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Project Finnanced by the Fiskeri - og havbruksnæringens forskningsfond (FHF)

Prosjekt nº: 900985 Dato: October, 2015.

Scope and Aims

Change in consumers' lifestyle has increased their interest for ready-to-use and less time-consuming products. This has motivated the cod industry to develop new products which match consumer needs, as is the case of light salted cod fillets and industrially desalted (previously heavy salted) cod products. These products, either commercialized in a frozen state or under refrigerated conditions (VP, MAP...), are frequently present at the market under an unspecific denomination which makes difficult for the consumer to recognize and differentiate. Several examples of cod products bordering or not complying regulations of unfair commercial practices and information to consumers have been detected.

The main project aims are the development of valid methodologies for the discrimination of light salted cod products from traditionally heavy salted and desalted products. Other novel advanced technologies will also be considered because of its potential to develop cheap, specific, fast and simple methodologies for this purpose. Project finally addresses the raise of awareness by the competent authorities/consumers for the settlement of a differentiated commercial status for both products.

- Determining and documenting general structural, compositional and sensorial differences between light salted and fully-cured /desalted cod. Scientific dissemination.
- Studies of novel advanced techniques with potential to discriminate cod products, as a basis for further development of simple tailored methods.
- Selection of appropriate (cost-effective, simple, widespread across control laboratories) discriminant methodologies.
- Method optimization based on widespread techniques for the differentiation of these cod products.
- In-house method validation. Development of a Standard Operating Procedure (SOP).
- Reporting to Authorities, and Food Sector of technical criteria for the different categorization in trade and market of these products. Roadmap for its legal implementation and consumer awareness campaign.

Summary

Project objectives aimed the development of valid methodologies for the discrimination of light salted cod products from desalted products. It also focuses on the sensitization of the competent authorities for the settlement of a differentiated commercial status for both products.

Executed tasks involved a first preliminary study of both cod products including as much variability as possible (origin, production methods, time of storage, etc...) and applying a wide variety of techniques. Results would determine the suitability of each of the methodologies, selecting the best ones to be further validated in a second stage. The performance of the methods has been evaluated and the ones with best discriminant potential, easy to implement and disseminate, simple and low-cost were selected. The methods were optimized and validated and Standard Operating Protocols were developed for the appropriate dissemination of the methods.

In parallel to purely analytical research activities, publications and technical reports specially designed for the sensitization of the Authorities have been prepared. Contact to Authorities has already been established and in order aware them of both the necessity of legal changes concerning the commercial differentiation of light-salted and desalted cod products, and the existence of appropriate instruments for the official control enforcement.

Project Technical Execution

1. SAMPLING ACTIVITIES (Work Package 1).

Sampling activities were initially rather complicated due to the international approach of the project and since the season of cod was nearly finished at the time to collect the materials. After the celebration of the Start meeting, the sampling was carefully designed to get the amount of materials and with the special characteristics essential to execute the project tasks. A plan for the task was defined (*Submitted Delivery 1.1*). This work package was divided in the collection of materials for internal processing at pilot plants and laboratories, and market samples. Detail of the materials involved is laid down in annexes from Delivery 2.1, Delivery 2.3 and Delivery 3.1.

Industry sampling.

It consisted in light salted and heavy salted samples from fresh and frozen raw materials. These products were supplied by Jangaard Export AS and Nils Sperre AS and directly shipped to centers. Since at the time for sampling there was no option to get light salted cod fillets from fresh raw materials, and some of the materials arrived at Italy in unappropriate conditions for the studies, these needed to be purchased locally to other producers (Icelandic, Henry Johansen AS).

Materials were internally processed according to the procedures for thawing and desalting laid down in the sampling plan for WP2. Materials were either vacuum packed, or MAP and submitted to High Pressure processing in some cases. Samples were frozen or a simulation of the shel-life in the market was carried out for chilled products.



Market sampling.

Market sampling was carried out by UNITE and ANFACO-CECOPESCA to create the set of samples used in WP2. Involved cod products in all available commercial formats that were purchased at different retail distributors and belonging to different producers and trademarks.









