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

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Summary of project results.

The updating of nutritional information, according to labelling criteria, has been carried out at ANFACO-CECOPESCA's laboratories in clipfish (cod, ling, saithe, tusk), saltfish (cod), stockfish (cod, ling, saithe, tusk and haddock) and dried cod heads, lutefisk and rehydrated cod stockfish, and proposed nutritional labels were introduced with the obtained data.

Certain divergences were found between reported data and criteria laid down in clipfish and saltfish production standards, so the updating of the norms might be appropriate. In addition microbiological quality of these products was confirmed by the analysis, as well as for organic pollutants (dioxins & PCBs), and heavy metals (Hg, Cd, Pb).

Moreover, a shelf-life test in MAP desalted fish (no additives) remained short since, after 17 days of 2°c storage, microbiological loads were still low. Despite lack of microbiological data at day 33, the sensory test at this extended end-point was still satisfactory.

Sammendrag av resultater fra prosjektets faglige sluttrapport:

Oppdateringen av næringsinformasjon er utført i henhold til merkingskriterier for klippfisk (torsk, lange, sei og brosme), saltfisk (torsk), tørrfisk (torsk,sei,lange,hei,hyse), tørkede torskehoder, lutefisk og bløytet torsk tørrfisk ved ANFACO-CECOPESCA sine laboratorier. Det er foreslått næringsinnhold på etiketter basert på analysert data.

Det ble funnet visse avvik mellom analyserte data og kriterier fastsatt i klippfisk- og saltfiskproduksjonsstandarder, slik at oppdateringen av norm kan være hensiktsmessig. I tillegg ble både mikrobiologisk kvalitet av disse produktene analysert samt organiske forurensninger (dioksiner og PCB) og tungmetaller (Hg, Cd og Pb).

Videre er det utført en litteraturstudie om utvanningsprosessen samt en holdbarhetstest i MAP for utvannet klippfisk (uten tilsetningsstoffer). Kimtall var fortsatt lav etter 17 dager, med lagring ved 2 °C. Selv uten mikrobiologiske data etter 33 dager, så var den sensoriske testen ved dette utvidede avslutningstidspunktet fortsatt tilfredsstillende.

Project Background & Objectives.

Nutritional labelling has become mandatory in the present EU regulation of information to food consumers (*Regulation UE 1169/2011*), and this regulation has fully entered into force (after transitory periods) on the 13th, December 2016. Thus, the nutritional information should be transferred across the trade chain in order to assure the consumers become informed about the product features.

There are two different present nutritional databases available for labelling purposes in Norwegian traditional products, which correspond to http://www.matvaretabellen.no/ (references in stockfish from 1958) and NIFES Sjomatdata (http://www2.nifes.no/sjomatdata/) (from 2003). Therefore, nutritional data labelled by companies should be accurate and needs be updated. The labelling regulation for the



Brazilian markets is defined in some legal documents¹, and has many similarities with the EU environment.

Complementary, producing companies must perform controls within their internal management to assure the safety, quality and traceability of their commodities. Some examples of this are hygiene, microbiological and contaminant analysis.

Microbiological criteria in food products across the EU have been derogated with the exception of the criteria included in Reg. CE 2073/2005, leaving 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.

Some criteria still could apply to heavy salted products. If we consider a heavy salted product as a product ready to be consumed, Reg. CE 2073/2005 determines a limit for Listeria monocytogenes. Producing companies still apply microbiological controls that had been performed before the derogation of the Regulation.

The updating of nutritional database and screening of food safety parameters is one of the key points in the priority issues of the Norwegian salted fish & dried fish industry. This project supports the industry in addressing their present necessities.

In view of the first results reported, extended project tasks were carried out. The objective of the extension was to evaluate the effect of desalting in nutritional values of saltfish and contaminant levels. In addition, taking advantage of the desalting trials and available materials a shelf-life test of MAP packed desalted whitefish was also implemented.

Project Organization.

ANFACO-CECOPESCA is the only organism participating in the project and therefore assumes all the planned tasks in close coordination to FHF representatives. Even though a Steering committee was not defined, the project progress and deliverables were submitted to revision to some Norwegian producers that had participated in providing the fish materials.

Project started at 01/02/2017. The expected end of the project was 30/09/2017 due to the initial delay in the project start compared to proposal. Since the tasks were further extended the end-date was one month re-scheduled up to 30/10/17.

Implementation.

Sampling

Sampling consisted in planning, gathering of fish products from several Norwegian producers, and shipment to ANFACO-CECOPESCA's laboratories in Vigo (Spain). Two shipments were carried out in March 2017. The first one for the clipfish materials (4 fish species) & saltfish (cod) supplied by four Norwegian producers, and the other for lutefisk, torrfisk (G. morhua), and rehydrated stockfish from three Norwegian companies. Different production batches from each producer were required. Materials were properly received and stored either in freezing (-21°c) or chilling (2-3°c) conditions until analysis.

¹ Resolução-RDC nº 359, de 23 de Dezembro de 2003 . Resolução-RDC nº 360, de 23 de Dezembro de 2003



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

The received materials were made laboratory samples and assigned an internal code for analysis, distributing materials and providers to maximize representation of the expected results.

