ANFACO-CECOPESCA

UPDATING OF ANALYTICAL DATA OF TRADITIONAL CLIPFISH, SALTFISH & STOCKFISH. PART 3: MICROBIOLOGICAL AND CONTAMINANT RESULTS.

FHF PROJECT 901307

ANFACO CECOPESCA

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1. SAMPLING. LABORATORY CHECK-IN. PREPARATION OF THE SAMPLES FOR ANALYSIS.

Sampling coordinated by FHF and ANFACO-CECOPESCA consisted in planning, gathering of fish products from several Norwegian producers, and shipment to ANFACO-CECOPESCA´s laboratories in Vigo (Spain). Two shipments were performed. Clipfish materials (140 kg. approx.) were expedited to Spain at the beginning of March 2017. The second shipment (35 Kg. approx.), containing stockfish (ST) (dried cod), lutefish (LU), dried cod heads (TH) and rehydrated stockfish (RS) samples took place at the end of March 2017. Materials were properly received and stored either in freezing (-21ºc) or chilling (2-3ºc) conditions until analysis.

Clipfish materials from 4 fish species were supplied by four different Norwegian producers. Dried, rehydrated and lutefisk cod materials were gathered from three Norwegian companies. Different production batches from each producer were required. From the overall received material set, fish products were selected, made laboratory samples and assigned an internal code for analysis.

Fig. 1: Consignment and sample check - in at ANFACO-CECOPESCA´s pilot plant.

Sampling was completed with an extra shipment carried out in June-July 2018, and consisting in five individuals of four species of dried fish (ling *(Molva molva)*, saithe *(Pollachius virens),* haddock *(Melanogrammus aeglefinus)*, and tusk *(Brosme brosme)*.

The numbers of replicates in each one of the selected products and parameters are shown in table 1 below.

	Hg	Cd, Pb	Microb. analysis.	Dioxins & PCBs
Wetsalted cod	1	1	1	1
Dried-salted cod 7/8 (type A)	1	*1*	1	-
Dried salted cod 7/8 (type B)	1	1	1	-
Dried salted tusk 7/8	6	1	2	-
Dried salted ling 7/8	6	1	2	-
Dried salted saithe 7/8	1	1	1	1
Total *analyses*	*16*	*6*	*8*	*2*

	Hg, Cd, Pb	Microb. analysis.
Dried Cod heads.	-	1
Stockfish cod	2	1
Rehydrated stockfish cod	-	1
Lutefisk	-	1
Total analyses	2	4

	Hg	Microb. analysis.
Stockfish haddock.	3	3
Stockfish ling	5	3
Stockfish saithe	3	3
Stockfish tusk	5	3
Rehydrated Stockfish ling	3	-
Rehydrated Stockfish tusk	3	-
Total analyses	2	4

Table1: Distribution of the samples and analysis.

The samples were processed according to general homogenization protocols and guidelines from regulations. Frozen materials were defrosted and homogenized. The dried cod heads were very difficult to homogenize with standard laboratory materials. Therefore, heads were cut in small pieces making use of an industrial saw. The released powder was collected and further manual and mechanical milling was carried out. For the case of stockfish and making use of a sharp knife, slices of skin and muscle were cut followed by milling.

For the preparation of the heavy-salted samples, we have followed the protocol described in the Portuguese regulation (*Decreto- Lei 25/2005*), where; after mechanical elimination of the surface salt excess, transversal sections in its length were selected and homogenized to get the analytical sample.

Dried fish was cut by using an industrial saw (Figure 2) allowing the selection of the edible part of the fish (muscle). Once at laboratories samples were prepared using standard protocols and submitted to analysis.

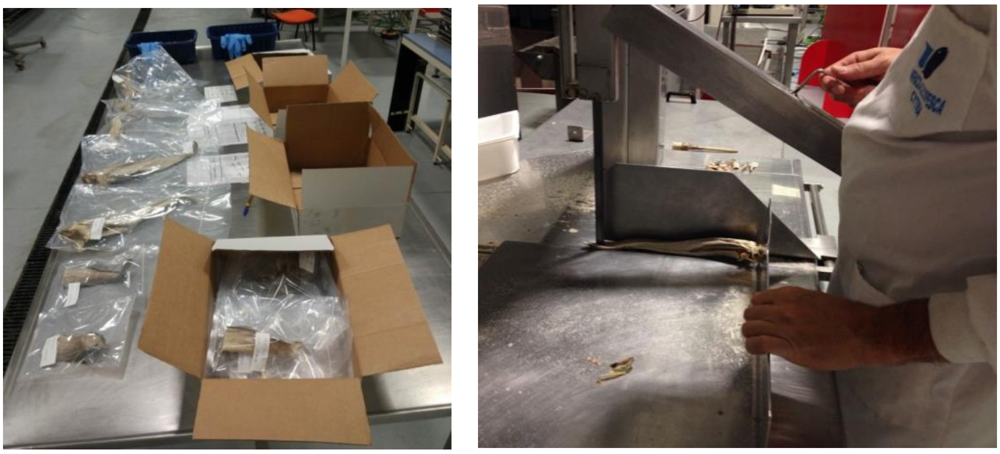

Fig. 2: Sample check - in at ANFACO-CECOPESCA´s pilot plant.

Three replicates of dried ling and dried tusk were submitted to rehydration in order to calculate the mercury level processing factor. The rear section of the fish was used for rehydration keeping the front of the fish for the analysis as received (dried). Rehydration consisted in putting fish individuals in small different containers immersed in a mixture of water and ice and stored in a cold chamber (1-2ºc). The fish: water ratio was higher than 1:10 in all cases, and controls made along the process indicated that temperature was maintained between (0,5 – 2,3°c) .

Fig. 3: Rehydration trials of tusk and ling replicates.

Rehydration trial was performed during 6 days with 4 water changes at days 2, 3, 4, & 5. The weight gain of fish at each water renewal was recorded, and presented in Fig.3. After 6 days of rehydration, samples were individually vacuum packed and frozen (-20°c) until analysis.

