ANFACO-CECOPESCA

PART 2: DESALTING TRIALS. NUTRITIONAL COMPOSITION AND SHELF LIFE OF PACKED DESALTED CLIPFISH.

FHF PROJECT 901307

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TABLA DE CONTENIDO

1. BACKGROUND.

It was considered to extend project tasks to determine the effect of desalting in the clipfish initial results, and define levels in the product as it is consumed for the parameters that are needed for nutritional labelling, and comparing these values to previous references. Moreover a small and simple shelf life study was carried out based on microbiological values. For this purpose a preliminary search on the scientific literature has been carried out and also presented in this report.

2. SUMMARY STATE OF THE ART ON THE MICROBIOLOGICAL STATUS OF DESALTED CLIPFISH.

Heavily salted cod (*Gadus morhua*) is an important ingredient in Mediterranean cuisine. Microbial load can be reduced with high salt content, which means a low water activity. Salted cod fillets are a very stable product from a microbiological point of view. However, the main disadvantage of salted cod is that it has to be desalted for approximately 48 h before being cooked, a fact that goes against the present trends of reducing cooking times. From this point of view, the development of a new ready-to-use desalted cod product could be interesting for industry and consumers. The fast spoilage of this product, mainly due to microbial growth, limits its shelf life, because the salt level of the desalted cod (1.5% in water phase) (Fernández-Segovia, 2003) is not low enough to preserve it for more than 4 or 5 d (Fernández- Segovia, 2000). To prolong its shelf life without changing the intrinsic features of the recently desalted cod, some kind of preservation treatment needs to be applied. The basis of hurdle technology is the combination of traditional and innovative preservation techniques in small doses with the aim of establishing a series of factors that interact cumulatively or synergistically to control the microbial population in food. In this way, the same inhibitory effect is reached with several lows intensity treatments, inhibiting or delaying the multiplication of the microorganisms. This fact contributes to a better preservation of the sensory properties after the treatment. The factors that are frequently combined are: heat, pH and water activity (Aw) reduction, chemical additives, vacuum or modified atmosphere packaging, electric pulses, high pressures, etc. The effectiveness of the different preservation methods can be studied through different parameters such as microbial growth, sensory analysis, changes in lipids or in proteins, or volatile compounds.

MICROBIAL SPOILAGE OF DESALTED COD

A few studies have been performed in relation to the spoilage of desalted cod by microorganisms. In cod fillets pile salted, dried and desalted, after 6 days of chilled storage, *Psychrobacter sp* represented 95–100 % of the total count in all samples (around 10^7 cfu/g), and a musty odor had developed. This situation was independent on freshness of raw material employed (0, 7 and 12 days stored in ice), although samples elaborated with the freshest raw material showed higher strain diversity, suggesting that use of very fresh raw material for salt-curing could lead to a more unpredictable spoilage pattern of desalted fish, as local salt-tolerant bacteria with spoilage potential are likely not to be diminished during the salting and drying step (Barat, 2006). Other report shows that *Psychrobacter* sp have been shown to be major spoilage bacteria in desalted cod, causing a musty off-odor being sensory detectable at approximately 10^6–10^7 cfu/g of fish (Bjorkevoll, 2003). These bacteria are predominant in most fresh cod, but are able to survive as non-growing cells during salting and drying, and recover during desalting (Bjorkevoll, 2003). Based on the obtained results, it could be concluded that the

freshness of the raw material have no significant effect on the storage stability of desalted cod products. Stored at 4 °C, salt-cured and dried salt-cured cod rehydrated using sterile water, were rejected due to musty odor after 7-10 days storage. 90 % of the total viable count (TVC) in all products studied, was identified as belonging to the genus *Psychrobactera* Gram-negative, oxidase- and catalase-positive, non-pigmented, halotolerant, psychrotolerant, facultative aerobe and non-motile bacterium. The morphology of the bacterium resembles coccobacilli and the cells occur most often in pairs. The bacterium was able to hydrolyze lipids, but not proteins. It did not produce H(2)S or TMA and the spoilage in rehydrated salt-cured and dried salt-cured cod is therefore different from what is observed in fresh cod. The bacterium, which is present in cod skin mucus, survived NaCl concentrations up to 25 % (w/v), stating its ability to survive during the salt-curing process. The dominating bacterium in rehydrated salt-cured and dried salt-cured cod seems to mainly originate from the fresh fish itself and not from contamination during processing. Similar results in plate count were obtained when incubating plates at 4, 12 or 20 °C and lower results were obtained when cultivating at 37 °C (*Bjørkevoll, 2003)*.
In the case of chilled raw cod packed in vacuum or in modified atmosphere (MA), *Photobacterium phosphoreum* could be the microorganism responsible of spoilage *(Dalgaard , 1993)*. For raw cod fillets, changes in the gas mixture induced changes in the bacterial community composition along storage. *Pseudomonas sp*. dominated in CO_2:O_2 packaged cod during storage, whereas *Photobacterium spp*., *S. putrefaciens* and *Pseudomonas spp*. dominated in the air and CO_2:N_2 packages. Shelf life for MA-packaged farmed cod, was at least 11 days at 0 °C. There was a significant difference between storage in air, and CO2:N2 and CO2:O2, where oxygen was suggested as the better gas mixture. (Hovda, 2007)

FOOD SAFETY CONCERN

An important bacterium in chilled products is *Listeria monocytogenes*. This microbe is not a spoilage microorganism, but a food safety concern. It has been shown that *L. monocytogenes* grows well in rehydrated salt-cured cod when it is introduced to the rehydration water and the bacteria may reach high levels within a few days. Long term exposure to very high salt concentrations does not eliminate *Listeria* spp., and hence *Listeria* being present in the fish prior to salt-curing can recover and grow in rehydrated salt-cured cod during chilled storage. Although rehydrated salt-cured cod is mostly used in dishes that are heat treated before consumption, the food safety risk of L. monocytogenes still has to be considered due to the risk of cross contamination and undercooking e.g. by microwave heating. Furthermore, raw rehydrated salt-cured cod is also used in some recipes, in Spain and Portugal, which are not heated prior to eating. The storage time to reach the legislated level of 100 cfu/g for ready-to eat products depends on several factors such as storage temperature and initial load, but low levels of *L. monocytogenes* in salt-cured cod may grow to infective levels for sensitive consumers before the rehydrated product is considered sensory unacceptable (Lorentzen 2010).
An important subject to be bear in mind is the risk of growth of clostridium botulinum type E in vacuum packed or MAP products. The use of salt concentration around 5% in the product contribute to the inhibition of C. botulinum growth (Graham, 1996) this concentration can be achieved by using a 7% NaCl solution in the last steps of rehydration (Fernández-Segovia, 2007).

