



OFFICIAL CONTROL OF POLYPHOSPHATE ADDITIVES AND ANALYTICAL STATE OF THE ART.

PREFACE

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

Phosphate additives approval in salting processes is now under discussion by the EU authorities, leading to technical and political discussion between representatives of different countries with confronted interests. Late scientific studies have documented new information about key technical points that could be introduced for discussion.

Likewise, there have been detected some problems in the official controls, currently being applied, for the phosphate additives legislation enforcement. Besides, available analytical methodology does not fully suit the legislation requirements yet. This is making that controls are not homogenous across Europe, and the interpretation of the results in several countries is frequently inaccurate.

This project tries to document last data available about the application of phosphates to salted products, the present official control situation in the EU, and an approach to the late analytical advances regarding phosphate additives.

This report has been financially supported by the Norwegian Seafood Research Fund (FHF).

TABLE OF CONTENTS.

1. INTRODUCTION.....	4
2. PRESENT PHOSPHATE ADDITIVES LEGAL FRAMEWORK.....	4
3. LATE STUDIES ON THE APPLICATION OF PHOSPHATES IN SALTING PROCESSES.	7
4. PHOSPHATES OFFICIAL CONTROL AND ANALYTICAL STATE OF THE ART.	11
4.1 ANALYTICAL STATE OF THE ART.	11
4.2 PRESENT SITUATION OF PHOSPHATE ADDITIVES OFFICIAL CONTROL IN THE EU.	15
5. FINAL DISCUSSION AND RESEARCH NEEDS.	18
6. REFERENCES.	19

1. INTRODUCTION.

Phosphates are very little toxic substances with acute health effects comparable to common salt. In fact, some common medical practices consist in the administration 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. Its abundance in many foods makes that almost no deficiencies are reported.

One of the sources for the intake of phosphorus in humans is phosphates additives which are included in several products for technological purposes.

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 from 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 that phosphate additives 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.

EU has implemented specific legislation for polyphosphates followed up by the introduction of these additives in their food surveillance programs for foodstuff. The application of this control measures relies in each Member Country Authorities leading to heterogeneity in the current analytical methods applied as well as the misinterpretation of the analytical results in several cases.

This situation worsens when considering the analytical state of the art in polyphosphates quantification, where the lacks of a common standard method and solutions to some analytical problems have not been accomplished yet.

Nevertheless, results from research trials about the application of polyphosphates in salting processes have been recently published, reinforcing knowledge in this topic that could be of great interest for the industry, administration and scientific forum.

2. PRESENT PHOSPHATE ADDITIVES LEGAL FRAMEWORK.

As it has been commented above, phosphates are not considered toxic, but when used improperly excessive moisture absorption can be interpreted as a fraud to consumer. This is the main reason why these additives, in some sectors of the fishing industry, have undergone thorough testing by government agencies in several countries.

Current EU additives legislation is set up in Regulation 1333/2008/EU repealing previous Directive 95/2/CE. This directive addresses all issues concerning the application of additives to foodstuff; from basic definitions and carry-over principle, to procedural and labeling criteria. Besides, not only it states the need of a common list of permitted additives, but 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/UE, and states are obliged to internally address this evaluation for phosphate additives before the expected deadline (December/2018).

Recent positive list of additives, complementing Regulation 1333/2008/UE, has been published 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 an additives maximum level of 5 g P₂O₅/kg in unprocessed frozen fillets, meanwhile FAO Codex Alimentarius Commission of WHO considers up to 10 g P₂O₅/kg. In contrast, USA and Canada allow free use under Good Manufacturing Practices. Concerning the use of phosphates the new legislation did not change the previous status.

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

Table 1: Allowed phosphate levels in seafood in the EU.

Light salted or fully salted cod are considered as a processed product since it substantially changes some of the natural characteristics of the product; so as it has been clearly shown in Table 1, 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 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.

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 as

from January/2011. Icelandic prohibition is not enforced in practice yet, probably waiting for an eventual permission via an amendment to Regulation 1333/2008/UE.

Users had exposed there was a need for the use of phosphates as processing aid, as color stabilizer and an antioxidant for preservation, and stated that practically the total amount of added phosphates are removed during rehydration, remaining only at trace levels. Companies also claimed that no increase in weight is obtained in the rehydrated product so there is no mislead to consumers.

The Commission Standing Committee on the Food Chain and Animal Health (SCFAH) (SANCO – DI (2011)D/310301) addressed this situation in March 2011 discussing the need and implementation of the phosphates additives in salt processing.

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. Literally, they emphasize that “the soaking process which removes the PPs is not done by the producer but by another company or the final consumer, hence the product is in trade with the added phosphates”, which reinforces the Portuguese thesis.

On the other hand, Portuguese “bacalhau” sector are against the permission of the use phosphates in wet salted products. Basically, they justify their position in:

- The lack of technological use in wet salted fish, apart from the mere factor of increasing the water content of primary processed cod, and therefore increasing the income from the sale of these products by the industry of the northern Europe countries (where the fishing and the first processing phase occur), and making more expensive and time consuming, the drying processing occurring subsequently (2nd phase), which is undertaken by Portuguese industry.
- Misleading to final consumers since cod in Portugal is sold in retail bulk without specific labeling and therefore without the information about the use of phosphates.
- Loss of traditional product characteristics. The introduction of additives in processing would modify the final characteristics of the product (color, flavor, texture,...) which are essential in the genuine Portuguese cod market.
- Not taking into account key factors for the additives approval such as the societal, economic, traditional, ethical and environmental consequences, the precautionary principle and the feasibility of controls.