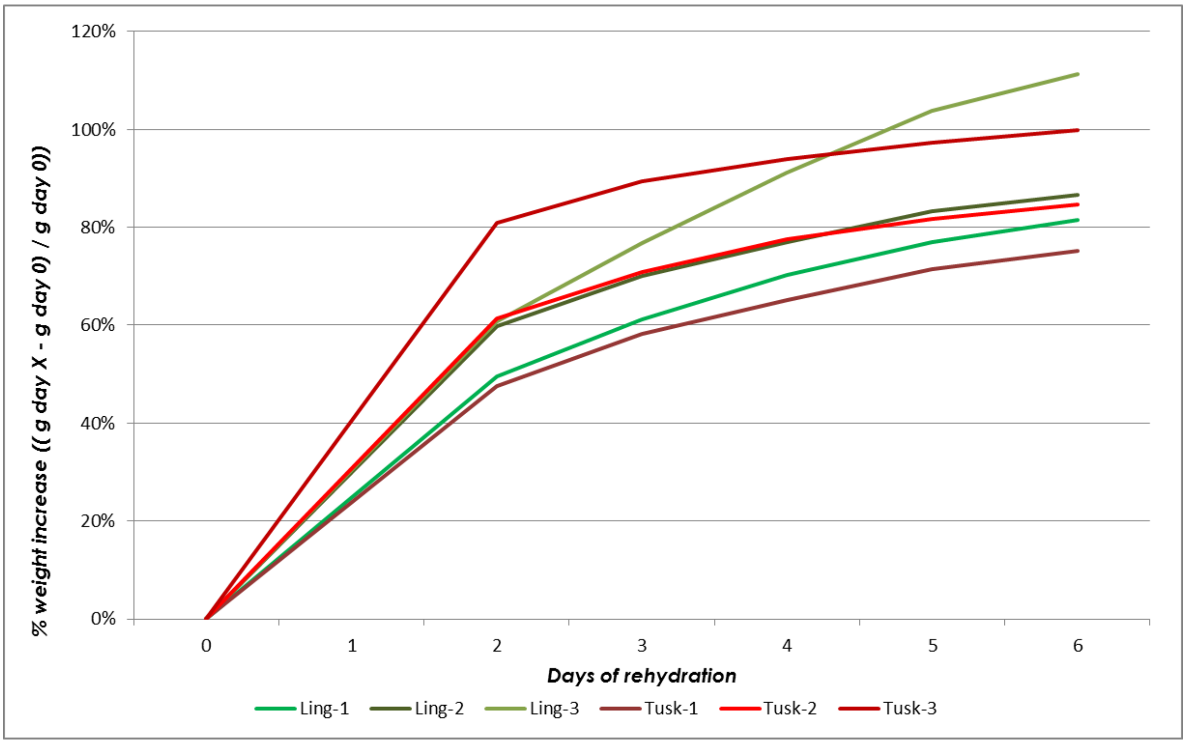

Fig. 4: Relative weight increase of fish replicates during rehydration (days).

Even though the rehydration rate has been significantly reduced at day 6, it seems it did not finish for the samples. Nevertheless, due to the low size of the fish and considering the rehydrated material corresponded

2. METHODS.

Microbiological analysis carried out were 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). All microbiological methods applied are accredited by the Spanish National Accreditation Body (ENAC).

Heavy metal analysis (mercury, lead and selenium) were performed following certified methodologies based on atomic absorption spectroscopy (DC-AAS, GF-AAS). Selenium determination required a higher sensitivity technique, so Inductive Coupled Plasma – Mass Spectrometry (ICP-MS) was used. The determination of PCBs & dioxins compounds was performed by gas chromatography with mass detection after extraction and purification of cod fat.

3. HEAVY METALS RESULTS.

Heavy metals; mercury, cadmium and lead were selected for analysis in some of the received materials. Food products commercialized in the EU have to comply with Reg. EC 1881/2006. The maximum limits for these elements are common for all the species and are presented in table 2 below. The Brazilian legislation in force [1] matches the maximum limits marked by the EU. In addition, it lays down a maximum limit for arsenic of 1 mg/Kg.

	Reg. EC 1881/2006		
	Mercury	Cadmium	Lead
Max. Limit (mg/Kg)	0,50	0,050	0,30

Table 2: Maximum heavy metal limits in the selected species according to Reg. EC 1881/2006.

As it is laid down in article 2 of the regulation, when the foodstuff (fish) are dried, diluted, processed or composed of more than one ingredient, these factors shall be taken into account:

- Changes of the concentration of the contaminant caused by drying or dilution processes.
- Changes of the concentration of the contaminant caused by processing.
- The relative proportions of the ingredients in the product.

The specific concentration or dilution concerned shall be provided and justified by the food business operator, when the competent authority carries out an official control. In case this factor is not provided, or is deemed inappropriate, the authority shall itself define that factor, based on the available information.
Since the analyzed products correspond to heavy-salted /dried-salted and stockfish, we should understand that transformation factors from industries should be applied. Nevertheless, these results give an indication of the potential risk of exceeding the legal limits.

[1] *Resolução - RDC No- 42, De 29 De Agosto de 2013 Dispõe sobre o Regulamento Técnico MERCOSUL sobre Limites Máximos de Contaminantes Inorgânicos em Alimentos.*

One sample of each of the following products was analyzed with the exception of stockfish (cod), dried salted ling and dried salted tusk were two samples were considered. As displayed in table below, **cadmium and lead** values seem not to represent any risk. The obtained values remained below the method quantification limit, far from the maximum limit.

	Cadmium (mg/kg)	Lead (mg/Kg)
Stockfish cod (n=2)	<0,010	<0,06
Wetsalted cod	0,010	<0,06
Dried-salted cod (7/8 - type B)	<0,010	<0,06
Dried-salted cod ((7/8 - type A)	<0,010	<0,06
Dried-salted ling (7/8) (n=2)	<0,010	<0,06
Dried-salted saithe (7/8)	<0,010	<0,06
Dried-salted tusk (7/8) (n=2)	<0,010	<0,06

Table 3: Cadmium & lead results.

Mercury gives higher and more varying values. One replicate of each of the salted products was analyzed with the exception of cod heads (no analysis), dried- salted tusk and ling (6 replicates each).

	Mercury (mg/kg)
Wet salted cod	<0,030
Dried-salted cod (7/8 - type B)	0,049
Dried-salted cod (7/8 - type A)	0,22
Dried-salted saithe (7/8)	0,14

Table 4: Mercury results in stockfish and heavy salted cod / saithe.

Reference values from literature are in line with the obtained results. Studies in 804 samples of wild cod from the northern coast of Norway and Barents Sea (*Julshamn et al. 2013)*[2] reported mean muscle mercury levels of 0,036 ± 0,023 mg/kg, with samples in the *range of 0,01 to 0,16 mg/Kg.* Other consulted references point out similar results, so mercury could be considered as a contaminant that does not put under risk the commercialization of cod products.

As it has been introduced before, the number of replicates in dried-salted ling and tusk (7/8) was extended, since the first obtained values were relatively high. Ling mean result (n=6) was 0,38 ± 0,24 mg/Kg. This value remains below the maximum limit for ling (0,5 mg/kg), but one of the replicates did not comply with legislation, as it can be seen in Fig. 5 & 6.