ENHANCEMENT OF DESALTED COD SHELF LIFE

Different strategies and additives have been tested to increase rehydrated salted cod shelf life:

- **Oxygen peroxide** can delay cod spoilage, but it was found to be unsuitable since it could involve unpleasant changes in the sensory characteristics, abnormal coloring of the skin, turning from grayish to brown. In addition, unpleasant changes in the appearance and texture of muscle were observed (Fernández-Segovia, 2007).

- **Adding NaCl** to the desalting water: this process leads to higher NaCl concentration in the rehydrated product which in turns means bacterial growth inhibition. Salted cod immersed 24 hours in distilled water followed by 24 hours in 3.5 % NaCl or 7 % NaCl desalting solution increased the salt content from 1.5 % in water phase of desalted cod without salt to 3.13 % and 5.78 % respectively. This salt increase induced *Pseudomonas* growth inhibition and presented a synergistic effect with the presence of additives (potassium sorbate and citric acid) on mesophilic and psychrophilic bacterial growth (Fernández-Segovia, 2003).

- **Use of additives**: the combination of citric acid with potassium sorbate. Potassium sorbate markedly reduced growth of *Photobacterium phosphoreum*, identified as the specific spoilage organism in modified-atmosphere-packed cod fillets (Fernández-Segovia, 2007). The use of NaCl (7 % in solution), citric acid (CA) (0.2 % in solution) and potassium sorbate (KS) (0.45 % in solution) enhanced shelf life up to 42 days compared to 14 days in controls without additives. It has been described that the use of these additives did not alter sensory analysis (Fernández-Segovia, 2007)

- **Water blanching**, which leads to a precooked product. The appearance of the final product using water blanching is different from the standard desalted cod, but after cooking there are no differences among both products. Water blanching induces a drop in the initial microbial counts and enhances the shelf life of the product (Fernández-Segovia, 2007).

- **MAP and vacuum packaging**, both reduced microbial growth when compared to air packaging, although did not prevent microbial spoilage. In this sense, CO_2 inhibits psychrophilic microorganisms growth, such as *Pseudomonas* (Fernández-Segovia, 2007). Anaerobic packaging did inhibit *Pseudomonas* and *Shewanella putrefaciens* in raw salmon filets (Sivertsvik , 2006) , and both, vacuum and MAP, improve shelf life in sardines (Özogul, 2004). Vacuum and MAP also reduced molds and yeasts growth in desalted cod. The use of vacuum and MAP did not alter sensory analysis. Similar results were obtained with vacuum and MAP, so vacuum can be chosen since is a cheaper solution (Fernández-Segovia, 2007).

- **The combination of additives** (citric acid and potassium sorbate) treatment **with modified atmosphere packaging** has been proposed as the best choice to develop a chilled product of ready-to-use desalted cod with a shelf life longer than 1 month (Fernández-Segovia, 2006; Magnússon, 2006).

Table 1 below, summarizes the shelf life of rehydrated salted cod depending on the procedure used and main rejection criteria, found in the bibliography.

Product	**Shelf life (days)**	**Rejection criteria**	**reference**	**Bacteria involved**
Rehydrated Salt-cured cod in plastic bags	7-10	Musty odour	Bjørkevoll 2003	genus *Psychrobactera*
Rehydrated dried salt-cured cod in plastic bags	7-10	Musty odour	Bjørkevoll 2003	genus *Psychrobactera*
Raw cod in MAP	13-20. Máximum shelf life obtained with 48%CO2	Off odour	Dalgaard 1993	
Rehydrated salted cod chilled air packaging	14	Mesophilic counts >10^6cfu/g	Fernández-Segovia 2007	
Rehydrated salted cod chilled vacuum or MAP	>42	Mesophilic counts did not reach 10^6cfu/g	Fernández-Segovia 2007	
Rehydrated salted cod chilled air packaging	14	Psychrophilic counts >10^6cfu/g	Fernández-Segovia 2007	
Rehydrated salted cod chilled vacuum	>42	Psychrophilic counts did not reach 10^6cfu/g	Fernández-Segovia 2007	
Rehydrated salted cod chilled vacuum	42	Psychrophilic counts >10^6cfu/g	Fernández-Segovia 2007	
Blanched Rehydrated salted cod chilled air packaging	28	Mesofilic counts >10^6cfu/g	Fernández-Segovia 2007	
Blanched Rehydrated salted cod chilled vacuum or MAP	>42	Mesofilic counts did not reach 10^6cfu/g	Fernández-Segovia 2007	
Rehydrated salted cod chilled air packaging	6-10	Sensory attributes and microbial counts	Magnússon 2006	
Rehydrated salted cod chilled MAP packaging	18-24	Sensory attributes and microbial counts	Magnússon 2006	
Rehydrated salted cod chilled MAP packaging and citric acid	24-28	Sensory attributes and microbial counts	Magnússon 2006	
Rehydrated salted cod chilled MAP packaging, citric acid and potassium sorbate	>33	Sensory attributes and microbial counts	Magnússon 2006	

Table 1: Shelf life in rehydrated clipfish.

3. PROJECT DESIGN, DESALTING AND PACKING AT THE PILOT PLANT.

The basic design of the project is detailed in Figure 1 below.