A draft (*Document SANCO/10145/2012*) for an amendment of the regulation 1333/2008/UE concerning the allowance of the use of polyphosphates in wet salted fish was submitted for discussion in a new meeting of the SCFCA (*SANCO – Ares (2012) 673323*) in February 2012. The proposal was tackled by the Portuguese representatives in the basis of the assumptions detailed above. Commission indicated that the technological need (antioxidant) was demonstrated as well as

the lack of mislead to consumers. The traditional “bacalhau” is protected since the approval doesn’t necessarily mean that phosphates shall be used in production of salted cod. Vote was postponed to the next meeting and a meeting between applicants and a delegation of Portugal was scheduled.

In contrast, the 44th session from the Codex Committee on Food Additives in March 2012, it was issued a recommendation concerning provisions for the use of phosphates in the sense of discontinuing their application for the food category 09.2.5 “Smoked dried, fermented, and/or salted fish and fish products, including mollusks, crustaceans and echinoderms”. Therefore, it should be expected that the draft proposal would be reassessed, in the light of the position/Codex Committee’s conclusions. Current situation is that Portugal has presented its allegations and this topic had to be issued in a new meeting from the DGSANCO-SCFCAH in November, but finally was postponed until the beginning of 2013.

3. LATE STUDIES ON THE APPLICATION OF PHOSPHATES IN SALTING

PROCESSES.

Phosphates are used in a wide range of food products mainly because of their water binding capacity, easy solubilization, pH buffering effect and ion chelating properties. In salting processes, the increase of water uptake by tissues in intermediate processing steps, and the reduction of the amount of drip during transport and storage are enough to justify companies interest in their application. Besides their chelating properties on metals like iron, copper and zinc, inhibit the development of oxidative processes which could lead to quality hitches related to flavor and surface yellowing (*Gonçalves,Ribeiro,2008*).

The 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 really low temperatures (0-4 °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 have been used just like 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 in muscle tissue, taking advantage of the osmotic diffusion to spread salt and phosphates in fillets (*Gonçalves, 2008*).

Several studies have been carried out in testing their effects during salting processes. Probably the most complete to date were carried out by *Thorarinsdottir et al.*, between 2001 and 2010 published in a series of scientific papers.

Cod fillet inesion in brines with 2,5% and 2% polyphosphates (BRIFISOL B512) resulting in a 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); but no differences were found after rehydration. Neither benefit in WHC nor muscle pH differences were detected. Moreover sensorial quality decreased for polyphosphate treated samples compared to controls in salted products, and this quality loss may not compensate obtained weight yield profits. These differences fade out after rehydration and cooking.

In this trials, phosphorus levels increased from raw material (0,44 gP₂O₅/100g) to cod after brining in phosphate treated samples (0,60 gP₂O₅/100g), meanwhile control levels decreased (0,33 gP₂O₅/100g) because of the salt-water exchange. 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 (0,10 gP₂O₅/100g). Quality evaluation showed better results in control than phosphate-treated fillets after salting,

Other studies in pacific cod salting (Schroder, 2010), with the injection of CARNAL 2110 (4%) prior brining (brines without PP) and standard dry-salting resulted in a slight decrease of humidity for PP treated samples compared to control after injection. This differences are not maintained in the subsequent salting steps. No yield studies were carried out. Schroder reported the loss of phosphates during frozen cod thawing from 0,44 g P₂O₅/100g to 0,36 P₂O₅/100g. After injection phosphate levels in phosphate-treated samples increased to 0,81 P₂O₅/100g meanwhile control decreased to 0,27 gP₂O₅/100g. Brining decrease these phosphate contents for both sets of samples supposedly because of a diffusion effect. In contrast to Thorarinsdottir results, during dry-salting the water leaked out from cod muscle did not contain significant levels of phosphates. Deep injection of additives, instead of phosphates absorption form brines, resulting in strong linkage to bound proteins was argued as the key factor to prevent phosphates loss in this step. Just like Thorarinsdottir studies, rehydration significantly reduced phosphorus levels even below natural contents, but in this case there is still a different final level in rehydrated samples between phosphate treated samples and control (0,29 gP₂O₅/100g and 0,07 gP₂O₅/100g respectively).

(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 to be placed in market as chilled. BRIFISOL 550 (sodium tripolyphosphate, monophosphate and short chained polyphosphates blend) showed the best results in yield gain probably by means of increasing muscle pH against other blends. All phosphate treatments increased general quality and tenderness but several differences between blends were obtained along product shelf-life. No possitive effects were detected in cooking and purge loss. 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 color development in fillets. BRIFISOL 550 again achieved best results in decreasing yellowness.

Recently, one study (M.Van Nguyen et al., 2012) carried out by a consortium of icelandic institutes has been published with very interesting data. Basically they studied the behaviour of phosphate species during cod salting. The application of phosphates was made by immersion, where control and treated samples were dry-salted and rehydrated, and several determinations were applied to each step. Results showed that diffusion of phosphates is characteristic for each one of the species, and depends on several factors; as the concentration gradients or presence of excessive sodium chloride as an antagonist. Therefore, the final levels in muscle are conditioned by the degradation of phosphates additives, muscle-brine interaction in phosphates diffusion, drip during dry-sating and storage, and rehydration.