[2] *Julshamn, K., Duinker, A., Nilsen, B. M., Frantzen, S., Maage, A., Valdersnes, S., & Nedreaas, K. (2013). A baseline study of levels of mercury, arsenic, cadmium and lead in Northeast Arctic cod (Gadus morhua) from different parts of the Barents Sea. Marine pollution bulletin, 67(1), 187-195.*

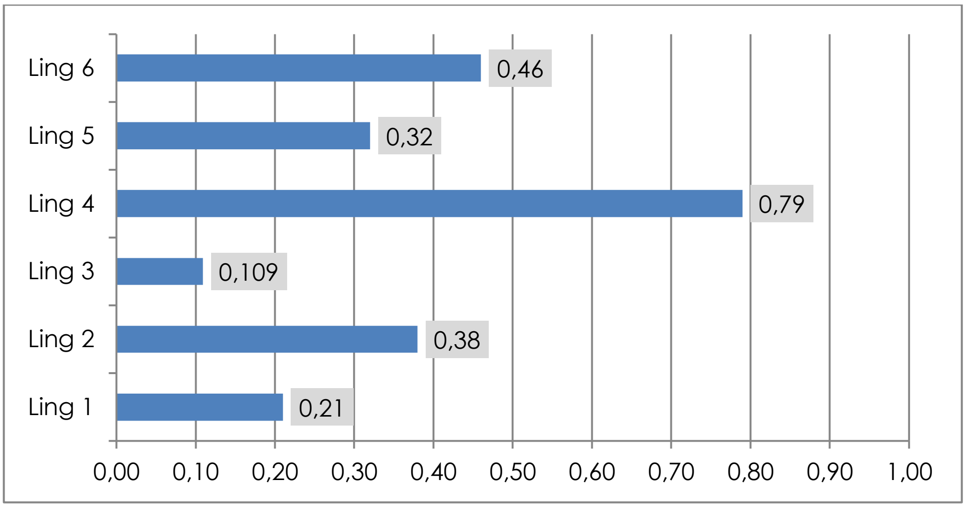

Fig.5: Mercury levels (mg/kg) in dried-salted ling (7/8).

A more disturbing situation seems to occur in dried-salted tusk. Mean value was 0,66 ± 0.26 mg/Kg, with half of the samples overriding the legal threshold and with the rest bordering it.

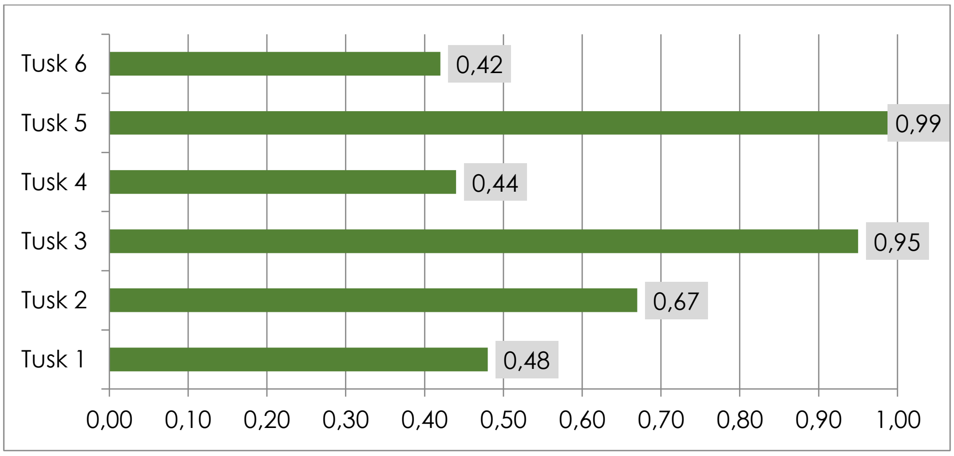

Fig.6: Mercury levels (mg/kg) in dried-salted tusk (7/8).

The results of the analysis carried out in dried fish (ling, saithe, tusk and haddock) confirm that mercury is associated to protein fraction so dehydration causes an increase of mercury levels (figure 7).

Haddock (0.093 ± 0.014 mg/kg), cod (0,076 ± 0,012 mg/kg) and saithe (0.086 ± 0.019 mg/kg) still show low and similar mercury contents, meanwhile ling (0.20 ± 0.05 mg/kg) has a higher average level but still within a safety distance to legal tolerance. On the other hand, average tusk levels (0.63 ± 0.19 mg/kg) go beyond the assigned 0,5 mg/Kg limit, with one of the samples reaching 0.94 mg/kg, a very similar situation as dried salted tusk results.