Additional market sampling was carried out in WP3 by UNITE in order to validate the FTIR method. Simultaneously, ANFACO-CECOPESCA also collected, and successfully shipped to Norwegian partners, the cod products in the Spanish market for the validation of the methods developed by NOFIMA and SINTEF.

2. <u>IDENTIFICATION AND SELECTION OF CRITICAL PARAMETERS (Work Package 2).</u>

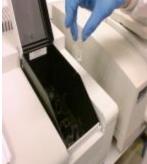
The analytical activities were divided in two consecutive stages. The first one (*corresponding to submitted Delivery 2.1*) consisted in an initial study of the characteristics of the materials and a preliminary screening of the methods to be developed. The second one was conditioned for the first stage results and was focused on thesting the effectiveness of certain techniques and parameters for the discrimination of cod products (*Delivery 2.2 -2.3*). Activities and results were scientifically discussed and presented to the Steering Committee in the Folow-up meeting held in Lisbon in January 2015. A summary of the results are shown in Table 1.

<u>Task 2.1: General comparative characterization of light salted and desalted cod products.</u>

A summary of the results is that there are some factors and parameters that have shown to develop significant variance between light salted products and heavy salted products. Market samples developed wider internal variance and did not respect the pattern that the industry samples, immediately analyzed after processing, had indicated. The ripening during storage, the use of different additives, the conditions of processing, quality of raw materials or even water and salt, and the type of packaging are posibbly behind big part of this variance. No big difference between qualities of raw materials has been detected in the samples. Results of yield, nutritional and mineral composition, textural, freshness and oxidation indicators, and microbiological quality are presented in detail in Deliverable.2.1.









As theorically expected, the levels of free nitrogenous compounds like taurine, and trimethylamine oxide (and derived compounds) in muscle are highly affected during processing but not in subsequent stages of product

shelf-life. The application of image and colorimetric methods to the samples seemed useful for the target discrimination.

Sensorial testing performed by UNITE showed that expert panels are highly efficient in discriminating cod product nature. A consumer panel at ANFACO-CECOPESCA was not able to discriminate light salted cod from desalted cod when not processed (at the time of purchase), but differences were noticeable when cooked. In any case, there was not a significant preference of the consumer panel for any of the products.







Task 2.1, as planned, was developed in cooperation by UNITE and ANFACO-CECOPESCA. The selected analyses were aimed to set a general basis on the characteristics of the materials and as a first approach to the subsequent tasks in the project and to the current method development. This task involves physical-chemical and production aspects. Sensory analysis both at ANFACO-CECOPESCA and UNITE have also been performed.

Task 2,2 – 2.3: Development of the potentially discriminant methodologies. Analysis of the samples.

The techniques included in the proposal which had been identified as potentially discriminant from literature were tested in this WP. Task 2.1 results helped to select and refine the techniques to be applied in Task 2.2 and task 2.3.

A simulation of the cod products fate has been carried out in pilot plants, industrial plants and laboratories in order to include the potential variability of the samples present at the market. Some real market samples have additionally been included in some of the implemented methods.

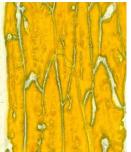
ANFACO-CECOPESCA studied the levels of some nitrogenous compunds (creatine, taurine and TMAO and derived volatile amines), the application of microbial fingerprinting by moleculary techniques (T-RFLP) and the study of the muscle structure by means of protein profiling using SDS-PAGE electrophoresis, and optical microscopy.

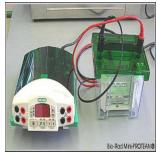
Results indicate that the methodology for the analysis of the level of TMAO +TMA is simple, cheap and sensitive, and showed good result when discriminating desalted and light salted samples. The packaging applied or the time of storage / time in market do not affect results. The analysis of peptides (creatine and anserine) as well as taurine seems also promising as discriminant methods in the same way as the TMAO method cited above. The levels also decay from raw materials to processed materials being this loss more prominent in desalted materials

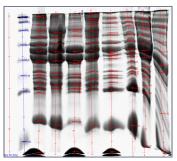
and allowing this segregation. Nevertheless, both methods involve the utilization of HPLC protocols including derivatization steps, and therefore they are more slow and expensive and not as easily transferable as the previous TMAO method.

