	0,18	<1,0	<0,5	4,30	< 0,25	2,00
		H.III.4.B	1109214	5,99	15,2	0,37	405	614	0,09	0,21	<1,0	<0,5	3,7	< 0,25	7,9
		H.III.4.C	1109215	6,71	17,1	0,36	412	431	0,1	0,23	2,1	<0,5	4,1	< 0,25	10,2

LIGHT SALTED

FRESH RAW MATERIAL

SMP	ANFACO CODE	Na (g/100g)	ClNa (g/100g)	K (g/100g)	Ca (mg/Kg)	Mg (mg/Kg)	P (g/100g)	P2O5 (g/100g)	Fe (mg/Kg)	Cu (mg/Kg)	Zn (mg/Kg)	Cd (mg/Kg)	As (mg/Kg)
L.V.1.A	1109216	2,01	5,1	0,29*	144	166	0,11	0,25	<1,0	< 0,5	2,5	< 0,25	2,5
L.V.1.B	1109217	1,80	4,6	0,26	136	174	0,11	0,25	2,36	< 0,5	2,5	< 0,25	13,1
L.V.1.C	1109218	1,97	5,0	0,31	139	186	0,12	0,27	<1,0	< 0,5	2,6	< 0,25	2,9
L.V.2.A	1109219	1,70	4,3	0,36	123	200	0,15	0,34	<1,0	< 0,5	2,6	< 0,25	3,5
L.V.2.B	1109220	1,96	5,0	0,36	112	194	0,14	0,32	1,82	< 0,5	2,5	< 0,25	6,3
L.V.2.C	1109221	1,98	5,0	0,36	129	195	0,14	0,32	<1,0	< 0,5	2,8	< 0,25	6,0
L.V.3.A	1109222	1,65	4,2	0,36	112	187	0,16	0,37	<1,0	< 0,5	3,6	< 0,25	3,1
L.V.3.B	1109223	1,78	4,5	0,38	117	181	0,15	0,34	<1,0	< 0,5	2,6	< 0,25	14,3
L.V.3.C	1109224	1,60	4,1	0,36	102	188	0,16	0,37	<1,0	< 0,5	2,7	< 0,25	1,1
L.V.4.A	1109225	1,72	4,4	0,41	112	186	0,18	0,41	<1,0	< 0,5	2,8	< 0,25	1,3
L.V.4.B	1109226	1,77	4,5	0,45	107	182	0,2	0,46	2,08	< 0,5	3,3	< 0,25	4,8
L.V.4.C	1109227	1,50	3,8	0,4	105	177	0,19	0,44	3,17	< 0,5	3,9	< 0,25	2,6

FROZEN RAW MATERIAL

SMP	ANFACO CODE	Na (g/100g)	ClNa (g/100g)	K (g/100g)	Ca (mg/Kg)	Mg (mg/Kg)	P (g/100g)	P2O5 (g/100g)	Fe (mg/Kg)	Cu (mg/Kg)	Zn (mg/Kg)	Cd (mg/Kg)	As (mg/Kg)
M.V.1.A	1111763	2,48	6,3	0,18	273	200	0,12	0,27	<1,0	<1	2,7	< 0,25	2,5
M.V.1.B	1111764	2,43	6,2	0,18	168	202	0,13	0,30	<1,0	<1	3,2	< 0,25	3,4
M.V.1.C	1111765	2,60	6,6	0,22	172	207	0,13	0,30	<1,0	<1	2,9	< 0,25	3,4
M.V.2.A	1111766	2,68	6,8	0,29	176	205	0,19	0,44	<1,0	<1	2,5	< 0,25	1,8
M.V.2.B	1111767	2,57	6,5	0,26	152	211	0,16	0,37	<1,0	<1	2,9	< 0,25	1,2
M.V.2.C	1111768	2,67	6,8	0,28	162	198	0,15	0,34	3,1	<1	3,0	< 0,25	<1,0
M.V.3.A	1111769	2,13	5,4	0,14	155	189	0,05	0,11	<1,0	<1	2,8	<0,25	2,6
M.V.3.B	1111770	2,53	6,4	0,16	206	166	0,04	0,09	1,8	<1	2,6	<0,25	3,1
M.V.3.C	1111771	2,25	5,7	0,11	209	167	0,04	0,09	<1,0	<1	4,0	<0,25	<1,0
M.V.4.A	1111772	2,27	5,8	0,27	213	197	0,10	0,23	2,1	<1	4,5	<0,25	<1,0
M.V.4.B	1111773	2,69	6,8	0,29	233	213	0,11	0,25	3,2	<1	4,1	<0,25	<1,0
M.V.4.C	1111774	1,76	4,5	0,23	1066	197	0,11	0,25	<1,0	<1	4,5	<0,25	<1,0