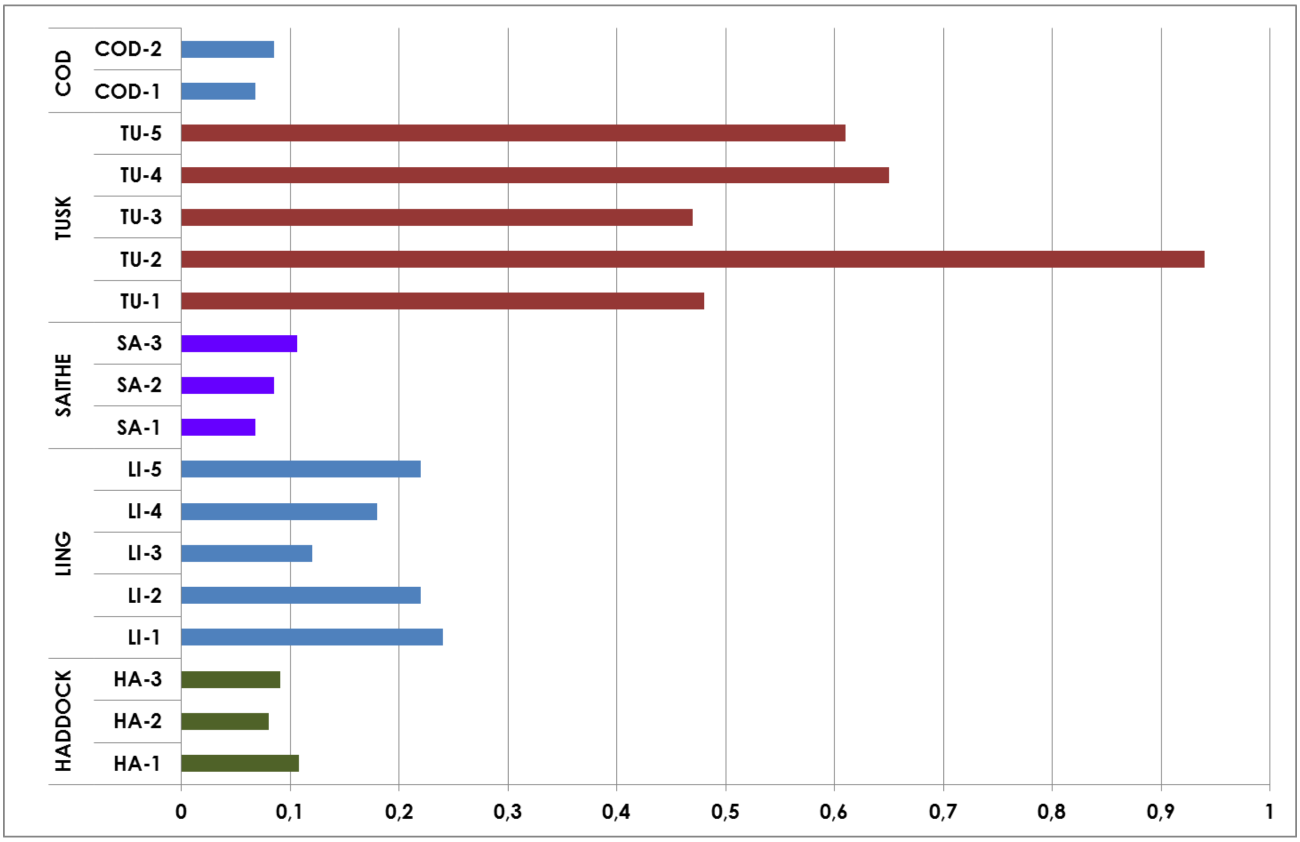

Fig. 7: Mercury levels (mg/kg) in stockfish samples.

High mercury values in tusk and ling have been reported in literature and some studies about this have been carried out[3]. Mercury is therefore a factor of concern for tusk producers and should be included in their HACCP systems. Since for food security reasons the commercialization of the product might be put into risk, the transformation factors for rehydration and desalting cited in Article 2 of the Reg. CE 1881/2006 were obtained.

- **MERCURY FACTORS AT DESALTING.**

Food products commercialized in the EU have to comply with Reg. EC 1881/2006. For cod, saithe ling and tusk a maximum 0,5 mg Hg/ Kg content has been determined. Nevertheless, and as it is laid down in article 2 of the regulation, when the foodstuff (fish) are dried, diluted, processed or composed of more than one ingredient, these factors shall be taken into account:

- Changes in the concentration of the contaminant caused by drying or dilution processes.
- Changes in the concentration of the contaminant caused by processing.
- The relative proportions of the ingredients in the product.

The specific concentration or dilution concerned shall be provided and justified by the food business operator, when the competent authority carries out an official control. In case this factor is not provided, or is deemed inappropriate, the authority shall itself define that factor, based on the available information.

[3] https://www.nifes.no/en/prosjekt/contaminants-in-tusk-ling-and-other-deep-sea-fish-from-norwegian-sea-areas/

Since no raw materials (fresh/frozen cod) have been received, it has not been possible to calculate the mercury factor for clipfish production, thus only desalting factors have been addressed. Nonetheless, the regulation refers to the original raw material instead of the final desalted products.

To determine these desalting factors, a 72 hours desalting time was selected with tap water at 2°c and a fish: water ratio of 1:5. Fish sample and water were weighed in the pilot plant weigh scale. 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 2°c all along the desalting time.

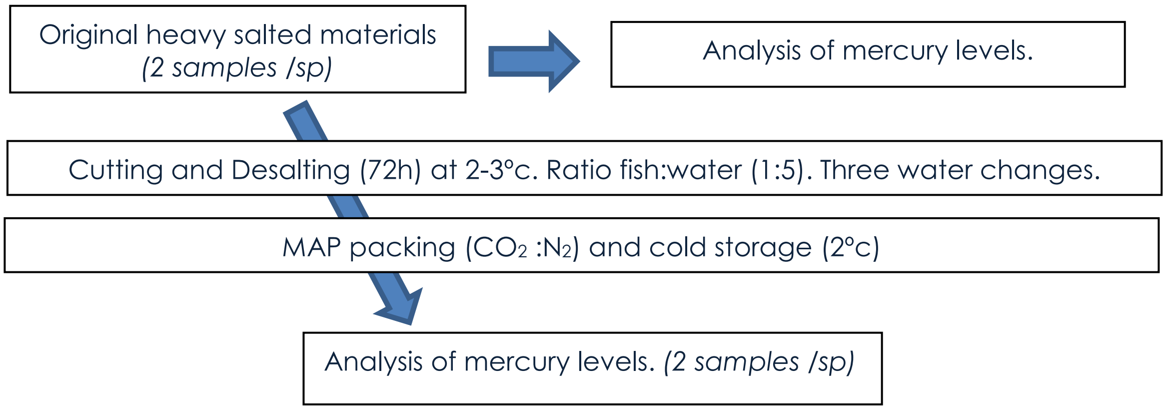

Figure 8: Desalting trial for the estimation of mercury factors.

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.
In table 5, the results from the mercury analysis of the same individuals before and after desalting are shown. The mean factors (after desalting /before desalting) of the two replicates have been calculated.

	Mercury (mg/Kg)			
	Clipfish	Desalted	Ratio Desalted / clipfish	*Mean factor (n=2)*
Saithe_1	0,156	0,161	1,03	**0,92**
Saithe_2	0,180	0,152	0,84	
Tusk_1	0,119	0,10	0,85	**0,92**
Tusk_2	0,160	0,158	0,99	
Cod_1	0,114	0,101	0,89	**0,82**
Cod_2	0,110	0,079	0,72	
Ling_1	0,353	0,327	0,93	**0,91**
Ling_2	0,171	0,145	0,85	

Table 5: Mercury results and calculated factors for clipfish desalting (n=2).

Even though the number of replicates is low, it can be seem that the mercury does not become considerably affected by the desalting process. Probably the slight increase in yield during desalting is behind the obtained factors. Therefore, the mercury levels arisen from the inspection of heavy salted fish by the sanitary authorities would not change significantly when ready to be consumed, so the potential risk of high mercury values would prevail after desalting.

In any case, the production of clipfish and the subsequent desalting to get final ready-to-prepare products involve mass transfer of mainly water and salt, a change in mineral composition and to a minor extent protein loss.

Literature reports that methylmercury seems to be linked to the protein fraction in fish products. Assuming that the variation of fat, carbohydrates is insignificant, we can calculate how the mercury levels would be affected by the mass transfer. We have assumed the theoretical case there is no significant mercury loss during the process, but his should be checked in future trials.

In order to make calculations we have selected available nutritional information from the present and previous projects, and completing absent data with reported values from the NIFES seafood database (*https://sjomatdata.nifes.no/#search/*) of the three products along the processing chain (raw materials, clipfish, desalted products).

Mean nutritional values for cod samples are the ones detailed in table 6 below. For cod, wet salted materials have been selected, but it should be mentioned that desalting from dried-salted materials instead, may lead to reduced humidity in final desalted products, as it has been the case of ling and cod.

In order to standardize data, the **residual mass factor** is defined as the difference between the total mass and the sum of humidity & ash (mainly salt). As an example the residual mass factor for wet salted cod would be: *1 – (0.577+0.201) = 0.222 or (22,2%)*. So the residual mass factor will bring information about the rest of the nutritional components after getting rid of humidity and ashes. In the absence of real yield values, the ratios between the calculated residual masses for wet salted cod & desalted cod vs. the reference (cod raw materials) could be interpreted as caused by the change in the total mass (yield).