T-RFLP technique (*Terminal restriction fragment length polymorphism*) has found some trends that could be signs of a discriminant potential but; since the optimization of the method was tedious, and as some other cheaper and faster techniques showed better resolution, the full amount of samples to unveil its real potential has not been analyzed yet. In any case, it has been detected that the complexity of the method does not match the project requirements for easily transferable methodologies. SDS-PAGE method has proven so far its almost universal applicability for discrimination among different species, and products. However, in this case market samples and more specifically product shel-life sgnifficantly affected the discrimination of the target samples. It was found that there were difficulties in assigning market samples to any of the processing groups on basis of the SDS-PAGE profile. It is the number of bands per sample, the only factor that could be used in a mathematical model, in a combination with other parameters to detect overall statistical difference between treatment types of cod samples.









The use of optical microscopy has not permitted a statistically significative differentiation among the industry panel samples due to inter-group variations of the studied parameters in the light salted samples. Only intercellular space measurements, in combination with another variable (like the previous number of bands), could be used for statistical difference between treatment types of cod samples. The development of a mathematical model, combining the results from both analytical methods, is presently ongoing.

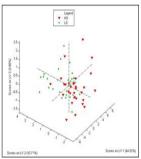
UNITE performed the microbiological studies and fingerprinting by classical methodologies and phenotype microarray. UNITE also tested the potential of an electronic nose to test whether the profile of volatiles was sufficient to differentiate these products. FTIR (Fourier-Transformed Infrared) spectra of cod samples and the development of discriminant models were also part of the Italian tasks in this part of the project.

Microbiological methods could not really discriminate among samples of different origin. The thesis (HS vs LS) was significant for some microbial groups, where thesis x time was more influencing. In particular, significant differences, for some groups, including specific spoilage organisms were observed, however their growth during time was often more affected by packaging conditions and storage time than by the kind of samples.

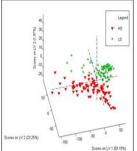
In the same way, also the phenotypic characterization of the microbiota, although showing differences among the samples depending on the variations in microbial groups load, added useful information but could not discriminate the samples according to their technological history.

The electronic nose could be considered as a valid tool to screening desalted and Light-salted samples, in many cases also being able to distinguish samples on the base of their technological history and storage. Results of sensitivy and specificity could be considered very good, considering that a 8-sensors Electronic Nose was used (a new 12 sensors EN is in phase of implementation). Until now, gas sensors have been used for the analysis of fish products, while this is the first report of the use of metalloporphyrins for this kind of evaluation. The volatile profiles of the samples appeared to be affected by the time of storage, therefore he electronic nose, coupled with a more simple method could give even better results not only in discriminating HS and LS samples, but also in differentiating them according to their shelf-life. The electronic nose is a fast analysis, which also has the advantage of the possibility of a remote control.





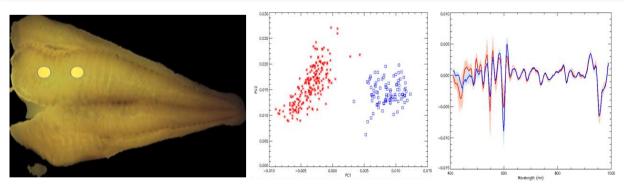




Surprising results were obtained as regards the FTIR analysis. The simple and fast sample preparation (homogenization) gave sufficient information to discriminate and differentiate light-salted from heavy salted samples, independently on other important parameters, such as kind of raw material, packaging conditions and storage time. The analyses performed confirmed 100% sensitivity and 100% specificity of this method, for the samples analysed, so it is the most interesting technique to include in WP3 in order to confirm the performance and the applicability of the method.