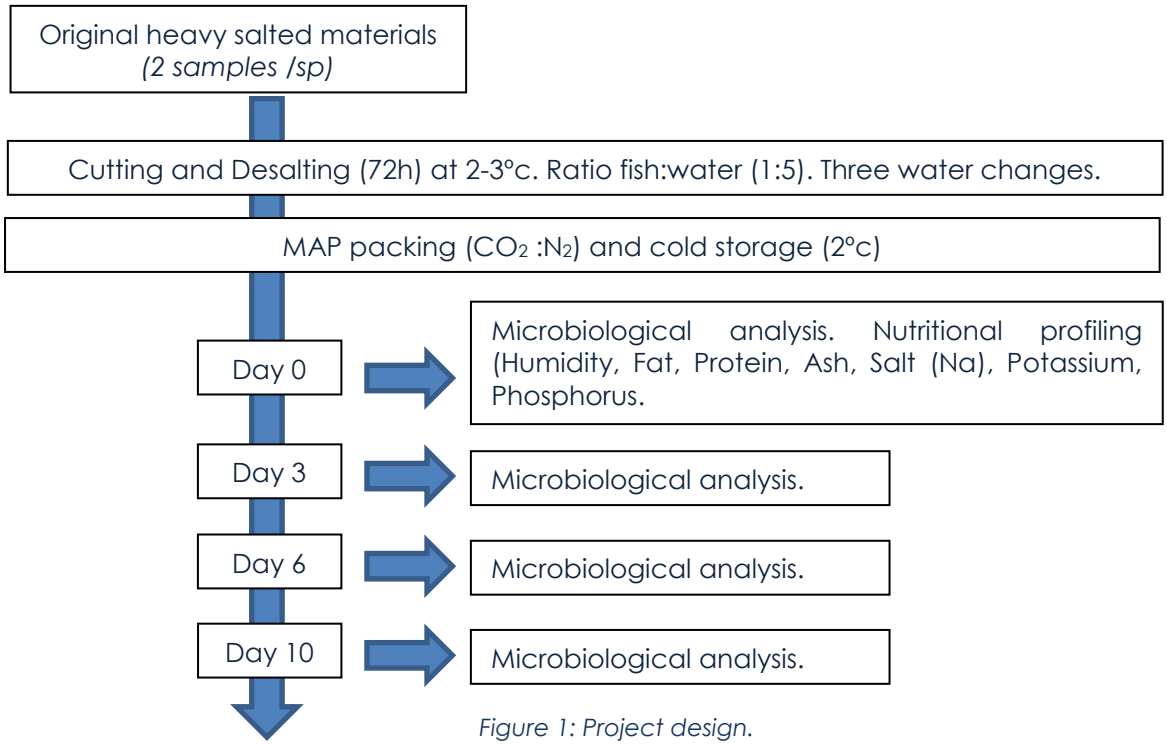

Figure 1: Project design.

Due to low unexpected microbiological loads at the proposed conclusion of the testing, the days of the shelf-life testing were partially extended.

- **Product handling and desalting.**

Each sample corresponds to one fish. Two samples of each one of the four species were selected; dry-salted ling, tusk and saithe and wet-salted cod. Head-end edges, wings and tails were discarded and fish were cut in relatively homogeneous sections of the loins using an industrial saw as shown in Figure 2 below. The resulted loins of each one of the eight fish were included in separately in 16 L. plastic containers. One of the loins was included in a plastic bag, then codified and sent to laboratory for analysis. The rest of the fish loins were immediately prepared for desalting. The surface salt was manually washed away by placing the loin under tap water for approximately ten seconds.

Figure 2: Left: Cutting of the clipfish loins. Right: Surface salt washing.

A 72 hours desalting time was selected with tap water at 2ºc and a fish:water ratio of 1:5. Fish sample and water were weighed in the pilot plant weigh scale, as shown in figure 3 below. Three water changes were made at 8h, 24h and 48h. No stirring was included in the containers and the samples were placed at the bottom of the container. Containers with samples were preserved in a cold chamber at 2ºc all along the desalting time.

Figure 3: Left: Weighing of fish and water. Right: Cold storage during desalting.

After 72h and three water changes the product was carefully displayed on a food tray with a soaking pad inside a plastic bag, and packed under MAP using a mixture of CO_2 and N_2 (50:50) using an ORVED 18N instrument coupled to gas supply.

One of the samples was codified and submitted to laboratory for the analysis (day 0), the rest of the replicates were stored in a cold chamber a 2ºc during the shelf-life testing. As it would be detailed below desalted fish was submitted for analysis after 3, 6, 10, 17 days. Three extra samples were additionally sensory evaluated after 33 days of cold storage.

Figure 4: Packed samples for the shelf-life testing.

4. ANALYTICAL METHODS.

Analysis of the **moisture, fat, and ashes** content was developed following under gravimetric procedures meanwhile **protein** was obtained after sample mineralization, Kjeldahl distillation and acid / base volumetric titration. The energy and carbohydrates content were calculated. All these methods are certified by the Spanish Accreditation body (ENAC).

The technique used for mineral analysis was ICP-OES (Varian Vista MPX) with a preliminar acid mineralization of the samples in pressurized vessels in a microwave oven. Analyzed minerals were Na, K, and P. The levels of Na will be used as the key to salt (NaCl) content making use of the Na:Cl factor.

Microbiological analysis of enumeration of aerobic mesophilic microorganisms, total enterobacteria, *Staphylococcus positive coagulase*, Coliforms and *E.coli* were carried out by automated MPN (TEMPO) whereas detection of *Salmonella* and *Listeria monocytogenes* were performed by ELFA (VIDAS). Enumeration of aerobic mesophilic microorganisms and of anaerobic sulphite-reducers at 37º & 50º C were performed by plate count based on ISO 4833-1:2013 and ISO 15213:2003 respectively. All microbiological methods applied are under 17.025 accreditation of ENAC.

5. SHELF LIFE / MICROBIOLOGICAL RESULTS.

The shelf life test turned out short because the quality of fish, under the selected conditions, was preserved longer than expected. The selection of days of testing (0, 3, 6, 10, 17) resulted in not the best approach to evaluate the deterioration of the test materials. The tests carried out at day 17 only consisted in one replicate for each species.

In general, the full set of microbiological analysis performed can be considered as satisfactory, since poor microbial loads were found.

Absence of *Salmonella* and *Listeria Monocytogenes* in 25 g product was obtained from testing at the start of the shelf-life test (day 0) and at the end (day 17).