	Humidity (%)	*Protein (%)*	*Ash (%)*	*Residual mass (%)*	*Ratio of residual mass to reference (Cod raw materials)*
Cod raw materials	81,9	16,7	1,5	**16,60**	**1,00**
Wet salted cod	57,8	21,5	20,1	**22,15**	**1,33**
Desalted cod	80,8	15,7	2,0	**17,19**	**1,04**

	Humidity (%)	*Protein (%)*	*Ash (%)*	*Residual mass (%)*	*Ratio of residual mass to reference (Ling raw materials)*
Ling raw materials	78,73	19,30	1,15	**20,12**	**1,00**
Dried salted Ling	51,21	29,21	18,78	**30,01**	**1,49**
Desalted Ling	74,30	22,55	2,85	**22,85**	**1,14**

	Humidity (%)	*Protein (%)*	*Ash (%)*	*Residual mass (%)*	*Ratio of residual mass to reference (Tusk raw materials)*
Tusk raw materials	78,97	19,10	1,11	**19,92**	**1,00**
Dried salted Tusk	51,49	28,54	19,24	**29,27**	**1,47**
Desalted Tusk	74,45	21,10	3,25	**22,3**	**1,12**

Table 6: Reference nutritional data used to calculate residual masses and their ratios.

Based in the assumptions commented before, and applying the corresponding ratios to mercury levels in wet salted/dried salted products, we can estimate the theoretical mercury content in raw materials and desalted products. In tables below we can see the obtained values for the ling and tusk replicates.

	Tusk raw materials (Calculated data)	Dried salted tusk (Data from analysis)	Desalted tusk (Calculated data)
Tusk- rep1	0,29	**0,42**	0,32
Tusk- rep2	0,67	**0,99**	0,75
Tusk- rep3	0,30	**0,44**	0,34
Tusk- rep4	0,65	**0,95**	0,72
Tusk- rep5	0,46	**0,67**	0,51
Tusk- rep6	0,33	**0,48**	0,37
Tusk- rep7	0,08	**0,119**	0,09
Tusk- rep8	0,11	**0,160**	0,12

	Ling raw materials (Calculated data)	Dried salted ling (Data from analysis)	Desalted ling (Calculated data)
Ling-rep_1	0,31	**0,46**	0,35
Ling-rep_2	0,21	**0,32**	0,24
Ling-rep_3	0,53	**0,79**	0,60
Ling-rep_4	0,07	**0,109**	0,08
Ling-rep_5	0,26	**0,38**	0,29
Ling-rep_6	0,14	**0,21**	0,16
Ling-rep_7	0,24	**0,353**	0,27
Ling-rep_8	0,11	**0,171**	0,13

Table 7: Real and calculated mercury results in ling and cod replicates.

Mean mercury result in wet salted cod mentioned above of 0,112 mg/kg, the factor, we can estimate the mercury concentration in **raw materials (0,084 mg/Kg)** and in **desalted cod (0,088 mg/Kg)**. We can see that this latter value matches the real mean value measured in the desalted materials (0,090 mg/Kg) presented above in table 5. A similar situation occurs for measured mercury values in desalted ling and tusk, so we can conclude that mercury factors seem only to be affected by the mass balance during desalting. As it has been commented before, the assumption that this effect also occurs during cod salting shall also be confirmed experimentally.

- **MERCURY FACTORS AT REHYDRATION OF STOCKFISH (Haddock, saithe, ling, tusk).**

The estimation of mercury factors at rehydration has been only carried out for stockfish ling and tusk, since stockfish cod, haddock and saithe levels were of a very low concern. Despite the extension of the rehydration trial up to 6 days, it seems that the moisture content in the rehydrated samples did not stop increasing. Nevertheless, and as seen in Fig. 4, the daily increase in weight was rather stable after 6 days. The swelling of fish muscle has been limited reaching only 57% (tusk) and 59,4%(ling) moisture content. Prolonged immersion as well as filleting, skinning and/or slicing of the sample would have been necessary in order to fully rehydrate dried fish, but it seems fish would not finally get the approximately (80%) moisture content of the fresh cod. Dehydration of stockfish surely affects the muscle capacity to soak up water at rehydration. In this section, the two options have been studied.

A considerable reduction of the mercury contents was observed in the samples o f this rehydration trial.

Id. Code	Humidity (%)	Rehydrated mercury (mg/kg)	Stockfish Mercury (mg/Kg)	
Ling-1	59,4	0,14	0,24	58,3%
Ling-2	[redacted]	0,098	0,22	44,5%
Ling-3	[redacted]	0,070	0,12	58,3%
			Mean	53,7%
			SD	8,0%

Id. Code	Humidity (%)	Rehydrated mercury (mg/kg)	Stockfish Mercury (mg/Kg)	
Tusk-1	57,0	0,35	0,48	72,9%
Tusk-2	[redacted]	0,58	0,94	61,7%
Tusk-3	[redacted]	0,26	0,47	55,3%
			Mean	63,3%
			SD	8,9%

Table 8: Mercury factors for rehydrated samples.

Despite haven´t been able to fully rehydrate samples, it has been detected that the mercury levels correlate well with the protein/water level in fish. This statement is based on the comparison of the experimental mercury levels obtained in the rehydrated samples, and the calculated mercury values after applying the ratio of the protein content (considering a linear variation of protein to humidity). As it can be seen in Table 9, and despite the error of applying the humidity level of replicate 1 to replicates 2 and 3, the estimated and observed mercury levels approach significantly.

	Dried Ling			Rehydrated ling			
	Humidity (%)	Protein (%)	Mercury (mg/kg)	Humidity (%)	Estimated Protein (%)	Estimated Mercury (mg/kg)	Observed Mercury (mg/kg)
Ling-1	23,5	68	0,24	59,4	36,1	**0,13**	**0,14**
Ling-2	20,6	71	0,22	59,4	36,3	**0,11**	**0,098**
Ling-3	17,2	78	0,12	59,4	38,2	**0,06**	**0,07**

	Dried tusk			Rehydrated tusk			
	Humidity (%)	Protein (%)	Mercury (mg/kg)	Humidity (%)	Estimated Protein (%)	Estimated Mercury (mg/kg)	Observed Mercury (mg/kg)
Tusk-1	24,6	66	0,48	57,0	37,6	**0,27**	**0,35**
Tusk-2	24,1	65	0,94	57,0	36,8	**0,53**	**0,58**
Tusk-3	20,9	70	0,47	57,0	39,9	**0,27**	**0,26**

Table 9: Comparison of observed and predicted mercury levels based on applying protein ratio.

By using the same premise, we can also calculate the expected mercury level for an theoretical final fully rehydrated sample of 80% humidity.