VIS/NIR spectroscopy has been studied by **NOFIMA** for the appropriate differentiation of light salted and desalted products. Nofima has performed the development and optimization of analytical techniques (Task 2.2) and the analysis of industrial samples (Task 2.3). Although the spectroscopy measurements have been performed using hyperspectral imaging, but as previously published, these measurements can also be performed by cheaper handheld spectroscopic devices.

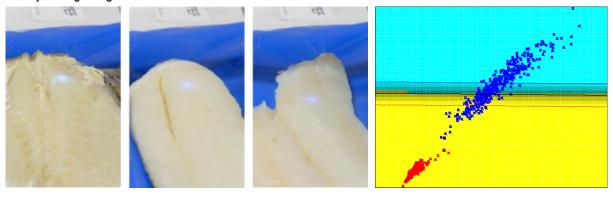
Hyperspectral imaging / VIS-NIR spectroscopy is a good alternative for differentiation of light salted and heavy salted cod. The classification made based on industry samples appeared to be robust and was able to differentiate between lightly salted cod (single and double frozen) and heavy salted cod (fresh and frozen/thawed raw material) with almost 100% accuracy. Furthermore, the classification could also handle heavy salted products that have been stored frozen after rehydration. Using the spectral data for deciding the status of raw material prior to salting (fresh or frozen/thawed cod) worked well for lightly salted cod (93,3% correct classification), but did not work for heavy salted cod. However, 88% of the heavy salted cod was correctly classified with respect to frozen storage or not after rehydration.



The inclusion of a limited number of real market samples (Spanish sampling) affected the previous model performance. After changing algorithm from KNN to PLS, the method results were significantly improved (correct classification for 94% (market) and 100% (industrial) of the test samples). More variability from market samples should be included in the models in order to enhance accuracy and robustness. The configuration used by NOFIMA for discrimination has some similar commercial versions (probes and detection). It could be possible that models could be also developed with presently available laboratory instruments, although these instruments are not much extended in standard laboratories.

SINTEF performed the development and optimization of the method for discrimination of the previously commented materials by means of a machine vision system using fluorescence scatter spectral data.

The simple machine vision setup, with a near-UV laser and imaging in fluorescence mode, and the developed models based on industry samples enabled near-perfect discrimination between heavy salted cod and light salted cod by use of a linear discriminant function on three simple features. The mechanism that explains the difference is not completely understood, although it is suspected that the light scattering ability of the desalted cod is different than light salted cod, and thus the fluorescence ability is affected. There may also be differences in fluorescence due to presence of bacterial cultures in heavy salted cod. Further investigation is necessary in order to optimize the algorithms and the machine vision setup, and perform a larger number of measurements in a factory setting using a hand-held device.



An external validation set of samples (Spanish sampling) was delivered to SINTEF for the confirmation of its good performance. The configuration used for market sample validation could have created a shift in the recordings of the spectra due to dissembling and re-assembling of the instrument. Good results were obtained in cross-validation, but further studies should be addressed on a larger set of samples to prevent overfitting of the models and assure its robustness in discriminating samples with a larger source of variance.

In any case, the use of specific devices or instrumental configurations also complicates the method intervalidation and fast implementation from official and standard control laboratories. That is the main reason why these methodologies have not been further studied in WP3.

Task 2,4 Selection of critical parameters and methodologies to be optimized in WP3.

The meeting discussion in Lisbon allowed the selection of the methods that not only showed best technical results, but were also more in line with the main objectives of the project (Simple, cost-effective, easy to incorporate and disseminate, use available instrumental, etc...). The FTIR and the analysis of nitrogenous compounds (TMAO amines, creatine/anserine, and taurine) were selected. Since taurine and creatine showed similar results, the method of creatine was finally selected due to its higher analytical simplicity.