Values below the quantification limit of the methods were also obtained along the whole shelf-life test for all samples in the microbiological parameters summarized below:

- Count of anaerobic sulphite-reducers at 37ºc. ---------- <10 cfu/g at days 0, 3, 6, 17.
- Count of anaerobic sulphite-reducers at 50ºc. ---------- <10 cfu/g at days 0, 3, 6, 17.
- Staphylococcus positive coagulase. ---------- <10 cfu/g at days 0, 3, 6, 17.
- Count of total coliforms. ---------- <10 cfu/g at days 0, 3, 6, 17.
- Count of E. coli. ---------- <10 cfu/g at days 0, 3, 6, 17.
- Count of total enterobacteria ---------- <10 cfu/g at days 0, 3, 6, 17.

There are some exceptions to general results mentioned above, in the shape of incidental and not significant values above the quantification limit found in some of the replicates. A summary table with the whole set of results can be seen in Annex I.

A maximum level of coliforms was found in the only replicate of saithe at day 17, but the 4,3 $x10^3$ cfu/g. Even though E. coli counts in the same sample remain at <10 cfu/g, and despite the lack of legal microbiological tolerances, this value should be considered. A maximum limit in frozen seafood of $1x10^3$ cfu/g was laid down in the derogated Spanish regulation. Nevertheless, this criterion for a frozen product would be too strict to be applied to a chilled sample during its shelf-life.

The only microbiological parameter with a significant change during the test was the enumeration of aerobic mesophilic microorganisms. Nevertheless, the variation has been lower than expected for the scheduled dates of analysis. Variation among replicates has been important but within the low range of the obtained values.
In Figure 5 below, we can see the results from the shelf-life testing for both products. Since the variation between replicates has been important, the maximum values and not the mean values of the replicates have been displayed in the diagram. It can be seen that there has not been an important growth of microbiota until day 10. The end values at day 17 are not high when, in absence of maximum

legal tolerances, are compared to the derogated criteria from a previous Spanish reference[4] in frozen seafood; which determined a tolerance of 1x10^6 cfu/g of aerobic mesophilic microorganisms.

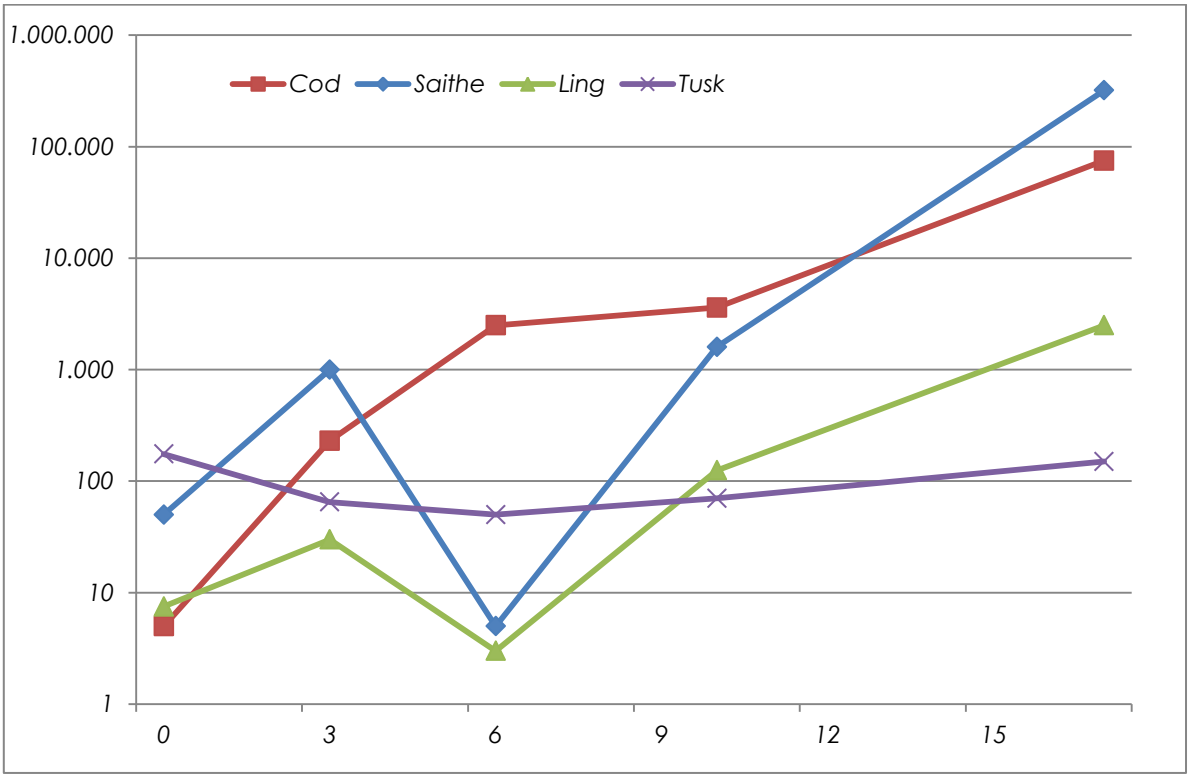

Figure5: Aerobic mesophilic microorganisms at the Shelf-life trial of the desalted whitefish samples.

The lack of significant microbiological loads in raw materials, the hygienic and appropriate production conditions, complemented by a fast processing where the temperatures of the materials have been maintained low, appropriate modified atmosphere packing (MAP) and finally, a low and steady temperature (2ºc) , have been the key factors to inhibit the spoilage of the desalted whitefish loins.

Figure 6: Detail of the samples at day 17.

In any case the shelf-life test has been unsatisfactory, because at day 17, the fish quality was still good and the endpoint, and therefore the expiring date could not be inferred. Considering these results, and since three packed samples (ling, cod, saithe) were still available, they were preserved inside the cold chamber until day 33.

The sensory evaluation at day 33 was still satisfactory in all samples. Images of the products before and after being cooked can be seen below, and the results from the small sensory test are shown in Table 3. Sections of the samples were cooked inside a plastic bag in a microwave oven at 900 W for 1min 40 s.