	Dried Ling			Fully rehydrated ling		
	Humidity (%)	Protein (%)	Mercury (mg/kg)	Humidity (%)	Estimated Protein (%)	Estimated Mercury (mg/kg)
Ling-1	23,5	68	0,24	80,0	17,8	**0,06**
Ling-2	20,6	71	0,22	80,0	17,9	**0,06**
Ling-3	17,2	78	0,12	80,0	20,4	**0,03**

	Dried tusk			Fully rehydrated tusk		
	Humidity (%)	Protein (%)	Mercury (mg/kg)	Humidity (%)	Estimated Protein (%)	Estimated Mercury (mg/kg)
Tusk-1	24,6	66	0,48	80,0	17,3	**0,13**
Tusk-2	24,1	65	0,94	80,0	17,0	**0,25**
Tusk-3	20,9	70	0,47	80,0	18,3	**0,12**

Table 10: Estimated mercury levels in fully rehydrated fish based on the application of the protein ratio.

The expected mercury values in a theoretical fully rehydrated fish (80% content) are far below the 0,5 mg/kg threshold. Even though, these results should be confirmed experimentally, it seems that mercury levels are merely affected by the weight increase during rehydration as strongly correlated to protein content in the product.

4. ORGANIC POLLUTANTS (Dioxins, furans and PCBs).

The presence of dioxins, furans and dioxin-like PCBs in certain foodstuff is regulated by the EU under Reg. CE 1881/2006. The level of dioxins and dl-PCBs are reported as the sum of the concentrations of the 17 different PCDD/F and 12 dl-PCBs compounds weighed by their respective toxicity (toxic equivalence factors (TEQ) set by the WHO). For muscle of fish and fishery products, a maximum sum of dioxins & furans has been set in 3,5 pg/g (PCDDD/F - TEQ). Including the contribution of dioxin-like PCBs this value increases up to 6,5 pg/g (PCDDD/F-PCBs - TEQ) of muscle meat. The EUs maximum limit for the sum of the six indicator non-dioxin-like PCBs in fish is 75ng/g.

Since these contaminants are lipophilic, and the total fat in the selected products (wet salted cod and dried salted saithe) was low, the expected concentrations were also low compared to legal thresholds. Only one replicate of each one of the two products was analyzed.

- **WET SALTED COD.**

As it can be observed in table 11, the levels of dioxins and furans congeners are below the quantification limit (LOQ) with the exception of OCDF.

Nombre	Conc(pg/g)	TEQ(pg/g)	limite de	detec.(pg/g)	Recup.(%)
			Conc.(pg/g)	TEQ(pg/g)	
TCDF	<LDC	0.0019	0.019	0.0019	97
PeCDF-1	<LDC	0.0003	0.011	0.00033	84
PeCDF-2	<LDC	0.0034	0.011	0.0033	85
HxCDF-1	<LDC	0.0022	0.022	0.0022	99
HxCDF-2	<LDC	0.0022	0.022	0.0022	97
HxCDF-3	<LDC	0.0024	0.024	0.0024	92
HxCDF-4	<LDC	0.0032	0.032	0.0032	88
HpCDF-1	<LDC	0.0002	0.021	0.00021	72
HpCDF-2	<LDC	0.0004	0.039	0.00039	60
OCDF	0.057	0.00002	0.039	0.0000117	34
TCDD	<LDC	0.016	0.016	0.016	88
PeCDD	<LDC	0.032	0.032	0.032	76
HxCDD-1	<LDC	0.0056	0.056	0.0056	90
HxCDD-2	<LDC	0.0055	0.055	0.0055	88
HxCDD-3	<LDC	0.0064	0.064	0.0064	100
HpCDD	<LDC	0.0009	0.090	0.0009	63
OCDD	<LDC	0.0001	0.35	0.0001038	34

Table 11: Dioxins and furans results in wet salted cod.

Following the "upper bound" criteria for the calculation, the numerical LOQ were used for congeners with levels below LOQ. The sum of PCDDD/F - TEQ was 0.082 pg/g which is far below the legal threshold of 3,5 pg/g. The sum of the contributions of the dioxin- like PCBs, detailed in table 12, was 0.028 pg/g (PCBs – TEQ).

Name	Conc.(pg/g)	TEQ(pg/g)	limite de	detec.(pg/g)	Recup.(%)
			Conc.(pg/g)	TEQ(pg/g)	
PCB 77	0.18	0.00002	0.14	0.0000143	78
PCB 81	<LDC	0.00007	0.24	0.0000714	61
PCB 126	<LDC	0.016	0.16	0.016	96
PCB 169	<LDC	0.011	0.37	0.01107	111
PCB 105	3.99	0.00012	0.64	0.00001929	82
PCB 114	<LDC	0.00002	0.55	0.00001656	90
PCB 118	9.71	0.0003	1.18	0.00003549	69
PCB 123	<LDC	0.00004	1.25	0.00003762	81
PCB 156	<LDC	0.00003	1.09	0.0000327	85
PCB 157	<LDC	0.00002	0.73	0.00002193	77
PCB 167	<LDC	0.00005	1.62	0.00004851	81
PCB 189	<LDC	0.00001	0.41	0.00001242	62

Table 12: Dioxin- like PCBs results in wet salted cod.

The total contribution of dioxins, furans and dioxin-like PCBs is therefore 0,11 pg/g (PCDDD/F-PCBs – TEQ) which is also far below from the 6,5 pg/g limit determined by European Standards.
All the not-dioxin-like PCBs (PCB28, PCB52, PCB101, PCB138, PCB153, PCB180) levels were under the quantification limit in this sample. The sum of total NDL-PCBS under upper bound criteria was therefore 0,18 ng/g.

- **DRIED SALTED SAITHE (7/8).**

In table 13, detail of the results of dioxins and furans is presented. With the exception of TCDF congener, the rest of the analyzed compounds remained at levels below the quantification limit.

Nombre	Conc(pg/g)	TEQ(pg/g)	limite de	detec.(pg/g)	Recup.(%)
			Conc.(pg/g)	TEQ(pg/g)	
TCDF	0.035	0.0035	0.019	0.0019	116
PeCDF-1	<LDC	0.0003	0.011	0.00033	107
PeCDF-2	<LDC	0.0034	0.011	0.0033	114
HxCDF-1	<LDC	0.0022	0.022	0.0022	114
HxCDF-2	<LDC	0.0022	0.022	0.0022	113
HxCDF-3	<LDC	0.0024	0.024	0.0024	108
HxCDF-4	<LDC	0.0032	0.032	0.0032	108
HpCDF-1	<LDC	0.0002	0.021	0.00021	94
HpCDF-2	<LDC	0.0004	0.039	0.00039	81
OCDF	<LDC	0.00001	0.039	0.0000117	55
TCDD	<LDC	0.016	0.016	0.016	106
PeCDD	<LDC	0.032	0.032	0.032	102
HxCDD-1	<LDC	0.0056	0.056	0.0056	107
HxCDD-2	<LDC	0.0055	0.055	0.0055	106
HxCDD-3	<LDC	0.0064	0.064	0.0064	100
HpCDD	<LDC	0.0009	0.090	0.0009	85
OCDD	<LDC	0.0001	0.35	0.0001038	55

Table 13: Dioxins and furans results in dried-salted saithe.