A table summary of the trials performed in WP2 and a summary of the obtained results is laid down below:

ANFACO-CECOPESCA	Conclusions		
Task 2.1 General characterization of cod products.			
Nutritional evaluation (n=40)	Different protein : water ratio in cod products.		
Yield (%) (n=34)	Desalting (+15%). Defrosting (-27%). Higher drip in frozen raw materials.		
pH (n=103)	Light salted closer to (pH=6,75) than desalted (6,48). Additives and market storage cause high variance.		
Water Holding capacity (%)(n=113)	Significantly higher in desalted samples from industry trials. More variance in market samples.		
Texture (n=36)	Harder texture in desalted samples. Poor method standardization. Not significant.		
Oxidation (TBARS)(n=40)	No significant differences between cod products.		
Salt levels (n=40)	Industry desalting (1.95%), market desalted (2,4%), market light salted (1.89%). Some market samples up to 4%.		
Minerals (n=40)	Ca /Mg related to salt and water used in processing. Sulfur steady values (179 ± 33 mg/100g). Fe,Mn,Zn, low values no interesting information. Phosphorus and potassium affected by industrial processing (levels drop from raw materials). More intense decay in desalted samples. Market additives may mask this effect.		
Sensory evaluation (11 panelists)	Differences only when cooked and not as raw. Basically beacause of texture and flavour. Panel showed no preference for any of the products.		
Non-protein nitrogen, TMAO and derived compounds and taurine. (n=40)	Important differences in cod products.Loss of compounds during processing. Further studies on TMAO, creatine and taurine in Tasks 2.2 and 2.3		
Task 2.2 and 2.3 Method development and analysis of the samples.			
TMAO and derived compounds. (n=73)	Very good discrimination. Selected for WP3.		
Taurine. (n=72)	Good discrimination. Considering selection to WP3.		
Creatine (n=47)	Good discrimination. Considering selection to WP3.		
T-RFLP. (n=16)	Costly method. Some trends but not very promising. Work not extended in the project.		
Optical microscopy (n=75)	Costly method and variable results. Work not extended in the project.		
Protein profiling. (n=103)	Good results in industry samples but affected by market storage. Work not extended.		
UNITE			
Task 2.1 General characterization of cod products.	Task 2.1 General characterization of cod products.		

Nutritional evaluation (n =15)	No significant differences between cod products.
Water Activity (n =45)	No significant differences between cod products. Only Refreshed samples showed lower aw values.
pH (n =45)	Good Discrimination between HS-desalted and LS.
Salt levels (n=34)	Refreshed and LS samples showed different NaCl% with respect to HS-desalted products.
Drip loss (n=15)	For HS-desalted MAP Chilled a significant difference of weight loss occurred within 48 hours.
Texture (n=15)	Good results affected by packaging and storage condition.
Water Holding capacity (n=15)	Good results affected by packaging and storage condition.
Image Analysis (n=15)	No significant differences between cod products.
Colorimetric Analysis (n=15)	Good Discrimination between HS-desalted and LS.
Sensory analysis (38 for Raw Fish and 33 for Cooked Fish)	Good ability of tasters to identify the different samples, both rae and cooked. Panelists showed preference for HS-desalted products.
Task 2.2 and 2.3. Method development and analysis of the samples.	
Classic microbiological analysis (n=60)	No significant differences between cod products. Generally LS products showed a higher total viable count than HS-desalted.
Microbial Phenotype microarray (n=60)	Preliminary results showed that substrate utilization was affected by packaging and storage condition. Potential for further studies in future projects. Still working on data analysis.
Electronic nose (n=60*)	Good results, especially to identify different packaging and storage condition. Potential application for screening analysis. Considering selection to WP3.
FTIR (n=56*)	Very good discrimination of cod products. This analysis showed 100% of specificity and sensitivity. Selected for WP3.
Colorimetric analysis (n=60*)	Good Discrimination between HS-desalted and LS, considering very simple use and fast analysis. Potential for further studies in future projects.
Water Activity (n = 54*)	Good discrimination between LS and HS-desalted both MAP Chilled and HPP Chilled.
pH (n =54*)	LS HPP Chilled and LS VP Frozen samples showed higher pH with respect to HS-desalted samples.
Image Analysis (n=60*)	Some differences were evidenced, but not always significant.
Texture (n=54*)	Some differences were evidenced amnong HS and LS products, but not always significant.
Drip loss (n=54)	Nosignificant differences.