Figure 7: Desalted saithe at day 33 (left: Uncooked / right: Cooked)

Figure 8: Desalted cod at day 33 (left: Uncooked / right: Cooked)

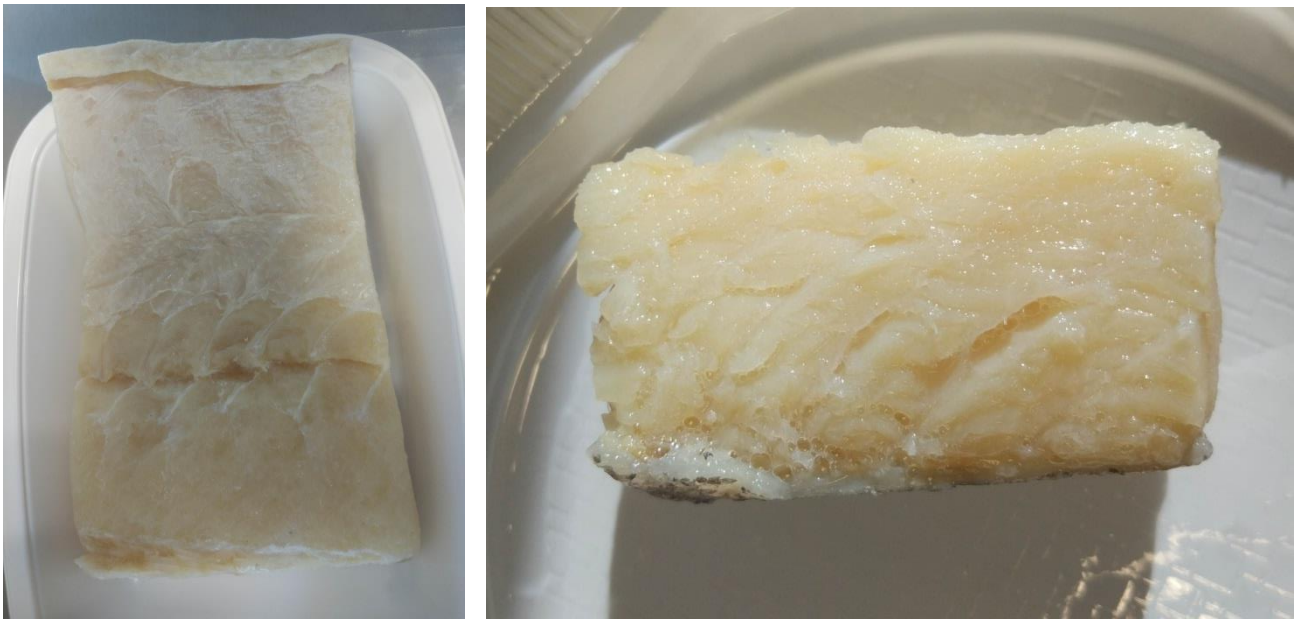

Figura 9: Desalted ling at day 33 (left: Uncooked / right: Cooked)

		Cod	Saithe	Ling
CHILLED	**General appearance**	Bright. No visible deffects.	Dry aspect. Heterogeneus and yellowish colour.	Very nice. Homogeneus.
	Color	White and shiny. Characteristic.	Yellowish, with surface lines of orange. Not appropriate.	Light yellow, but typical of matured products.
	Odour	Slight fishy odour but not unpleasant.	Slight fishy aroma.	Intense matured aroma. Characteristic.
	Texture	Keeps structure when pressure is applied. No gaping.	Dry and solid.	Typical. Solid, elastic and juicy. No gaping.
AFTER COOKING	**General appearance**	Typical white. Laminated.	Does not seem dry and a more whity colour.	A bit yellowish but bright. Pleasant.
	Texture	Soft but fibrous when being chewed	Dry but not affecting chewiness.	Very nice. Juicy and firm.
	Taste	Typical matured cod. Tasty. Appropriate salt level.	Salty, but not unpleasant.	Tasty. Typical.
	Mean evaluation (0-3 scale)	**2,6**	**2,1**	**2,7**

Table 2: Sensory evaluation and mean scores of desalted samples at day 33.

6. NUTRITIONAL EVALUATION OF DESALTED SAMPLES.

The nutritional values of the desalted products are presented in figure 9 below, and the complete results dataset is shown in Annex II. The humidity content of the desalted samples was around 75% in saithe, ling and tusk meanwhile cod showed higher values (81%). It should be reminded that cod was the only species that was desalted from wet salted material (*mean humidity of 57,8%)* instead of 7/8 dry-salted materials (*mean humidity of tusk: 51,5%, ling:51,2, saithe: 53,6%*). There is correlation between the initial humidity and final humidity in the different materials.