Levels found are straightforward to previous studies. Natural phosphates diffuse to brines and final natural levels are reduced in heavy salted samples, except when phosphates additives were included in brining, as it is obvious. As in Schroeder studies, if phosphates had been injected in pre-salting, probably they would have been partially lost in brining as well.

Dry-sating leads to a significant loss of phosphate additives that were applied during brining; but not a significant reduction of natural phosphates was observed in control samples since levels were already low after brining. This probably corresponds to natural phosphate strongly bound to muscle structure not affected by diffusion and drying. As it was argued in Schroeder studies, maybe the injection of additives in pre-salting would not lead to this results since it seems to establish a stronger link of phosphates to proteins.

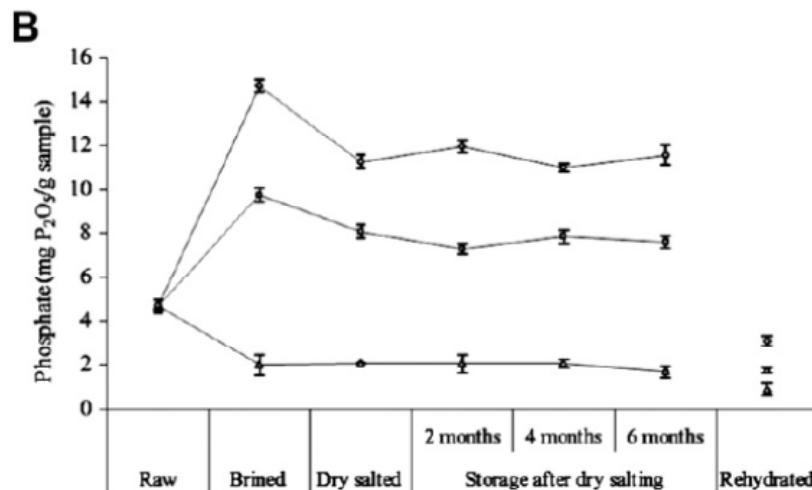


Fig. 6. Changes in total phosphate content of salted cod during salting, storage and rehydration determined by (A) IC method and (B) Spectrophotometric method [CN-OP (-Δ-), CN-3P (-□-) and CN-6P (-◇-)].

Fig.1 : Total phosphates in cod salting (CN-OP (control) , CN-3P (3%phosphates in brine), CN-6P (6%phosphates in brine)). (M.Van Nguyen et al., 2012)

During 6 month storage, total phosphate levels remained still in all samples, even though significant degradation of polyphosphates to ortophosphate was observed in treated samples. Rehydration

reduces the final content of phosphates far below the initial values because of the diffusion to surrounding water. There were still some additives present in the treated rehydrated products, even though results were very low. This undoubtedly reinforces the thesis of considering phosphates in salting processes as additives instead of processing aids, as it was determined by DGSANCO (commented above).

Recent studies carried out within the project collaboration between MOREFORSKING MARIN and ANFACO-CECOPECA have been focused on the effect of phosphates in cod salting applied under different processing methodologies and raw material qualities. As it has been more in depth reported by Moreforskning (*Report MA 12/15: Phosphate treatment of light and heavy salted cod products.*), trials demonstrate important differences in the absorption of phosphates depending on the salting process.

Pre-salting injection seems the most effective way to introduce these additives in cod, specially followed by brining with additional phosphates. Effective improvements in yield were obtained and related to phosphates absorption in samples. Pro-oxidant cations levels were neither affected by the phosphate addition nor salting process; so at least, chelation effect if present, did not ease these metals leaking out. Almost no differences between samples with phosphates and controls were observed concerning chemical oxidation. Only some sensorial improvements were achieved in light salted samples treated with phosphates.

Phosphate levels during processing undergo the same pattern as the previously commented studies. Nevertheless, these levels in raw materials were lower and more variable ($0,26 \pm 0,09$ gP₂O₅ /100g) than in the reference papers. Light salting seems not to affect phosphate levels ($0,27 \pm 0,02$ gP₂O₅ /100g), if we pay attention to average data from light salted samples without additives use. When phosphates are injected, the final amount will obviously depend on the quantity of additives introduced and the weight yield obtained. This is not consistent to Schroeder studies, where thawing significantly lead to natural phosphates loss. Freezing/thawing and drip may be behind differences found in raw material natural levels.

Data from NIFES (*Nutrients reports NIFES*) show that natural contents of cod raw materials, both from aquaculture or wild, are in the range of 140-220 mg P/100g, which corresponds to 0,32 – 0.50 gP₂O₅ /100g) though, as in other studies, it does not specify if material underwent freezing-thawing prior analysis.

Diffusion during heavy-salting leads to a sharp downfall in phosphate contents ($0,16 \pm 0,05$ gP₂O₅ /100g) which could be even enhanced if a more intense dry-salting step would have taken place, as references have stated. When phosphates are introduced in brining the final contents would depend on the balance between the natural or previously injected phosphates leaking out, and the incoming polyphosphates from brines. The concentration of phosphates in brines, time of immersion, temperature, and other factors will condition additives absorption and therefore final contents.