	2nd trial													
	SMP	ANFACO CODE	Na (g/100g)	ClNa (g/100g)	K (g/100g)	Ca (mg/Kg)	Mg (mg/Kg)	P (g/100g)	P2O5 (g/100g)	Fe (mg/Kg)	Cu (mg/Kg)	Zn (mg/Kg)	Cd (mg/Kg)	As (mg/Kg)
J.0	J.0.A	17592	0,22	0,56	0,34	245	388	0,10	0,23	3,7	<0,25	5,8	-	-
	J.0.B	17593	0,29	0,74	0,26	365	491	0,09	0,21	3,6	<0,25	7,1	-	-
	J.0.C	17594	0,23	0,58	0,34	100	368	0,09	0,21	<2	<0,25	<2	-	-
	J.0.D	17595	0,17	0,43	0,38	103	348	0,10	0,23	3,1	<0,25	4,1	-	-
	J.0.E	17596	0,33	0,84	0,36	1276	578	0,14	0,32	<2	<0,25	5,4	-	-
G.6	G6.1.A	17597	6,59	16,75	0,18	512	576	0,05	0,11	<2	<0,25	5,4	-	-
	G6.1.B	17598	5,73	14,57	0,20	556	504	0,04	0,09	11,2	<0,25	5,8	-	-
	G6.1.C	17599	6,18	15,71	0,20	466	451	0,04	0,09	<2	<0,25	4,9	-	-
	G6.1.D	17600	6,38	16,22	0,20	570	591	0,04	0,09	<2	<0,25	5,5	-	-
	G6.1.E	17601	7,94	20,18	0,23	577	480	0,04	0,09	<2	<0,25	5,4	-	-
	G6.2.A	17602	8,12	20,64	0,21	344	443	0,05	0,11	<2	<0,25	5,5	-	-
	G6.2.B	17603	8,07	20,51	0,21	325	539	0,06	0,14	6,6	<0,25	3,9	-	-
	G6.2.C	17604	8,04	20,44	0,21	245	377	0,04	0,09	<2	<0,25	<2	-	-
	G6.2.D	17605	7,49	19,04	0,25	429	410	0,04	0,09	<2	<0,25	4,1	-	-
	G6.2.E	17606	7,32	18,61	0,23	372	401	0,05	0,11	2,0	<0,25	4,7	-	-
	G6.3.A	17607	8,06	20,49	0,28	454	607	0,06	0,14	<2	<0,25	<2	-	-
	G6.3.B	17608	6,86	17,44	0,25	382	500	0,06	0,15	19,6	<0,25	<2	-	-
	G6.3.C	17609	6,58	16,73	0,26	352	524	0,06	0,13	64,9	0,94	<2	-	-
	G6.3.D	17610	7,89	20,06	0,26	425	717	0,07	0,16	14,3	<0,25	<2	-	-
	G6.3.E	17611	7,19	18,28	0,27	376	489	0,06	0,13	39,5	0,53	<2	-	-
G.7	G7.1.A	17612	6,47	16,45	0,22	417	695	0,06	0,13	<2	<0,25	<2	-	-
	G7.1.B	17613	6,52	16,57	0,17	553	741	0,05	0,12	<2	<0,25	<2	-	-
	G7.1.C	17614	7,08	18,00	0,18	605	631	0,06	0,13	4,7	<0,25	<2	-	-
	G7.1.D	17615	6,95	17,67	0,23	389	635	0,05	0,11	5,1	0,35	<2	-	-
	G7.1.E	17616	6,90	17,54	0,19	535	710	0,05	0,11	<2	0,25	<2	-	-
	G7.2.A	17617	6,73	17,11	0,19	689	541	0,07	0,17	87,5	2,11	60,8	-	-
	G7.2.B	17618	6,25	15,89	0,20	420	541	0,06	0,13	8,9	<0,25	7,6	-	-
	G7.2.C	17619	6,74	17,13	0,19	372	532	0,05	0,12	7,6	0,47	7,9	-	-
	G7.2.D	17620	7,33	18,63	0,20	372	543	0,06	0,13	13,5	<0,25	11,1	-	-
	G7.2.E	17621	5,77	14,67	0,22	361	481	0,05	0,11	<2	0,26	4,2	-	-
	G7.3.A	17622	7,39	18,79	0,32	473	448	0,05	0,11	2,4	0,37	4,4	-	-
	G7.3.B	17623	7,52	19,12	0,27	371	462	0,05	0,11	4,4	<0,25	5,3	-	-
	G7.3.C	17624	8,02	20,39	0,27	1881	512	0,09	0,20	8,9	0,61	4,5	-	-
	G7.3.D	17625	7,85	19,95	0,18	1099	532	0,08	0,17	17,2	0,52	5,7	-	-
	G7.3.E	17626	7,54	19,17	0,21	217	288	0,03	0,07	<2	0,38	2,9	-	-