NOFIMA		
Task 2.2 and 2.3 Method development and analysis of the samples.		
Hyperspectral imaging	Very good discrimination of cod products. Good discrimination of raw materials (fresh/frozen) used in light salting. Potential for further studies in future projects.	
SINTEF		
Task 2.2 and 2.3 Method development and analysis of the samples.		
Machine vision	Very good discrimination of cod products. Potential for further studies in future projects.	

^{*}each sample was analysed in 3 or 4 replicates, depending on the ethod.

Table 1: Summary of results of the analytical methodologies applied.

3. OPTIMIZATION OF THE SELECTED ANALYTICAL METHODS AND VALIDATION.

Further studies of the selected methods, in this case under a purely analytical perpesctive were carried out in WP3. Several changes were applied to the methodologies for their optimization and finally Standard Operating Protocols (*Delivery 3.2*) were developed for a rapid dissemination of the methodologies. Activities carried out in WP3, method characteristics and the derived validation reports are detailed in Delivery 3.1.

The TMAO+TMA method was valued as very easy to disseminate because it is easy to implement, low cost and the fact that could be applied with instrumental which is available at every laboratory of control. Results from validation guaranteeing the analytical quality of the method were presented. Method is accurate, precise, sensitive and specific and linear in the application range in compliance with Decission 657/2002 CE regarding criteria and procedures for the validation of analytical methods. Validation has been made based on spiked samples as no reference material is still available for these compounds. Method robustness has also been confirmed based on the results obtained in this period. The creatine method also allows the analysis of the dipeptides anserine and carnosine in cod samples. Anserine also has some discriminant potential but it has not been considered for discrimination since creatine itself showed enough discriminant potential. Carnosine was not present in cod samples. The method was successfully validated in compliance with Decission 657/2002 CE and SOP was developed.

The previous two methods are based on available references with significant modifications by ANFACO-CECOPESCA. It was clear evident that there is a significant difference in the levels of the target compounds. The threshold considered for discrimination of light salted and desalted samples was determined based on the analysis performed. These thresholds have been calculated by statistical modelling of the moderate set of data; and therefore, would necessary be more thoroughly calculated in a larger set of samples at the moment of intervalidation by means of data sharing among centers performing the analysis.

FT-IR analysis was able to positively discriminats the products on a PLS-DA based data treatment. However the method was validated for sensitivity and specificity, percentage of correct assignation in cross validation and in external validation. The results of the two different validation performed were shown, also demonstrating the robustness of the method. 30 market samples from 8 different producers were analysed according to the method developed. The method demonstrated to present indisputable advantages that rely on the samples preparation, which only requires mincing and homogenizing, and on the cost-effective procedure, very fast and cheap. As the sample could be directly analyzed, it also gives the advantage of simplifying analytical procedures among different laboratories, reducing errors linked, i.e. to sample pre-treatment or to analytes extraction. In addition, also the instrument is not at all expensive, and it is commonly present in analytical laboratories. The method developed appears to be energy-saving and environment-friendly, not requiring expensive and/or dangerous chemical reagents. In contrast, the main disadvantage of the method could be the need for a recalibration when

incorporated to a new laboratory and the fact that it cannot be applied to other Gadus species apart from *G. morhua*.

Three Standard Operative Protocols (SOPs) have been developed and are presently available. Methods have been validated and ready to be easily







incorporated by control laboratories worldwide.

Coordination and Dissemination Activities.

1. COORDINATION

Since the project involved four research centers, and intense activity of coordination was maintained along the project. Constant communication between project leader and project delegates at associated centers, using email, phone and Skype was necessary. The coordination also involved a direct contact with the FHF delegate Lorena Gallart Jornet for the adjustment of the project to sector interests and compliance with FHF requirements.