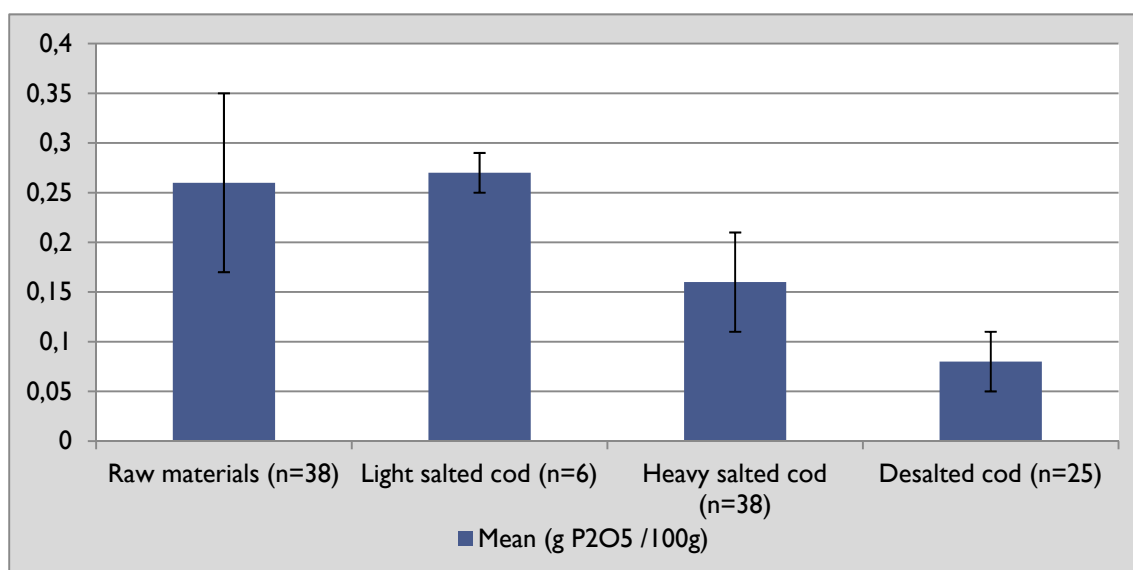


Fig.2: Total phosphates average results from "no phosphate treated samples" along cod salting. ANFACO-CECOPESCA/MOREFORSKING trials.

Finally, consumer or industrial desalting by cod immersion in water washes the majority of the remaining phosphates, either natural or from additives, leaving residual phosphate contents ($0,08 \pm 0,05$ gP₂O₅ /100g). Although our results did not directly address additives detection in desalted/rehydrated samples, a slight increase in these phosphate levels straightforward to the intensity of phosphate addition has been detected. Therefore there must be still some additives present in the final product, either present as polyphosphates or as orthophosphate from degradation. This pattern was also observed in the Icelandic trials mentioned above.

4. PHOSPHATES OFFICIAL CONTROL AND ANALYTICAL STATE OF THE ART.

Although legislation clearly establishes the allowed dosage of additives it needs to be enforced by means of official controls and the appropriate analytical methodology. Several approaches to polyphosphate additives quantification have been reported and are currently being used by delegate laboratories for official control. Unfortunately, there are still some problems with their performance and the technical interpretation of the results, which affect the appropriate control of these food additives.

4.1 ANALYTICAL STATE OF THE ART.

The dosage of phosphate additives should respect current legal limits, determined by the combination of all phosphate additives, and expressed as mg P₂O₅ /kg or equivalent units. Diphosphates, triphosphates and polyphosphates may only be present in foodstuff due to their use as additives, while monophosphate may come from both technological and natural sources.

There are some methodological limitations in the differentiation of natural phosphorus contents in food 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 several factors (temperature, pH, enzymatic activity etc...) resulting in an increasing level of monophosphates straightforward to the additives breakdown.

There are two analytical strategies in literature for the determination of phosphate additives. The first one uses the total phosphorus as the key method. The second group of techniques involves an extraction phase, and specific separation/expression of natural and added phosphates.

The seafood **total phosphorus contents** might be determined by different techniques. The most extended is the AOAC official method (*AOAC Official method 995.11*), probably because it does not need specific expensive instrumental. It basically performs a sample mineralization hydrolyzing phosphates, followed by the measurement of the yellow color produced by their reaction with a molybdate-vanadate solution in a UV-VIS spectrophotometer at 436 nm. It is an accurate, cheap and robust method, but slow, since mineralization is prolonged at least for 24h, unless microwave digestion is applied.

Other methods currently applied are based on atomic absorption/emission spectrometry. These methods include an acidic digestion phase in microwave digesters. Flame Ionization Atomic Absorption Spectrometry methods (FI / AAS) keep on being used because instrumental is available in most laboratories, and sensitivity is good for phosphorus common contents. Optical Inductive Coupled Plasma Emission methods (ICP-OES), are probably the best options because of its accuracy, sensitivity and lack of interferences. Its higher cost of use can be somehow mitigated because of its multi-elemental character, allowing the simultaneous determination of several elements.

In theory, the total phosphorus content might be an easy tool to discriminate excessive dosage of phosphates but it does not differentiate natural from additives phosphate and this is not consistent with present legislation, which establishes limits for the sum of phosphates from additives, excluding natural variations.

Other techniques are based on the assumption that muscle **phosphorus and nitrogen** are present in a **permanent ratio**. Basically this method performs a Kjendal distillation of the sample to determine its nitrogen/protein content. After that, a factor is directly applied to obtain the natural phosphate expected to be present. A total phosphorus analysis is performed aside. Added phosphate is calculated as the difference between both results.