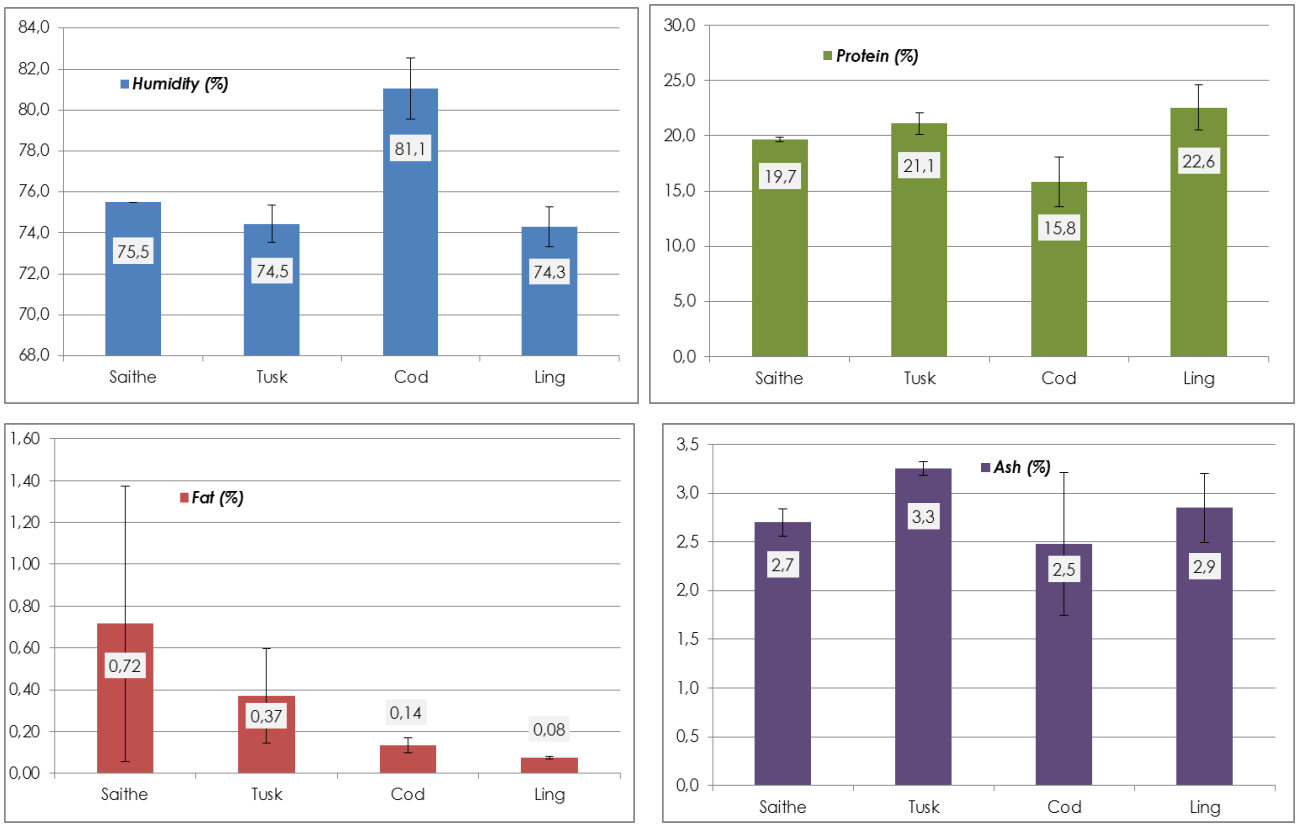

Figure 9: Nutritional values in desalted samples.

The protein level is inversely proportional to humidity and the level of fat is very low in all samples with the exception of saithe, where one of the replicates reached a 1,2 % fat content. The fat level had also been variable to the same extent within fat samples of dry-salted saithe, so the desalting process seems not to affect significantly to initial fat content of fish.

Since it is a low fat and carbohydrates product, the energy supported by each of the desalted materials *(table 3)* is highly dependent of the protein.

	Energy (Kj / 100 g)	***Energy (Kcal / 100 g)***
Saithe	368	87
Tusk	385,5	91
Cod	283,5	66,8
Ling	395	93

Table 3: Energy values in desalted whitefish products.

The ash level present greatly depends on the salt content. The desalting process was satisfactory according to the 2-3% salt level in desalted fish as shown in Fig. 10.

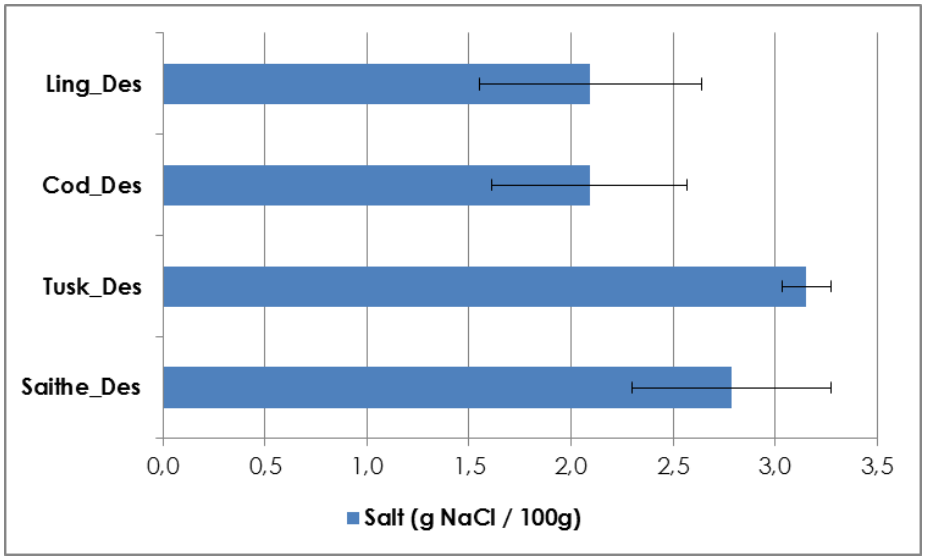

Figure 10: Salt content after desalting.

A proposal for labelling based on the obtained data is presented in Fig.11 below. The fat values in the samples may vary considerably between individuals up to around 1,5%, and therefore affecting the energy value supported by the fish product. Besides, the final protein value in cod is dependent of the final humidity content which might be different depending on whether wet salted cod, dried salted cod or extra-dried salted cod is used for desalting.

Nutritional information	Saithe	
	Per 100g	
Energy	368 kJ	87 kcal
Fat*	0,7*	g
of which		
saturates	0,2*	g
monounsatured	0,3*	g
poyunsaturated	0,2*	g
Carbohydrates	<0,5	g
of which		
sugars	<0,5	g
Protein	19,7	g
Salt	2,8	g

Nutritional information	Tusk	
	Per 100g	
Energy	386 kJ	91 kcal
Fat*	<0,5	g
of which		
saturates	<0,1	g
monounsatured	<0,1	g
poyunsaturated	<0,1	g
Carbohydrates	<0,5	g
of which		
sugars	<0,5	g
Protein	21,1	g
Salt	3,2	g

Nutritional information	Per 100g	Cod
Energy	284 kJ	67 kcal
Fat*	<0,5	g
of which		
saturates	<0,1	g
monounsatured	<0,1	g
poyunsaturated	<0,1	g
Carbohydrates	<0,5	g
of which		
sugars	<0,5	g
Protein	15,8	g
Salt	2,1	g

Nutritional information	Per 100g	Ling
Energy	395 kJ	93 kcal
Fat*	<0,5	g
of which		
saturates	<0,1	g
monounsatured	<0,1	g
poyunsaturated	<0,1	g
Carbohydrates	<0,5	g
of which		
sugars	<0,5	g
Protein	22,6	g
Salt	2,1	g

Figure 11: Proposed nutritional labels for the desalted products.