The sum of the dioxin and furan toxic equivalents (PCDDD/F - TEQ) was very low (0.084 pg/g). The contribution of dioxin-like PCBs (*table 14)* was 0.038 pg/g for a final PCDDD/F-PCBs – TEQ of 0.122 pg/g.

Name	Conc.(pg/g)	TEQ(pg/g)	limite de	detec.(pg/g)	Recup.(%)
			Conc.(pg/g)	TEQ(pg/g)	
PCB 77	0.46	0.00005	0.14	0.0000143	68
PCB 81	<LDC	0.00007	0.24	0.0000714	50
PCB 126	0.24	0.0024	0.16	0.016	101
PCB 169	<LDC	0.011	0.37	0.01107	123
PCB 105	0.69	0.00002	0.64	0.00001929	74
PCB 114	49.5	0.0015	0.55	0.00001656	74
PCB 118	6.88	0.0002	1.18	0.00003549	74
PCB 123	4.23	0.00013	1.25	0.00003762	76
PCB 156	1.14	0.00003	1.09	0.0000327	77
PCB 157	3.16	0.00009	0.73	0.00002193	73
PCB 167	<LDC	0.00005	1.62	0.00004851	78
PCB 189	<LDC	0.00001	0.41	0.00001242	65

Table 14: Dioxin- like PCBs results in dried salted saithe.

Concerning the non-dioxin –like PCBs the sum concentration was also very low (0,37 ng/g) and far below the 75 ng/g legal threshold). The congeners PCB-138 and PCB-153 have been the main contributors to the sum.

Non dioxin-like PCBs	PCB28	PCB52	PCB101	PCB138	PCB153	PCB180
	<LQ	<LQ	0,054	0,12	0,1	<LQ

Table 15: Non-Dioxin- like PCBs results in dried salted saithe.

5. MICROBIOLOGICAL RESULTS.

The microbiological analysis carried out in salted fish and clipfish seem not to have microbiological concern. Its low water activity and cool storage prevents from the growth of microorganisms. As it can be seen in table 16, all the analysed samples showed microbiological counts below the quantification limits of the methods applied.

	Aerobic mesophilic microorganisms (cfu/g)	Total Enterobacteria (cfu/g)	*Staphylococcus* positive coagulase (cfu/g)	Count of total coliforms (cfu/g)	Count of *Escherichia coli* (cfu/g)
Wet salted cod	<10x10exp2	<10	<10	<10	<10
Dried-salted cod (7/8) (type B)	<10x10exp2	<10	<10	<10	<10
Dried-salted cod (7/8) (type A)	<10x10exp2	<10	<10	<10	<10
Dried salted Ling_rep1	<10x10exp2	<10	<10	<10	<10
Dried salted Ling_rep2	<10x10exp2	<10	<10	<10	<10
Dried salted Tusk_rep1	<10x10exp2	<10	<10	<10	<10
Dried salted Tusk rep2	<10x10exp2	<10	<10	<10	<10
Dried salted Saithe	<10x10exp2	<10	<10	<10	<10

Table 13: Microbiological results in saltfisk and clipfish.

The results obtained in dried products (no salting) are similar to the salted products, with the only difference of higher aerobic mesophilic bacteria (table 14). Hygiene criteria were satisfactory. The higher mesophilic load of the dried products seems to correspond to the natural flora of cod which may have increased during the first stages of drying until become steady in the final water activity levels of the product.

	Aerobic mesophilic microorganisms (cfu/g)	Total Enterobacteria (cfu/g)	*Staphylococcus* positive coagulase (cfu/g)	Count of total coliforms (37º) (cfu/g)	Count of *Escherichia coli* (cfu/g)
Stockfish cod.	2,1x10exp4	<10	<10	<10	<10
Dried Cod heads.	1,1x10exp4	<10	<10	<10	<10

Table 14: Microbiological results in dried cod /heads.

No pathogen bacteria (*Salmonella* & *Listeria monocytogenes)* were found in 25 g of any of the samples from table 9 and 10.

The microbiological load of dried fish is very high. All samples of haddock and saithe showed results over 10^6 cfu/g. It was also the case of one of the three replicates of ling and tusk, meanwhile the other remained at a lower variable level. In addition, with the exception of two saithe replicates the enterobacteriaceae are of low. Probably these two replicates have not been properly manipulated. The same occurs when looking at coliforms but in this case only one of the samples showed high values. *Escherichia coli* has not been detected in any of the samples. Ling and tusk contained lower microbiological loads and no enterobacteriaceae microorganisms.

Id. Code	Count of total aerobic microorganisms (cfu/g)	Count of total enterobacteriacea (cfu/g)	Count of total coliforms (cfu/g)	Count of *Escherichia coli* (cfu/g)
Stockfish haddock -1	>4,9E+06	33	<10	<10
Stockfish haddock -2	>4,9E+06	2400	5300	<10

Stockfish haddock - 3	>4,9E+06	<10	69	<10
Stockfish ling - 1	1000	<10	<10	<10
Stockfish ling - 2	2,7E+05	<10	<10	<10
Stockfish ling - 3	>4,9E+06	<10	<10	<10
Stockfish saithe - 1	>4,9E+06	33	33	<10
Stockfish saithe - 2	>4,9E+06	>4,9E+06	640	<10
Stockfish saithe - 3	>4,9E+06	>4,9E+06	>4,9E+06	<10
Stockfish tusk - 1	1000	<10	<10	<10
Stockfish tusk - 2	>4,9E+06	<10	<10	<10
Stockfish tusk - 3	9500	<10	<10	<10

Table15: Microbiological results in stockfish (ling, saithe, haddock and tusk).

Microbiological criteria in food products in Spain have been derogated since the putting into force of the Reg. EC 2073/2005[4], which has become the key reference across Europe. Nevertheless, and unlike Spain, other EU countries may still preserve extended national microbiological criteria not derogated. This regulation leaves the responsibility of the safety of food products to the producers, who shall apply their internal control in accordance to the known and potential risks. For example, in the case of Spain, food industries still take into account, within their HACCP systems, microbiological criteria in Spain [5] before the derogation. In this Spanish regulation the maximum limits for salted, dried-salted and dried fish products were:

- **Count of Aerobic mesophilic microorganisms (31 °C ± 1 °C):** 1x10^5 cfu/g.
- **Total Enterobacteriacea:** 1x10^2 cfu/g.
- **Salmonella-Shigella:** Absent/25 g.