This method is easy to be implemented, but the assumption of a permanent N/P ratio to all kind of food materials is not well-founded, and as it was previously detailed phosphorus contents are affected by several industrial processes. Besides, this is an indirect determination and the

uncertainties of the protein and total phosphorus determinations are transmitted to the final results. Nevertheless, these methods are not affected by the polyphosphates degradation, which it is the major problem from an analytical point of view, and which critically affects the other group of techniques.

The second kind of analytical methodologies, involve the extraction and separation of natural from added phosphates. We are making emphasis in the separation phase because it is critical for all methods detailed below. As the polyphosphates are unstable, since they become affected from pH, temperatures, enzymes, etc..., it is not easy to extract these compounds from such a complex matrix like fish tissues, causing minimal alteration in these analytes.

Apart from the extraction, sampling is another analytical point which could considerably affect the final results. Depending on the type of sample, the time and conditions of storage, polyphosphates present in the sample at the moment of the sample arrival to laboratories may have been partially or completely degraded because of enzymatic activity and physical-chemical reactions.

In frozen products, in addition prior to final glazing some of the baths with additives applied generates an external layer containing phosphates which could end up in result distortion. Besides, the penetration of phosphates into tissues is rather diffuse and the surface/volume ratio in the sample may sum up uncertainty to these results.

Extraction methods vary but frequently involve contact from sample to a polar solvent (water, trichloroacetic acid...), ultrasonic leaching and some short cold storage to ease solubilization. After that, centrifugation and/or filtering steps are carried out, frequently under controlled pH conditions to prevent chemical degradation of phosphates. Finally, these extracts are introduced in several instrumental techniques.

Making use of the different polyphosphate degradation kinetics under controlled conditions, another method was developed known as **Thermo-differential photometry** (R.Kruse et al., 2005). In contrary to the fast monophosphate anion PO_4 , condensed polyphosphates (P_2O_7 and P_3O_{10}) react very 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 straightforward to polyphosphates' 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. In the latest studies, it has been demonstrated that high amounts of salt in the product, critically conditioned the polyphosphates extraction step and the color development.

Methods based in **thin layer chromatography** have also been applied for polyphosphates detection (*P. Reece, V. Russell, 1994*) (*H. Marescot et al., 1998*), which are closely related to ISO 5553-1980 for the detection of polyphosphates in meat products by TLC. ANFACO-CECOPECA laboratories have adapted this method for the quantification of monophosphates, diphosphates, triphosphates and hexametaphosphate in fishery products. In this context, it has been proved that, alike similar methods, the extraction step is not fully efficient, but it is still a good alternative for the evaluation (semi-quantification) of polyphosphates. Robustness is another factor to be improved, considering the general low robustness inherent to the TLC methods.

Some approaches based on **capillary electrophoresis**, not intended for food quality control, for the analysis of polyphosphates have been reported. (*Stover FS, 1997*)(*Lee A, Whitesides G.M, 2010*). Authors detail that methodology could be adapted for routine quality control but no attempts have been made yet.

Ion-exchange chromatography techniques for the polyphosphates separation and quantification have been reported in literature, but references of their application in routine controls from laboratories are scarce. In any case, it seems to be the most promising technique for this purpose regarding last published scientific documentation.

A post-column derivatization of the samples in an indirect photometric detection (*Shamsi S.A, Danielson N.D, 1993*) (*Svoboda et al. 1997, 2002*) has been carried out using naphthalenetrisulfonate and molybdovanadate respectively for the detection of polyphosphates

(*Cui et al., 2000*) and (*Sekiguchi Y. et al., 2000*) firstly experimented with the application of this technique for the determination of condensed phosphates in food products with a hydroxide gradient. Results indicate that methods were precise as well as sensitive enough for the proposed purpose, but information of the accuracy was not detailed.

(*Kaufmann A. et al. 2005*) performed some trials about the enzymatic degradation of phosphates with polyphosphate detection by IC comparing carbonate and hydroxide gradients. This last method seems to show better performance in seafood samples but it also did not go through a standard validation protocol.

Ion chromatography instrumental companies (*Dionex, Application Note 71, 2011*) have internally developed some application studies for commercial purposes but they did not address the extraction procedures from real samples, but the standard separation and quantification.

Based in previous documents and using DIONEX instrumental, previously cited Icelandic trials (*M.Van Nguyen et al., 2012*) developed a method application for seafood. Although separations may be successful, final recoveries when comparing to total phosphorus contents were still low, evidencing that phosphates extraction needs to be necessary improved.

The most complete and best performing of the method references was published in 2012 by a group of Italian researchers (Ianmarino M., Di Taranto A., 2012). This method seems to overcome all previously detailed analytical problems, even in extraction, in a wide variety of food samples where polyphosphate are common additives. Intense validation has been carried out following Directive 657/2002/EC criteria for specificity, linearity, accuracy, LOD and LOQ. Besides, the method seems neither to be time costly (simple extraction and clean-up) nor expensive. Spiked and commercial samples have shown good recoveries confirming the effectiveness of this method for quality and official control. The method has also been tested by the participation in several proficiency tests since 2006.

A **method based in common HPLC** is under development in ANFACO-CECOPESCA's laboratories, taking advantage of the performance of the CORONA CAD detector. According 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. Although the detector allows the separate identification of the compounds when using commercial standards, the chromatography of the samples is difficult, and there have not been achieved satisfactory results yet. A pre-column ion-suppressor coupled to standard columns might be necessary to obtain clean chromatograms for these analytes, but trials have not been still carried out.