Desalted cod results could be compared to a previously analyzed sample set from the SALDICOD project *(FHF n: 900985)* where 10 wet salted cod samples (5 from fresh raw materials & 5 from frozen raw materials) underwent in-house desalting with the same procedure. 6 additional products from the Spanish cod market were also analyzed.

The mean ± SD results of this sample set were highly coinciding to the ones of present project. Mean humidity was 81,4 ± 1,3 %, fat levels were low and variable (0,11 ± 0,09 %), protein was 15,8 ±1,2 % and final salt after having carried out the same desalting procedure was 1,9 ± 0,6 %, almost the same values as the mean 2,1 ± 0.5 % level that cod samples resulted in the latter trials.

As it can be seen in Fig. 12, phosphorus and potassium levels undergo a significant drop during desalting, very intense for potassium and moderate to phosphorus. In both cases mineral contents remain below the 15% of the dietary reference intake threshold that would allow nutritional or health claims in labelling.

Similar phosphorus and potassium end levels had been obtained after desalting of wet salted cod samples during the previous SALDICOD trials. In that case the mean phosphorus and potassium values were respectively 67 ± 28 mg/100g, and16 ±13 mg/100g.

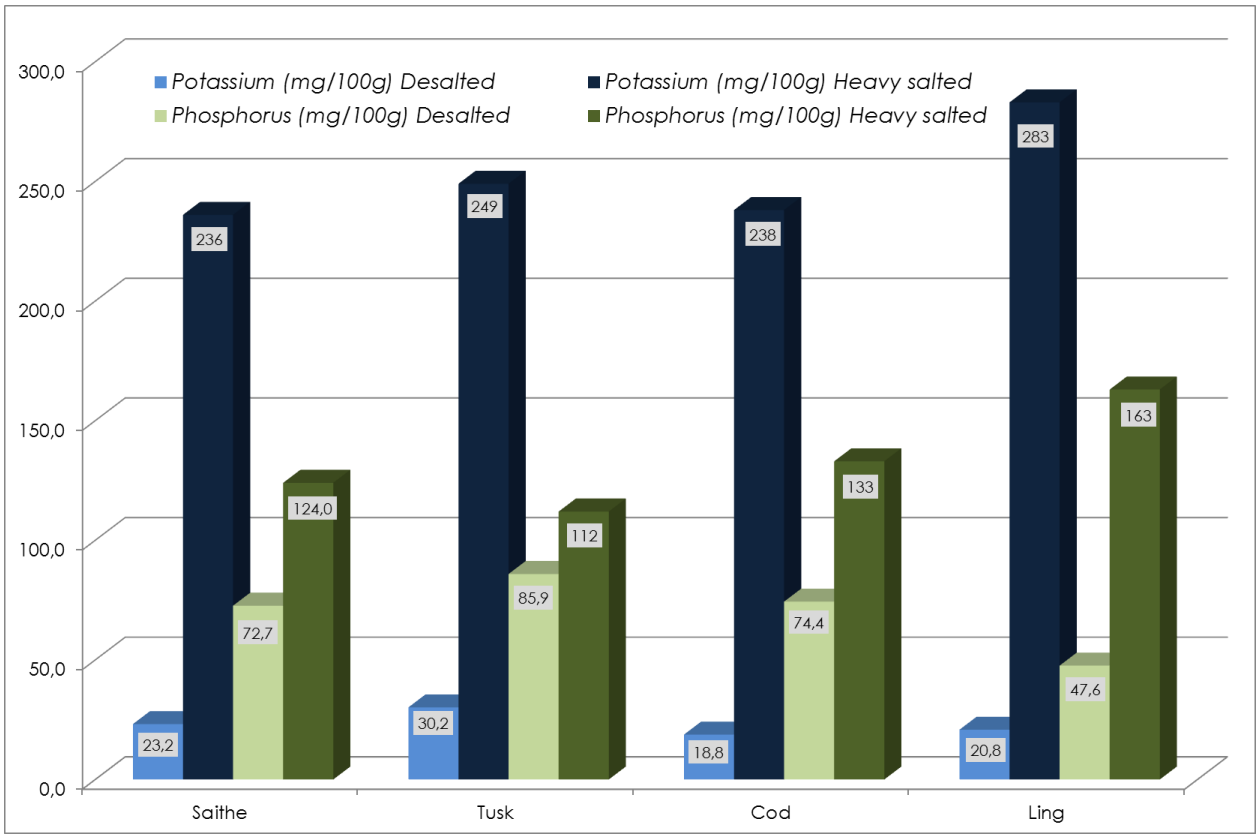

Figure 12: Phosphorus and potassium levels after and before desalting.

7. DISCUSSION AND CONCLUSSIONS.

Shelf life of desalted cod in MAP (CO_2:50% / N_2:50%) has been longer than previously expected. Consulted references that used MAP had extended shelf life up to 24 days with no additives, but when combined to citric acid and sorbate it was at least of 33 days (*Magnusson,2006)*. Other references using MAP in desalted cod without additives reached 10^6 cfu/g of mesophilic microorganisms at day 21. These results were greatly reduced when combined to blanching of fish products or using additives (sorbate & citric acid) extending shelf life to at least 35 days (*Fernández-Segovia, I., 2007)*[1].

To the contrary to what was expected because of the non-use of additives, the microbiological loads were very low at the end-point of the study (day 17), and therefore, the shelf-life test of the present project remained short in time. In order to deal to this eventuality, a sensory test was additionally carried out at day 33, but product quality despite being affected was still satisfactory.

The quality of raw materials, the steady and lower temperature (2ºc) during desalting and preservation compared to references, as well as the appropriate mixture of gases selected, may have been key factors in the obtained results. It is possible that under real trade conditions, temperature fluctuations

may have led to poorer results. Nevertheless, MAP under the conditions proposed has been a very good method to preserve the quality of desalted fish.

The nutritional value of the desalted products reveals that the major components in heavy salted products are only affected by the mass balance between salt and water. The final salt content under the selected procedure was within the expected range 2-3%. Fat level is very low and does not undergo a significant change, depending on the natural variation of fish individuals, especially for saithe in the samples studied in this project. Since carbohydrates remain insignificant, the protein and humidity in fish become inversely correlated.