7.3 Oxidation results

1st trial

		SMP	ANFACO CODE	PEROXIDES INDEX (meq.O2/Kg.fat)	TBA INDEX (mg/Kg muscle tissue)
HEAVY SALTED - FRESH RAW MATERIAL	G.O	G.0.A	1105442	<2,00	0.5
		G.0.B	1105443	<2,00	0.3
		G.0.C	1105444	<2,00	0.6
		G.0.D	1105445	<2,00	0.4
		G.0.5	1105446	<2,00	0.4
	G.1	G.I.1.A	1105447	129,6	0,3
		G.I.1.B	1105448	<2,00	0,8
		G.I.1.C	1105449	127,8	1,3
		G.I.2.A	1105450	41,0	1,3
		G.I.2.B	1105451	<2,00	2
		G.I.2.C	1105452	33,9	0,6
		G.I.3.A	1105453	<2,00	1,7
		G.I.3.B	1105454	<2,00	2,2
		G.I.3.C	1105455	<2,00	1,2
		G.I.4.A	1105456	37,0	2,1
		G.I.4.B	1105457	12,5	1
		G.I.4.C	1105458	13.16	1.2
	G.2	G.II.1.A	1105459	<2,00	1
		G.II.1.B	1105460	<2,00	1,1
		G.II.1.C	1105461	<2,00	1,7
		G.II.2.A	1105462	<2,00	0,9
		G.II.2.B	1105463	16,5	1,7
		G.II.2.C	1105464	<2,00	1,1
		G.II.3.A	1105465	14,7	1,3
		G.II.3.B	1105466	<2,00	0,80
		G.II.3.C	1105467	<2,00	0,8
		G.II.4.A	1105468	43,5	0,7
		G.II.4.B	1105469	41,7	1,2
		G.II.4.C	1105470	45.45	0.9
	G.3	G.III.1.A	1105471	<2,00	1,2
		G.III.1.B	1105472	<2,00	1,2
		G.III.1.C	1105473	<2,00	0,8
		G.III.2.A	1105474	49,0	1,4
		G.III.2.B	1105475	25,0	2,5
		G.III.2.C	1105476	45,5	1,2
		G.III.3.A	1105477	23,8	1,5
		G.III.3.B	1105478	25,0	2,2
		G.III.3.C	1105479	43,1	0,9
		G.III.4.A	1105480	16,7	0,90
		G.III.4.B	1105481	10,0	1
		G.III.4.C	1105482	13.51	1.0