4.2 PRESENT SITUATION OF PHOSPHATE ADDITIVES OFFICIAL CONTROL IN THE EU.

Regulation (EC) 882/2004 addresses the official control across Europe for food and feed law compliance. It establishes procedures for a common harmonized European framework. Basically, this document relays member countries the responsibility of the implementation of these control measures, leaving the communitarian administration the international supervision, and coordination of each country duties. It also establishes a list of **Community Reference Laboratories (CRL)** in different topics of the feed and food control, which are responsible for providing national reference laboratories assistance on the analytical methods, including reference methods; organizing comparative testing under internationally accepted protocols, when available; and staff training from national laboratories. The Community Reference Laboratory shall also play a role in dispute settlements between Member States in official control issues. The Institute for Reference Materials and Measurements (IRMM) in Geel (Belgium) has taken up the task on feed additives but there is not still any CRL focused on food additives.

The role of the designated **Competent Authority in Member Countries** is to ensure the effectiveness and appropriateness of official controls, by selecting National Reference Laboratories. Besides, they shall ensure that laboratories have enough capacity and experience to effectively fulfill

the requirements assigned, avoiding any conflict of interest. These laboratories shall be accredited in EN ISO/IEC 17025 on "General requirements for the competence of testing and calibration", be appropriately equipped and use validated methods.

As this regulation clearly states, "Official controls should take place on the basis of documented procedures so as to ensure that these controls are carried out uniformly and are of a consistently high quality". Moreover, Article 11 addressing methods of sampling and analysis it states that standard and internally recognized protocols should be used. In their absence, other methods fit for the intended purpose or developed based in scientific criteria and with appropriate internal validation following international validation criteria. Annex III mentions the points this validation should include which are further technically detailed in Commission Decision 2002/657/CE implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results.

The official control of phosphates across the European Union is far from homogeneous, methods are diverse and the interpretation of the results is inaccurate. The information to date is that the phosphate analytical problem has not been approached yet in an international level, for the homogenization, method applicability and validation.

As an example, laboratories in the Spanish Official Control apply total phosphorus methods which allow the identification and quantification of the total amount of phosphorus either coming from natural or from industrial processing sources. The use of this method to control and penalize producers is unfair and inconsistent with the legislation criteria, which clearly addresses that the limits are just for the combination of added phosphates up to 5 g P₂O₅/kg. Natural variation of phosphorus content in shrimp exceeded legal thresholds (*Crawford, 1980*), and frequently, other seafood stuff develop natural phosphorus levels bordering or even exceeding the marked thresholds, and thus generating false positives. The natural variation of phosphorus in the species is widely known in the industries, because it generates problems to standardize the additives dosage to achieve the desired technological effect complying with regulation.

An amendment to the Spanish Authorities about the application of this method for this purpose was submitted by ANFACO-CECOPECA. It was unofficially accepted and today Spanish authorities, in practice, do not create any trade obstacle to food products unless the dosage of phosphates is extremely high, evidencing an inappropriate use of additives. Arguments from the industry sector explaining the situation to inspectors usually end up in freight release, despite positive results.

This controversial method is also being officially applied in other member countries, like the case of the Icelandic national system which delegates this official control to a center of international prestige like MATIS. The Food Safety Authority of Ireland has recently issued a review of the appropriateness of this methodology for the official control of phosphates.

Some laboratories from other countries like Denmark (*Phosphat i ferske og frosne fisk og fiskerivarer Rapport. Projekt 2009-20-64-00163: <http://www.foedevarestyrelsen.dk/SiteCollectionDocuments>*) and Italy (*Rapporti INTISAN 96/34*) have carried out the previously commented methods based on Nitrogen/Phosphorus permanent natural ratios to establish natural phosphorus content, and then calculate added phosphates by differences to total phosphorus.. 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. In fact, factors applied for protein to natural phosphorus conversion are different in these countries (Denmark: 0.0106 and Italy: 0.025).

Danish laboratories carrying out this determination for official control have recently implemented an official detention of chilled plaice from Netherlands, and placed an alert on the RASFF system (*Notification detail-2012.0090*).

In fact, the lack of a valid reference method, and the inappropriateness of the applied methods was admitted by the Italian Ministry of Health which solicited their laboratories the development of an alternative method. (*Ianmarino M., Di Taranto A., 2012*). A working group composed of several Italian laboratories is carrying out an interlaboratory validation of the ion chromatography method, since the proficiency tests on the present official method (Thin Layer Chromatography based on ISO 5553:1980) gave not optimal results. This validation should be completed in 1 year and the procedure will be adopted as Italian official method for the determination of polyphosphates in seafood.

Some contacted private laboratories in Norway subcontract total phosphorus determinations to German and Swedish laboratories for screening services to seafood producers. NIFES laboratories are in charge of the official control in Norway implementing the majority of their analysis in crustaceans. NIFES does not have still a specific method for polyphosphate, so this official control is carried out by using the total phosphorus determination.

Other methods trying to separate and quantify just the phosphate additives apart from natural phosphorus, basically by chromatographic techniques, might be being used by EU laboratories. All these analytical methods cannot avoid the degradation of phosphate additives prior the arrival of the sample to the laboratory and during storage, and 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 always represents a real positive.