The activities extension implied desalting clipfish and saltfish. 72 hours desalting time was selected with tap water at 2°c and a fish: water ratio of 1:5. Three water changes were made at 8h, 24h and 48h. Containers with samples were preserved in a cold chamber at 2°c all along the desalting time. After desalting, product was carefully displayed on a food tray with a soaking pad, and inside a plastic bag, and packed under MAP using a mixture of CO2 and N2 (50:50) using an ORVED 18N instrument coupled to gas supply.

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 (1-2°c). 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). 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. After 6 days of rehydration, samples were individually vacuum packed and frozen (-20°c) until analysis.

Analysis.

The set of analysis was carried out. For the preparation of the heavy-salted samples, the protocol described in the Portuguese regulation (Decreto-Lei 25/2005)² was applied. Analysis of the **moisture**, **fat**, **and ashes** content was developed following gravimetric procedures meanwhile **protein** was obtained after sample mineralization, Kjeldahl distillation and acid / base volumetric titration. Fatty acid profiling was assessed using a standardized gas chromatography method. The energy and carbohydrates content were calculated. All these methods are certified by the Spanish Accreditation body (ENAC). **Water activity** was determined by using the Aqualab 4TE instrument.

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, Ca, Mg, Zn, and P. The levels of Na will be used as the key to salt (NaCl) content making use of the Na:Cl factor. Selenium levels were determined by ICP-MS.

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 under accreditation of ENAC (Spanish National accreditation organism).

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² Ministério da Agricultura, Pescas e Florestas. Decreto-Lei n.º 25/2005. Condições de comercialização de bacalhau seco. Diário da República: I Série; N.º 20. 2005-01-28, 696-703 p..Série:l.: 2005-01-28



Heavy metal analysis (mercury, lead and selenium) were performed following ENAC certified methodologies based on atomic absorption spectroscopy (DC-AAS, GF-AAS). The determination of PCBs & dioxins compounds was performed by gas chromatography with mass detection after extraction and purification of fish fat.

Nutritional Results

The nutritional results are available in the analytical results report (part 1) that can be downloaded at the web of the project 901307 in the FHF site. Proposed labels can be seen at Annex I.

In general it was registered that there are several divergences between mean reported values in some parameters (humidity, minerals) in saltfish and clipfish compared to Norwegian production standards (*Produksjon av saltfisk og klippfisk (Norsk Bransejenorm for fisk) / Standard Saltfisk-og klippfiskprodukter.* Norsk Bransjestandard for fisk). Wet salted cod contains in average 57,8 ± 1.6 % humidity. The 7/8 drying applied to wet-salted cod reduces humidity and consequently leads to an increase of around 4 percentage points in protein and half a point in salt. As expected, fat levels are very low but may vary in specific individuals up to approximately 1% content. The same effect occurs to other clipfish species whose humidity level varies from 51-54%. Humidity of dried salted cod (7/8) for the Portugal market remains at 50,5%. Even though the number of replicates has been limited, the intensity of the drying process varies between producers, but this variation may be explained by the different requirements of the market countries to where the products are intended to be exported.

Models of nutrition labels, including the EU minimum requirements for each one of the products, have been presented and expressed as required.

From the analyzed minerals, only selenium has significant levels in the product (as consumed) that may lead to the inclusion of a nutritional claim and/or derived health claims accepted by the EU. The rest of the minerals, in wet / dried-salted products shall be re-analyzed after being desalted before making any interpretation, but it seems that remaining levels might not be important. This can also be applied to fat related nutrients (EPA&DHA).

The effect of desalting on nutritional values was investigated and the results were consistent with available data from previous studies. 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).

Not only sodium is affected by the osmotic balance but also other minerals. Phosphorus and especially potassium levels fall down during desalting as a result of a transfer from muscle tissue to rehydrating water.



Microbiological and contaminant results.

The microbiological and contaminants results have been presented in a specific report that will not be made public, but internally addressed and discussed by the Norwegian industry. As a summary, the full set of results was satisfactory to legislation presently into force at the EU and Brazil. Microbial loads were minimal in clipfish; but stockfish (*G. morhua*), lutefisk and rehydrated stockfish contained moderate levels of aerobic mesophilic microorganisms which seem to be related to original fish bacteria strains and to its particular prolonged drying process and no hygiene concern can be interpreted from the obtained results.

Shelf life test performed in desalted whitefish packed under modified atmosphere 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. In general, the full set of microbiological analysis performed can be considered as satisfactory, since poor microbial loads were found. Only aerobic mesophilic microorganisms at day 17 can be considered for discussion, but still not of any safety concern. 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).

Despite any additional microbiological results at longer preservation times, the sensorial test performed on three packed samples (ling, cod, saithe) at day 33 was still satisfactory evidencing that long shelf-life can be achieved with the combination of appropriate packaging and low chilled preservation temperatures.