A more strict reference could be the quality criteria for the "Portuguese Style curing process" (Traditional Specialities Guaranteed TSG)[6], where more strict limits were included for clipfish:

– ***Total de aeróbios mesofilos*** — < 10^3 cfu/g;
– ***Contagem de coliformes totais*** — *< 10 cfu/g;*
– ***Contagem de clostrídios sulfito -redutores*** — *< 10 cfu/g;*
– ***Contagem de Staphylococcus aureus*** — *< 10^2 cfu/g;*
– ***Pesquisa de Listeria monocytogenes*** — *negative.*

[4] *Commission Regulation (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs. http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32005R2073&from=EN*
[5] *Orden de 2 de agosto de 1991, por la que se establecen las normas microbiológicas de los diferentes productos de la pesca y acuicultura, así como los límites del contenido en metales pesados y los métodos analíticos para la determinación de éstos.(Derogated in 2010). https://www.boe.es/boe/dias/1991/08/15/pdfs/A27153-27155.pdf*
[6] *Commission Implementing Regulation (EU) No 409/2014 of 23 April 2014 entering a name in the register of traditional specialities guaranteed (Bacalhau de Cura Tradicional Portuguesa (TSG))……. & ….. Diário da República, 2.ª série — N.º 68 — 6 de Abril de 2011. Ministério Da Agricultura, Do Desenvolvimento Rural E Das Pescas. Gabinete do Ministro. Despacho n.º 6006/2011.*

On the other hand, we can compare to criteria determined by the Brazilian legislation[7]. For heavy salted, dried and dried-salted fish maximum limits of $5x10^2$ cfu/g of *Staphylococcus* positive coagulase, 10^2 cfu/g of thermotolerant coliforms (45°c), and absence of *Salmonella* in 25 g have been set. The obtained results are in compliance with the criteria of all the references above.

For the case of frozen rehydrated stockfish and lutefisk (*table 16)* we can see a different situation. The rehydration of stockfish leads to an increase from the $21x10^3$ cfu/g to$15x10^5$ cfu/g of aerobic mesophilic microorganisms, and the presence of *Enterobacteria, Staphylococcus* positive coagulase and coliforms.
Lutefisk only showed low –moderate levels of aerobic mesophilic microorganisms, probably derived from the final steps of the NaOH neutralization and freezing. The rest of the parameters were below the quantification limit of the method.

	Aerobic mesophilic microorganisms (cfu/g)	**Total Enterobacteria (cfu/g)**	***Staphylococcus* positive coagulase (cfu/g)**	**Count of total coliforms (cfu/g)**	**Count of *Escherichia coli* (cfu/g)**
Rehydrated Stockfish cod	1,5x10exp6	10	20	160	<10
Lutefisk	1,5x10exp4	<10	<10	<10	<10

Table 16: Microbiological results in rehydrated stockfish and lutefisk.

No pathogen bacteria (*Salmonella* & *Listeria monocytogenes)* was found in 25 g of rehydrated stockfish and lutefisk.

Again, there are not criteria for these products across at an EU level. Nevertheless, we can compare this result to previous Spanish reference[4] in frozen seafood, which determined:

- **Count of Aerobic mesophilic microorganisms (31 °C ± 1 °C):** $1x10^6$ cfu/g.
- **Total Enterobacteriacea:** $1x10^3$ cfu/g.
- **Salmonella-Shigella:** Absent/25 g.

Rehydrated stockfish cod levels exceeded the level of aerobic mesophilic microorganisms marked in this derogated regulation, meanwhile lutefisk levels are below this threshold. This relatively high levels had been previously found in FHF project 900879 [8]. Nevertheless, in rehydrated stockfish it can be seen that total *Enterobacteria* levels are rather low compared to the same limits in the same legal document. Since there are no references to these types of seafood for the rest of hygiene indicators, we can only compare the obtained values to other seafood limits (cooked peeled /beheaded crustaceans and mollusks and from Reg. CE 2073/2005: *E. coli*: 1-10 cfu/g, *Staphylococcus* positive coagulase: 100-1000 cfu/g). Taking in consideration that cod products are uncooked, we can conclude that there hygiene has been satisfactory and these products are safe.

We can also check compliance to Brazilian regulation that defines maximum limits of; 10^3 cfu/g of *Staphylococcus* positive coagulase and absence of *Salmonella* in 25 g for frozen or chilled fish, that also includes an additional criteria of 10^3 cfu/g of thermotolerant coliforms (45°c), in the cases of

[7] *Resolução nº 12 de 02/01/2001 / ANVISA - Agência Nacional de Vigilância Sanitária. Regulamento técnico sobre padrões microbiológicos para alimentos.*
[8] http://www.fhf.no/prosjektdetaljer/?projectNumber=900879

manufactured fish products (hamburgers, …). Despite the category the rehydrated stockfish and lutefisk are considered in, results are in compliance to Brazilian regulation.

6. CONCLUDING REMARKS.

The analyses have been carried out in the products as received and commercialized. In saltfish, clipfish and dried samples, the comparison of heavy metals results to maximum regulated limits is merely indicative, since processing (salting/drying) has affected the initial concentration of these elements. Besides the final concentration (as it is consumed) should be measured in the product once rehydrated or desalted. This makes necessary to define a transformation factor for these samples so the interpretation of the contaminants risk of the product would be more accurate.

The cadmium and lead levels seem not to represent a risk in these products. Moreover, desalting and rehydration would probably dilute the detected levels. Further investigation regarding mercury levels should be made, especially in dried salted tusk and ling, and in dried tusk. The calculated transformation factors are indicative, so should be calculated in a larger sample set and in real industrial production in order to evaluate the effect of not only desalting and rehydration, but also salting and drying processes in mercury levels, especially for the case of ling and tusk, which seem to be higher. Both clipfish desalting and stockfish rehydration seems correlated to the mass balance, so a very small reduction of mercury contents was observed during desalting and a remarked reduction for the case of stockfish rehydration. These factors could be applied by operators, in case of goods detention at official control as laid down in article 2 of the Reg. EU 1881/2006.

Organic pollutants (PCBs, furans and dioxins) seem not to be a considerable risk, in part due to low fat levels of the products (lipophilic contaminants).

The salted and dried-salted products analyzed do not have relevant microbial loads. Dried fish contain high levels of aerobic mesophilic microorganisms, but are related to original fish bacteria strains and as a result of its particular prolonged drying process. Previous studies seem to indicate that these levels are typical of this product; however they may condition the shelf-life whenever these products are rehydrated and put into market chilled or defrosted. With the exception of two dried saithe replicates, probably during manipulation during packing, transport and reception, in general, no hygiene concerns arise from the obtained results.

Attending to microbiological legal references available and cited in this report, there is not legal concern that may affect their trade. Nevertheless, the initial high microbiological loads in dried fish will definitely compromise the product shelf-life once rehydrated.