Other issue that should be considered in the official control of salted/dehydrated fish is whether the analysis should be performed on the fully-salted/dehydrated product or the final rehydrated material. Regulation 1333/2008 states in article 11:3 that marked levels are applicable to final traded products,

but when referring to concentrated/dehydrated that need re-hydration before final consumption, a practical dilution factor should be applied. This point assumes that all components present in the product are diluted in the final foodstuff, which is not the case of phosphates, where rehydration leads to significant losses of phosphates in fish tissue.

For the case of salted products it is not still a big problem since presence of phosphates is not permitted yet and any detected polyphosphates is always a positive, if the carry-over argument is not put forward. At the moment, it is not known if the national reference laboratories across Europe are considering this point, even when the analysis in rehydrated product could be producing false negatives.

5. FINAL DISCUSSION AND RESEARCH NEEDS.

The possible utilization of phosphate additives during heavy-salting may soon be approved according to last meeting reports and draft presented in the DGSANCO-SCFCAH. Although there are still confronted arguments between countries under discussion, from a pure technical point of view, it seems that the permission might be effective soon. The use of polyphosphates seems to be reasonable in heavy-salting; and as the committee agreed, the permission does not necessary involve the obligation of use for producers. Besides, last studies documented that fair trade in final consumption is not under risk.

EU additives legislation has not taken in consideration the analytical state of the art for the practical implementation of polyphosphates official control. Although it defines maximum limits for the added phosphates, there were not validated analytical methods, in practice, which could differentiate, and effectively quantify these additives separately from natural phosphorus fluctuations.

Official controls for polyphosphate additives are not well defined in the EU. There is not still a Reference Laboratory for additives in foodstuff across the EU which could deal with the homogenization of Member State Laboratories protocols. In most cases, these national laboratories do not have suitable accredited methods for this purpose, using the lightweight and meaningless traditional total phosphorus determination for the discrimination of the appropriate use of phosphate additives.

The development of methods based in ion-chromatography in the last years, overcoming the some extraction problems, may have got closer to an end to these analytical problems, but this methodology needs to be tested and accepted at an international level. In any case, meanwhile all these measures do not take place; the analytical results in official inspections should be carefully interpreted taking into account present drawbacks, preventing the implementation of unfair trade restrictions for consignments.

On the other hand, considering the lack of toxicity of these additives and although methods based in N:P ratios have been criticized in this document; establishing more accurate ratios for each group of species, could be a good alternative probably not as a confirmation but as a screening method, because of its simplicity and low cost.

Alike, the evaluation of natural fluctuations and the effect industrial processing in each of the potential food products could be useful to establish an admissible range for a reasonable application of phosphate additives.

The need of international methodology harmonization by means of scientific analytical forums, workshops, staff training, community proficiency testing, and the settlement of accepted community protocols and appropriate techniques is evident and should be implemented as soon as possible. Further on, the setting of a community reference laboratory for food additives should be strongly considered.

6. REFERENCES.

- Commission Regulation (EU) No 257/2010 of 25 March 2010 setting up a program for the re-evaluation of approved food additives in accordance with Regulation (EC) No 1333/2008 of the European Parliament and of the Council on food additives.
- Commission Regulation (EC) No 1333/2008 of the European Parliament and of the Council by establishing a Union list of food additives.
- Commission Regulation (EU) No 1129/2011 of 11 November 2011 amending Annex II to Regulation (EC) No 1333/2008 of the European Parliament and of the Council by establishing a Union list of food additives.
- Commission Regulation (EC) No 1331/2008 of the European Parliament and the council of 16 December 2008 establishing a common authorisation procedure for food additives, food enzymes and food flavourings.
- Commission Regulation (EC) 882/2004 of the European Parliament and the Council of 29th April 2004 on official controls performed to ensure the verification of compliance with feed and food law, animal health and welfare rules.
- Summary Record of the Standing Committee on the Food Chain and Animal Health held in Brussels on 14 March 2011. Section "Toxicological Safety on the Food Chain". SANCO – DI(2011)D/310301.
- AOAC Official method 995.11, 17th Edition, vol II, chapter 45, (2002) p 42-45. Total Phosphorus in Foods.
- Andrew J. Alpert (1990).Hydrophilic-Interaction Chromatography for the separation of peptides, nucleic acids and other polar compounds. Journal of Chromatography, vol. 499: p. 177-196.
- Arnesen G, Dagbjartsson B. 1973. Saltfiskrannsóknir. Tæknitíðindi nr. 36. Reykjavík: The Icelandic Fisheries Laboratories. p 1-22.