It has been detected a significant difference between final humidity content in cod (81,1%) and the rest of the species (75% approx.) . The reason for this could be in the type of raw material used for desalting that was wet salted (57,8% humidity) for cod, and 7/8 dry salted ling, saithe and tusk (around 52% humidity). Both materials increase around 23 percentage points, but intense drying applied to salted materials may have caused some irreversible change in the fish muscle that affects the ability of the product to be rehydrated to the same extent as the case of wet salted materials.

As it has been previously reported, not only sodium is affected by the osmotic balance but also other minerals *(Van Nguyen,2012) (FHF project 900592)*. Phosphorus and especially potassium levels fall down during desalting as a result of a transfer from muscle tissue to rehydrating water. Therefore, the use of phosphate additives in salted cod would not necessary entail an increase in the final phosphorus levels after desalting. It should be also noted that the use of phosphate additives in desalting water is not permitted by the EU legislation.

ANNEX I: MICROBIOLOGICAL RESULTS.

		Count of total aerobic microorganisms (cfu/g)	Count of total entherobacteria (ufc/g)	Count of Staphylococcus positive coagulase (cfu/g)	Count of anaerobic sulphite-reducers at 37°c (cfu/g)	Count of anaerobic sulphite-reducers at 50°c (cfu/g)	Count of total coliforms (cfu/g)	Count of Escherichia coli (ufc/g)	Detection of Salmonella (Inmunofluorescence)	Detection of Listeria monocytogenes (Inmunofluorescence)
Day 0	Saithe_Des1_0	<40	<10	<10	<10	<10	<10	<10	Absence	Absence
	Saithe_Des2_0	50	<10	<10	<10	<10	<10	<10	Absence	Absence
	Tusk_Des1_0	340	10	<10	<10	<10	<10	<10	Absence	Absence
	Tusk_Des2_0	<40	<10	<10	<10	<10	<10	<10	Absence	Absence
	Cod_Des1_0	<40	<10	<10	<10	<10	<10	<10	Absence	Absence
	Cod_Des2_0	<10	<10	<10	<10	<10	<10	<10	Absence	Absence
	Ling_Des1_0	<40	<10	<10	<10	<10	<10	<10	Absence	Absence
	Ling_Des2_0	<10	<10	<10	<10	<10	<10	<10	Absence	Absence
Day 3	Saithe_Des1_3	<40	<10	<10	<10	<10	<10	<10		
	Saithe_Des2_3	1000	<10	<10	<10	<10	<10	<10		
	Tusk_Des1_3	90	<10	<10	<10	<10	<10	<10		
	Tusk_Des2_3	40	<10	<10	<10	<10	<10	<10		
	Cod_Des1_3	220	<10	<10	<10	<10	<10	<10		
	Cod_Des2_3	230	<10	<10	<10	<10	<10	<10		
	Ling_Des1_3	<40	<10	<10	<10	<10	<10	<10		
	Ling_Des2_3	50	21	<10	<10	<10	21	<10		
Day 6	Saithe_Des1_6	<40	<10	<10	<10	<10	<10	<10		
	Saithe_Des2_6	<10	<10	<10	<10	<10	<10	<10		
	Tusk_Des1_6	90	<10	<10	<10	<10	10	<10		
	Tusk_Des2_6	<40	<10	<10	<10	<10	<10	<10		
	Cod_Des1_6	2500	<10	<10	<10	<10	<10	<10		
	Cod_Des2_6	230	<10	<10	<10	<10	<10	<10		
	Ling_Des1_6	<10	<10	<10	<10	<10	<10	<10		
	Ling_Des2_6	<10	<10	<10	<10	<10	<10	<10		
Day 10	Saithe_Des1_10	1600	<10	<10	<10	<10	<10	<10		
	Saithe_Des2_10	230	<10	<10	<10	<10	<10	<10		
	Tusk_Des1_10	130	<10	<10	<10	<10	<10	<10		
	Tusk_Des2_10	<40	<10	<10	<10	<10	<10	<10		
	Cod_Des1_10	3600	<10	<10	<10	<10	<10	<10		
	Cod_Des2_10	<40	<10	<10	<10	<10	<10	<10		
	Ling_Des1_10	240	<10	<10	<10	<10	<10	<10		
	Ling_Des2_10	<40	<10	<10	<10	<10	<10	<10		
Day 17	Saithe_Des1_17	320000	<10	<10	<10	<10	4300	<10	Absence	Absence
	Tusk_Des1_17	150	<10	<10	<10	<10	<10	<10	Absence	Absence
	Cod_Des1_17	75000	<10	<10	<10	<10	<10	<10	Absence	Absence
	Ling_Des1_17	2500	<10	<10	<10	<10	<10	<10	Absence	Absence

ANNEX II: NUTRITIONAL RESULTS.

		Humidity (%)	*Fat (%)*	*Protein (%)*	*Ash (%)*	*Carboh. (%)*	*Energy (Kj / 100 g)*	*Energy (Kcal / 100 g)*	*Fat Energy (Kj / 100 g)*	*Fat Energy (Kcal / 100 g)*	*Salt (g NaCl / 100g) via Na analysis*	*Potassium (mg/100g)*	*Phosphorus (mg/100g)*
Saithe_Des1_0	1721453	75,5	0,25	19,8	2,6	<2,0	352	83	<37	<9	2,44	21,3	66,8
Saithe_Des2_0	1721454	75,5	1,18	19,5	2,8	<2,0	384	91	44	10,6	3,13	25,0	78,5
Tusk_Des1_0	1721455	75,1	0,53	20,4	3,2	<2,0	381	90	<37	<9	3,07	26,4	83,9
Tusk_Des2_0	1721456	73,8	0,21	21,8	3,3	<2,0	390	92	<37	<9	3,24	33,9	87,9
Cod_Des1_0	1721457	80,0	0,16	17,4	1,96	<2,0	310	73	<37	<9	1,75	13,0	53,8
Cod_Des2_0	1721458	82,1	0,11	14,2	3,0	<2,0	257	61	<37	<9	2,43	24,5	94,9
Ling_Des1_0	1721459	73,6	0,08	24,0	2,6	<2,0	416	98	<37	<9	1,71	17,2	47
Ling_Des2_0	1721460	75,0	0,07	21,1	3,1	<2,0	374	88	<37	<9	2,48	24,4	48

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