Project involved the celebration of 3 scientific coordination meetings which were scheduled in parallel to the presentation of results to the Steering Committee. The meetings were also scheduled coinciding with the celebration of parallel events (organized by the Norwegian Seafood Council) realted to the project topic. These meetings were:

- Start meeting (Scientific and Steering Committee): Alesund (Norway), March 25th-26th, 2014.
- Follow-up meeting (Scientific and Steering Committee): Lisbon (Portugal), January 21st-22nd, 2015. In line with the event "O futuro do bacalhau" (http://www.mardanoruega.com/Articles/Portugal/Saiba-mais/Artigos/O-Futuro-do-Bacalhau-voltou-a-Lisboa)
- Closure meeting (Scientific and Steering Committee): Milan (Italy), June 3rd- 4th, 2015. In line with the event "The future for Norwegian seafood in Italy" (http://www.seafood.no/Nyheter-og-media/Konferansekalender/Norwegian-Seafood-Seminar-in-Milan,-Italy)

More detail of the meeting discussion is available in the meetings minutes (*Deliveries 4.1 – 4.5 and Delivery 4.8*).

The objectives were not modificated along the project, and only certain tasks did change to some extent. The project underwent considerable delay in execution, due to the initial sampling problems. This was occasionated because of the end of the season of cod which complicated the delivery of fresh raw materials to centers.

2. DELIVERIES AND DISSEMINATION ACTIVITIES.

Standard Operative Protocols

The submitted deliveries of the project have been:

Delivery 3.2

Delivery 1.1	Sampling plan.
Delivery 2.1	Report of the comparative analytical study of both cod products.
Delivery 2.2	Procedures of the analytical methods.
Delivery 2.3	Report of the analytical results.
Delivery 2.4	Minutes of Technical Committee meeting with method selection for WP3.
Delivery 3.1	Methods procedures and in-house validation results report.

Delivery 4.1	Final coordination plan. Committee Members, dates and locations.
	Steering Committee Opening Meeting Minutes.
Delivery 4.2	Technical Committee Opening Meeting Minutes.
Delivery 4.3	Steering Committee Follow-up Meeting Minutes.
Delivery 4.4	Technical Committee Follow-up Meetings minutes.
Delivery 4.5	Technical Committee Closing meeting.
Delivery 4.6	Final report of the project technical activities including goals and
	objectives to be addressed in a second phase.
Delivery 4.7	Final report on the Dissemination Activities to Authorities and general
	consumers carried out along in the project.
Delivery 4.8	Steering Committee Closing Meeting Minutes.
Delivery 4.9	Technical paper for the appropriate labelling of cod products.

The dissemination activities were limited to the moment because the results of the project have been recently achieved. In view of the potential of the recent results to be used in favour of the Steering Committee interests, dissemination plan will be more intensively executed in the next months. The Steering Committee has supervised in all cases the plan for dissemination to be executed.

The dissemination plan is focused in three different pathways; scientific and institutional and general media:

- Scientific: Results of the project derived from the research activities will be published in peer-reviewed journals of considerable impact within the research community. The methods developed and their fitting to present regulation will be submitted to publications like "Food Control", "Food analytical methods" or "Food Policy". The results with interest not purely analytical will be submitted to other publications like "Food Research iInternational", "Journal of Food Sciene", or "LWT-Food Science and technology". At least 3 publications will be submitted including present project results.
 - Scientific dissemination has also been carried out by presentations to international congresses or Workshops realted to the topic. Appropriate events could be:
 - 5th TAFT (WEFTA) 2015 in Nantes, France. 12th -15th October 2015 (Attending Karsten Heia NOFIMA).

Other events were results of the project have been communicated were:

- Rodrigo González (ANFACO-CECOPESCA): Oral presentation at the FHF Seminar for hvitfisk industrien (Tromsø 22nd October, 2015).
- Carlos Ruiz (ANFABASA-ANFACO-CECOPESCA): Oral presentation at the event "Claves para incentivar el consumo de pescado" (Madrid, 16th September 2015).
- Karsten Heia (NOFIMA): Oral presentation at the Faggruppe konvensjonell meeting (Tromsø,12th February, 2015).
- Rodrigo González (ANFACO-CECOPESCA): Oral presentation at the Workshop "Innovación tecnológica en el complejo mar-industria: Iniciativas de interés empresarial" (Vigo, 29thOctober, 2015).