		SMP	ANFACO CODE	PEROXIDES INDEX (meq.O2/Kg.fat)	TBA INDEX (mg/Kg muscle tissue)
HEAVY SALTED - FROZEN RAW MATERIAL	H.O	H.0.A	1109175	431,82	2,4
		H.0.B	1109176	148,15	2,5
		H.0.C	1109177	300,00	1,4
		H.0.D	1109178	416,67	2,10
		H.0.E	1109179	236,84	1,90
	H.1	H.I.1.A	1109180	70,0	1,6
		H.I.1.B	1109181	37,0	1,3
		H.I.1.C	1109182	93,2	1,3
		H.I.2.A	1109183	37,5	1,7
		H.I.2.B	1109184	24,0	1,5
		H.I.2.C	1109185	22,7	1,3
		H.I.3.A	1109186	27,8	0,5
		H.I.3.B	1109187	23,5	0,6
		H.I.3.C	1109188	32,1	0,4
		H.I.4.A	1109189	7,0	0,4
		H.I.4.B	1109190	5,8	0,8
		H.I.4.C	1109191	6,1	0,4
	H.2	H.II.1.A	1109192	267,9	0,5
		H.II.1.B	1109193	343,1	0,7
		H.II.1.C	1109194	142,9	0,4
		H.II.2.A	1109195	203,9	0,9
		H.II.2.B	1109196	158,7	0,6
		H.II.2.C	1109197	131,6	0,4
		H.II.3.A	1109198	63,8	0,6
		H.II.3.B	1109199	121,2	1,10
		H.II.3.C	1109200	79,6	0,3
		H.II.4.A	1109201	68,5	0,7
		H.II.4.B	1109202	35,7	1
		H.II.4.C	1109203	36,4	0,80
	H.3	H.III.1.A	1109204	17,9	0,8
		H.III.1.B	1109205	19,2	0,9
		H.III.1.C	1109206	18,5	0,7
		H.III.2.A	1109207	61,2	1
		H.III.2.B	1109208	82,0	0,6
		H.III.2.C	1109209	125,0	1
		H.III.3.A	1109210	189,4	0,8
		H.III.3.B	1109211	59,3	0,9
		H.III.3.C	1109212	49,3	0,8
		H.III.4.A	1109213	32,0	0,90
		H.III.4.B	1109214	39,0	0,8
		H.III.4.C	1109215	35,7	0,8

2nd trial

LIGHT SALTED

FRESH RAW MATERIAL TRIAL

SMP	ANFACO CODE	PEROXIDES INDEX (meq.O2/Kg.fat)	TBA INDEX (mg/Kg muscle tissue)
L.V.1.A	1109216	216,22	0,7
L.V.1.B	1109217	205,88	0,5
L.V.1.C	1109218	74,07	0,6
L.V.2.A	1109219	115,38	0,6
L.V.2.B	1109220	94,59	0,7
L.V.2.C	1109221	406,25	0,8
L.V.3.A	1109222	62,50	0,3
L.V.3.B	1109223	104,17	0,4
L.V.3.C	1109224	43,48	0,8
L.V.4.A	1109225	62,50	0,3
L.V.4.B	1109226	125,00	0,5
L.V.4.C	1109227	152,78	0,6

FROZEN RAW MATERIAL TRIAL

SMP	ANFACO CODE	PEROXIDES INDEX	TBA INDEX (mg/Kg muscle tissue)
M.V.1.A	1111763	21,74	14,6
M.V.1.B	1111764	55,56	9,2
M.V.1.C	1111765	17,24	8,3
M.V.2.A	1111766	19,23	5,1
M.V.2.B	1111767	29,41	8,4
M.V.2.C	1111768	25,71	12,7
M.V.3.A	1111769	45,45	0,9
M.V.3.B	1111770	27,78	1,1
M.V.3.C	1111771	33,33	0,7
M.V.4.A	1111772	16,67	0,4
M.V.4.B	1111773	29,41	0,7
M.V.4.C	1111774	25,00	0,4