- Arnesen G, Dagbjartsson B. 1974. Saltfiskrannsóknir. Tæknitíðindi nr. 54. Reykjavík: The Icelandic Fisheries Laboratories. p 1-13.
- Bjørkevoll, I. (2004). Hospitering i Salt- og klippfisknæringa. Fiskeriforskningsrapport (Konfidensiell).
- Cui E, Cai F, Xu Q. 2000. Determination of tripolyphosphate in frozen cod and scallop adductor by ion chromatography. *Journal of Chromatography A*, 884:89–92.
- Gonçalves AA, Ribeiro JLD. 2008. Do phosphates improve the seafood quality? Reality and legislation. *Pan-Am J Aquat Sci* 3:237–47.
- Kaufmann A., K. Maden, W. Leisser, M. Matera, T. Gude. Analysis of polyphosphates in fish and shrimps tissues by two different ion chromatography methods: Implications on false-negative and positive findings. *Food Additives and Contaminants*, November 2005; 22(11): 1073–1082.
- Krzynowek J, Panunzio J. 1995. Practical application of thin-layer chromatography for detection of polyphosphates in seafood. *Journal of AOAC* 78:1328–1332.
- Lee A, Whitesides GM (2010) Analysis of inorganic polyphosphates by capillary gel electrophoresis. *Anal Chem* 82:6838–6846.
- ISO 17025 (2005). General requirements for the competence of testing and calibration laboratories.
- ISO 5553-1980. Meat and meat products. Detection of polyphosphates.
- INTISAN Reporti 96/34. Metodi di analisi utilizzati per il controllo chimico degli alimenti.
- Kin S, Schilling MW, Silva JL, Smith BS, Jackson V, Kim T. 2009. Effects of phosphate type on the quality of vacuum tumbled catfish fillets. *Inst. of Food Technologist Annual Meeting*, Anaheim, Calif., June 7-10, 155-09.
- Kin S., M.W. Schilling, B.S. Smith, J.L. Silva, V. Jackson, T.J. Kim. Phosphate Type Affects the Quality of Injected Catfish Fillets. *Journal of food Science—Vol. 75, Nr. 1, 2010.*
- Kim J.S, Schnee R., Park JW. Chemical and functional properties of various blends of phosphates. *Journal of Food Quality*.32 (2009) 504-521.
- Kruse R. Nieders. Landesamt f. Verbraucherschutz u. Lebensmittelsicherheit, Institut für Fischkunde Cuxhaven.2005. Bestimmung von Polyphosphaten in Fischen durch Kinetische Thermo-Differential-Fotometrie.
- Lampila, L.E., 1993. Polyphosphates rationale for use and functionality in seafood and seafood products. In: *Proceedings of the 18th Annual Tropical and Subtropical Fisheries Technological Conference of the Americas*. Virginia, USA, pp. 13–20.
- Lauritzsen K, Akse L, Johansen A, Joensen S, Sorensen NK, and Olsen, R.L. (2004). Physical and quality attributes of salted cod (*Gadus morhua* L.) as affected by the state of rigor and freezing prior to salting. *Food Research International*, 37(7), 677-688.
- Marescot H., B. Giorgio , R. Maillard, "Dosage des polyphosphates dans les produits de la mer". *Bilan d'activité des laboratoires de la DGCCRF (1998)*, p 85-87.
- Bjorkevoll et al. Phosphate treatment of light and heavy salted cod products. Report MA 12/15: Moreforking marin Reports. (<http://www.moreforsk.no/>)
- Reddy, B.R. and Finne, G. 1986. Hydrolytic and enzymatic breakdown of food grade condensed phosphates in white shrimp (*Penaeus setiferus*) held at different temperatures. *Eleventh annual tropical and subtropical fisheries technical conference of the Americas*. Jan 13-16, Florida. 201-212.
- Reece P., V. Russell, "Separation and identification of added polyphosphates in fish". 24th Western European Fish Technologist's Association Meeting 25-29 september 1994, Nantes, France.

- Schröder, U. (2010). Changes in Phosphate and Water Content during Processing of Salted Pacific Cod (*Gadus macrocephalus*). *J. of Aquatic Food Product Technology*, 19, 16-25.
- Sekiguchi Y, Matsunaga A. 2000. Analysis of condensed phosphates in food products by ion chromatography with an on-line hydroxide eluent generator. *Journal of Chromatography A* 81: 639–644.
- Sutton, A.H. The hydrolysis of sodium triphosphate in cod and beef muscle. *Journal of Food Technology*.8.185.1973.
- Shamsi SA, Danielson ND (1993) Ion chromatography of polyphosphates and polycarboxylates using a naphthalenetrisulfonate eluent with indirect photometric and conductivity detection. *J Chromatogr A* 653:153–160.
- Sutton AH, Ogilvie JM. 1967. Uptake of sodium and phosphorus, and weight changes in prerigor cod muscle dipped in sodium tripolyphosphate solutions. *J Fish Res Bd Canada* 25(7):1475-1484.
- Stover FS (1997) Capillary electrophoresis of longer-chain polyphosphates. *J Chromatogr A* 769:349–351.
- Svoboda L, Schmidt U (1997) Single-column ion chromatography of linear polyphosphates. *J Chromatogr A* 767:107–113.
- Svoboda L., Hynkova E., Kovárová M. Simplified post-column derivatization method for ion chromatography of linear polyphosphates. *Journal of Separation Science*. Volume 25, Issue 10-11, pages 715–718, July 2002.
- Tenhet, V., Finne, G., Nickelson, R., Toloday, D., 1981. Phosphorous levels in peeled and deveined shrimp treated with sodium tripolyphosphate. *Journal of Food Science* 46, 350–356.
- Thorarinsdottir, K.A., Arason, S.G., Gogason, S.G. and Kristbergsson, K. (2001) 'Effects of Phosphate on Yield, Quality and Water-Holding Capacity in the Processing of Salted Cod (*Gadus morhua*)', *Journal of Food Science* 66: 821–26.
- Thorarinsdottir, K.A., Bjorkevoll, I., Arason, S.G., (2010). "Production of salted cod in the Nordic countries. Variation in quality and characteristics of the salted products." *NORA (Journal 510-036)*.
- Thorarinsdottir, K.A., Arason, S., Bogason, S.G. and Kristbergsson, K. (2001). Effects of Phosphate on Yield, Quality and Water-Holding Capacity in the Processing of Salted Cod (*Gadus Morhua*). *J. of Food Science*, vol 66, No. 6.