Institutional: Direct contact to National and international Authorities has been carried out.

The methods (SOP) are now public. Direct contact to Spanish official control laboratories has already been made; in particular with the "Centro de Investigación y Control de la Calidad (AECOSAN- Spanish Ministry of Consume and Health Affairs)" which stands for the prevention of unfair comercial practices, and "Laboratorio Arbitral Agroalimentario (Ministry of Agriculture, Fisheries and Environmental affairs)" which deals the food safety control and quality of foodstuff. Dialogue is open for the inclusion of project related activities like the organization of Collaborative validation Studies within their future programs of activities.

As part of the dissemination activities, a technical document has already been prepared regarding legal

concepts affecting the present situation of the cod products marketing, and a proposal for an appropriate definition of cod product cathegories. The document (Additional Delivery 4.9), prepared in cooperation by ANFACO-CECOPESCA and ANFABASA has already been submitted to Spanish Authorities in order to start discussion for the implementation of internal regulation.

ANFABASA and ASSOITICA have maintained contact to their respective national Authorities and transmitted the need of an appropriate differentiation of cod products trade. Contact will be strengthened in the next dates with the participation of ANFACO-CECOPESCA and ASSOITTICA delegates in sectorial meetings with the administration. ANFACO-CECOPESCA has had a meeting at 13th October, 2015 with representatives of the Secretary of maritime Affairs and delegates of the Spanish Consumption Institution (AECOSAN)



to discuss the development of new regulation regarding cod products market in Spain. Meeting resulted

in the plan for methods intervalidation trials with official laboratories in Spain as well as other potential partner laboratories. It has been agreement on the necessity of the development of a technical quality regulation regarding cod products in Spain. The Spanish Ministry of Agriculture and Fisheries accepted the responsibility of leading this task.

After national dissemination the topic will be issued within the discussion forums of the AIPCE (EU Fish Processors and Traders Association), in order to sensitize the European



Authorities of the necessity of a better cod product market environment in the EU. Contact to Portuguese and Italian Associations towards the development of similar regulations will also be maintained.

General media:

To the moment only local dissemination in the general media was carried out in Spanish industrial publications like "ruta pesquera", "industrias pesqueras", or "Industria Conservera"

For the dissemination of the project results and objectives to the general consumer; and in special to increase of knowledge of the general consumer of the quality particularities of light salted and desalted cod products, it has been planned to involucrate the Norwegian Seafood Council, as steering committee participants (Italy and Spain). In this sense discussion has been prepared for a common dissemination strategy to consumers in Spain, Italy and Portugal. As part of this strategy ANFACO-CECOPESCA has also discussed with the Portuguese Association of Cod Producers (AIB) their interest in participating in this program.

Project Impact.

As mentioned in the dissemination section above, the project has succeed in raising awareness of authorities towards the modification of the current legal background concerning the putting on the market of new easy-to-use cod products. The potential development of new regulation will definitely protect traditional cod producers from unfair competence of unmatured cod products. This will turn the present market situation to a more balanced and complying with regulation retail environment. A plan for collaborative analytical studies between official control laboratories has been laid down and will be executed in the next months. ANFACO-CECOPESCA is actually preparing the tasks and finding finnanciation to support these activities. Once the methods have been validated the Spanish Ministry of Agriculture will take on the development of specific technical regulation regarding the commercialization of Cod products in Spain.

Complementary an impulse has been given to the scientific background regarding cod products characteristics and behavior during processing and trade. Several techniques have been applied to cod products which have brought very valuable information, which might enable future development of more simple and cheap tailored methodologies.

Three Standard Operative Protocols have been developed and validated and are now available for control laboratories. As soon as new regulation regarding cod product categorization, the industry and Authorities can take advantage of these tools for the market control enforcement.

The transnational context of this project would facilitate homogenization of market criteria and global trade of cod products. This will definitely increase the chances for the success of this project concerning the implementation of new measures in the commercialization of cod products by European Authorities. The strengthening of transnational collaboration among laboratories, research centers, producers and trade companies and respective national associations is also a matter to be underlined.