HEAVY SALTED - FROZEN RAW MATERIAL

	SMP	ANFACO CODE	PEROXIDES INDEX (meq.O2/Kg.fat)	TBA INDEX (mg/Kg muscle tissue)
J.0	J.0.A	17592	40,00	0,4
	J.0.B	17593	38,46	0,5
	J.0.C	17594	51,72	0,4
	J.0.D	17595	29,41	0,20
	J.0.E	17596	33,33	0,20
G.6	G6.1.A	17597	18,18	0,9
	G6.1.B	17598	27,78	0,8
	G6.1.C	17599	14,71	0,9
	G6.1.D	17600	23,81	0,6
	G6.1.E	17601	24,69	0,7
	G6.2.A	17602	20	0,9
	G6.2.B	17603	33,33	0,4
	G6.2.C	17604	25	0,7
	G6.2.D	17605	31,25	0,9
	G6.2.E	17606	17,24	0,8
	G6.3.A	17607	23,26	0,3
	G6.3.B	17608	17,24	0,3
	G6.3.C	17609	16,95	0,4
	G6.3.D	17610	23,81	0,3
	G6.3.E	17611	33,9	0,4
G.7	G7.1.A	17612	23,81	0,6
	G7.1.B	17613	78,43	0,9
	G7.1.C	17614	20,83	0,7
	G7.1.D	17615	47,62	0,4
	G7.1.E	17616	27,03	0,7
	G7.2.A	17617	26,32	1,0
	G7.2.B	17618	25,64	0,6
	G7.2.C	17619	27,03	0,5
	G7.2.D	17620	21,28	0,5
	G7.2.E	17621	22,22	0,6
	G7.3.A	17622	95,24	0,9
	G7.3.B	17623	92,11	0,6
	G7.3.C	17624	129,63	0,6
	G7.3.D	17625	72,92	0,7
	G7.3.E	17626	92,59	0,6

7.4 Fat content and fatty acids sample profile

	G.0.A 1109175	G.0.B 1109176	G.0.C 1109177	G.0.D 1109178	G.0.E 1109179	Average	SD	Fillet 205 J.0.A 1117592	Fillet 207 J.0.B 1117593	Fillet 215 J.0.C 1117594	Fillet 210 J.0.D 1117595	Fillet 213 J.0.E 1117596	Average	SD
Humidity (%)	82,6	82,7	83,0	80,9	83,1	82,5	0,9	81,0	81,1	81,2	80,5	80,5	80,9	0,3
Fat (%)	0,1	0,1	0,1	0,1	0,04	0,09	0,03	0,1	0,1	0,1	0,1	0,1	0,10	0,00
Fatty Acids Profile (%)														
C14:0 (Myristic)	1,38	1,46	3,32	3,53	1,1	2,16	1,17	1,31	1,3	1,76	1,71	0,55	1,33	0,48
C16:0 (Palmitic)	15,93	16,09	16,38	17,86	15,2	16,29	0,98	15,91	15,54	17,14	17,54	18,28	16,88	1,14
C16:1(n-7+n-9)	2,09	1,89	3,3	4,1	1,85	2,65	1,01	2,29	3,19	2,92	2,56	1,5	2,49	0,65
C18:0 (Stearic)	7,44	6,77	6,27	6,83	5,62	6,59	0,68	7,49	6,66	6,99	6,81	8,33	7,26	0,68
C18:1n-9 (Oleic acid)	34,32	37,3	15,04	25,43	49,75	32,37	13,03	16,04	23,42	20,41	16,86	39,52	23,25	9,56
C18:1n-7 (Vaccenic)	0	0	4,85	6,24	0	2,22	3,08	5,00	7,24	5,96	5,4	0	4,72	2,77
C18:2n-6 (Linoleic acid)	5,66	4,55	11,82	3,79	2,46	5,66	3,64	3,23	2,47	3,87	2,26	6,42	3,65	1,67
C18:3n-3 (Linolenic-ALA)	0,22	0,26	0,73	0,23	0,2	0,33	0,23	0,27	0,68	0,28	0,20	0,87	0,46	0,30
C20:1	4,44	4,02	3,31	3,9	3,37	3,81	0,47	3,57	2,58	3,94	3,84	2,05	3,20	0,84
C20:5n-3 (EPA)	5,44	4,53	7,27	4,52	3,15	4,98	1,52	6,01	6,18	6,38	7,03	2,54	5,63	1,77
C24:1 (Nervonic)	1,39	0,91	1,32	1,32	1,18	1,22	0,19	2,07	3,27	2	2,28	1,34	2,19	0,70
C22:5n-3 (DPA)	0,81	0,58	1,06	0,54	0,44	0,69	0,25	1,28	1,6	1,23	1,39	0,75	1,25	0,31
C22:6n-3 (DHA)	13,41	14,65	14,81	15,35	7,12	13,07	3,40	24,58	9,56	14,88	18,89	7,25	15,03	7,01
Saturated	26,24	25,79	28,49	29,79	23,36	26,73	2,50	26,58	26,32	28,17	28,22	28,98	27,65	1,15
Mono -insaturated	44,74	46,93	29,25	43,24	58,42	44,52	10,41	30,78	42,27	37,3	33,3	45,39	37,81	6,07
Poli-insaturated	26,71	25,45	38,72	25,03	14,7	26,12	8,53	37,88	22,87	28,41	31,45	20,16	28,15	7,02
Omega-3	20,58	20,49	25,86	20,92	11,34	19,84	5,26	33,14	18,87	23,88	28,45	12,57	23,38	8,04
Omega-6	5,66	4,55	12,26	3,79	2,95	5,84	3,72	3,75	2,91	4,2	2,73	7,47	4,21	1,92
Trans fatty acids (%)	0,67	0,6	1,7	0,86	0,76	0,92	0,45	2,48	3,55	2,61	3,64	1,04	2,66	1,05

	H.I.4.A 1109189	H.I.4.B 1109190	H.I.4.C 1109191	Average	SD	G.6.3.A 1117607	G.6.3.B 1117608	G.6.3.C 1117609	G.6.3.D 1117610	G.6.3.E 1117611	Average	SD
Humidity (%)	60,6	60,4	59,5	60,2	0,6	58,2	58,9	58,5	58,5	58,8	58,6	0,3
Fat (%)	0,2	0,1	0,1	0,13	0,06	0,2	0,1	0,1	0,1	0,1	0,12	0,04
Fatty Acids Profile (%)												
C14:0 (Myristic)	2,07	2,19	1,63	1,96	0,29	3,69	1,65	1,75	1,36	1,55	2,00	0,96
C16:0 (Palmitic)	15,95	25,31	18,6	19,95	4,82	17,37	16,78	16,87	17,03	18,2	17,25	0,58
C16:1 (Palmitoleic)	3,34	3,22	2,68	3,08	0,35	4,25	2,68	2,97	2,77	2,44	3,02	0,71
C18:0 (Stearic)	6,32	9,10	8,34	7,92	1,44	5,84	7,52	8,6	8,41	9,28	7,93	1,33
C18:1n-9 (Oleic acid)	20,34	24,52	21,52	22,13	2,16	18,98	16,97	14,98	17,71	18,9	17,51	1,65
C18:1n-7 (Vaccenic)	6,65	7,14	8,16	7,32	0,77	5,19	6,10	5,77	6,07	5,58	5,74	0,38
C18:2n-6 (Linoleic acid)	4,79	3,85	3,9	4,18	0,53	5,52	3,06	2,91	3,46	3,01	3,59	1,10
C18:3n-3 (Linolenic-ALA)	0,37	0	0,22	0,20	0,19	1,08	0,40	0,39	0,29	0,27	0,49	0,34
C20:1 (Eicosenoic)	6,44	5,15	3,99	5,19	1,23	2,84	2,72	3,57	2,37	3,00	2,90	0,44
C20:5n-3 (EPA)	6,85	2,93	5,47	5,08	1,99	7,20	6,78	7,11	5,20	4,49	6,16	1,23
C24:1 (Nervonic)	1,2	1,53	1,54	1,42	0,19	1,45	1,95	1,95	2,66	2,94	2,19	0,60
C22:5n-3 (DPA)	0,89	0,53	0,98	0,80	0,24	1,44	1,38	1,5	1,32	0,99	1,33	0,20
C22:6n-3 (DHA)	15,02	4,94	13,39	11,12	5,41	13,89	19,58	20,17	18,47	17,75	17,97	2,47
Saturated	26,09	38,17	30,28	31,51	6,13	29,3	28,19	29,33	29,51	31,59	29,58	1,24
Mono -insaturated	41,29	43,79	39,87	41,65	1,98	35,29	32,24	31,08	33,26	34,76	33,33	1,74
Poli-insaturated	29,64	13,61	26	23,08	8,40	31,47	33,25	34,43	30,43	28,93	31,70	2,19
Omega-3	23,58	8,99	21,12	17,90	7,81	25,33	29,25	30,3	26,24	24,64	27,15	2,49
Omega-6	4,79	3,85	3,9	4,18	0,53	5,89	3,6	3,3	3,88	3,4	4,01	1,07
Trans	1,23	0,67	1,37	1,09	0,37	2,35	2,99	3,82	2,78	2,21	2,83	0,64

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