Organic pollutants were far below the legal threshold, as well as cadmium and lead results. Nevertheless, some moderately high mercury results in some replicates of dried salted ling and tusk were recorded as it has been previously recorder by NIFES studies (https://nifes.hi.no/en/prosjekt/contaminants-in-tusk-ling-and-other-deep-sea-fish-from-norwegian-sea-areas/. The mercury values seems not to be affected by desalting and the obtained desalted factors (0,82-0,92) can be attributed to the change in desalting yield.

Discussion, main achievements and project impact.

Updated nutritional facts of the analyzed materials have been laid down including suggestions for labelling. Some reference values and definitions included in the Norwegian saltifsk / klippfisk production standards (Standard Saltfisk-og klippfiskprodukter.- Norsk Bransjestandard for fisk & Produksjon av saltfisk og klippfisk - Norsk Bransejenorm for fisk) are not consistent to some of the obtained results, so probably this standards would need to be updated.

Some differences in the degree of drying were detected between providers, so the nutritional values should be made specifically. Other option would be the setting of more strict technical criteria regarding the final humidity content for each type of product (degree of drying), that would necessary force the industry to adapt and homogenize criteria regarding the drying applied to fish.

It has been detected a significant difference between final humidity content in cod (81,1%) and the rest of the species (75% approx.) after desalting that can be explained by 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). Therefore, the use of either dry-salted or wet-salted as raw materials for desalting leads to



final results, and this factor should be considered in addition to the final quality of the desalted products (texture, aroma,...), yields and raw materials costs prior desalting.

From the analysed minerals, only selenium may lead to levels that permit the development of some nutritional in labelling, but this should be further investigated in the product as consumed (desalted). Alike the drop in the sodium levels, phosphorus and potassium experiment a considerable loss during desalting remaining at very low concentration in desalted products. This is important since the use of phosphates in heavy salted cod would not generally lead to phosphate excess in the final desalted product (as it is consumed). In this sense, it should be reminded that the use of phosphate additives during desalting is not permitted by the EU legislation.

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 analyzed heavy metals in clipfish were far below EU legislation levels, but further investigation regarding mercury should be made in tusk and ling. Probably the feeding behavior and also bigger sizes/ages of fish may lead to the moderate-high levels found in some of the individual samples. Mercury seems not to be significantly affected by desalting process. The opposite occurs to rehydration of stockfish in ling and tusk, where mercury values become reduced proportionally to the mass balance. Calculated factors should be taken into account by producers to a higher extend into their HACCP system.

The salted, dried and dried-salted products analyzed do not have relevant microbial loads and these levels are still below quantification limits soon after the end of the desalting trials. MAP ($CO_2:50\%$ / $N_2:50$) packaging of desalted products without additives has been longer than previously expected. The microbiological loads were very low at the end-point of the shelf-life study (day 17). The sensorial test additionally carried out at day 33 was still satisfactory for these products.

To the contrary, stockfish (G. morhua) contains low-moderate levels of aerobic mesophilic microorganisms, but seem to be related to original fish bacteria strains and to 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 were rehydrated and put into market chilled or defrosted. No hygiene concerns can be interpreted from the obtained results.

Key project achievements:

- Updated basic nutritional database for clipfish (saithe, ling, tusk, cod), saltfish (cod), desalted products, stockfish (cod, haddock, ling, saithe & tusk) and rehydrated cod stockfish, dried cod heads and lutefisk. Effect of desalting. Suggestions for labelling of the products mentioned above.
- Reporting of the safety of the previous seafood products regarding microbiological and contaminants hazards based in international criteria. Stockfish 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.
- Mercury factors during stockfish rehydration and clipfish desalting in ling and tusk are related to mass balance.



- Revision of literature for published microbiological studies in desalted products.
- Evidences of extended shelf-life of desalted products (without additives) up to 17 days, or even
 33 days (sensory testing without microbiological data), by using MAP (CO₂/N₂) packaging and low temperature (2°c) of storage.

List of deliverables.

The deliverables submitted were:

- D 1: Updating of analytical data for the nutritional labelling of traditional clipfish, saltfish & stockfish.
- D.2. Updating of analytical data of traditional clipfish, saltfish & stockfish. Microbiological and contaminant results.
- D.3: Report of extended tasks: Desalting trials. Nutritional composition and shelf life testing of packed desalted clipfish.
- D.4: Information sheet / Faktaark.



ANNEX I: NUTRITIONAL LABELS.

Nutritional information	Wet salted cod	
	Per 1	100g
Energy	378 kJ	89 kcal
Fat	<0,5	g
of which		
saturates	<0,1	g
monounsatured	<0,1	g
polyunsaturated	<0,1	g
Carbohydrates	<0,5	g
of which		
sugars	<0,5	g
Protein	22	g
Salt	18,0	g

Nutritional information	Dried salted cod (7/8) Type B	
	Per	100g
Energy	448	106
Lifeigy	kJ	kcal
Fat	<0,5	g
of which		
saturates	<0,1	g
monounsatured	<0,1	g
polyunsaturated	<0,1	g
Carbohydrates	<0,5	g
of which		
sugars	<0,5	g
Protein	26	g
Salt	18,7	g

Nutritional information	Dried salted cod (7/8) type A Per 100g	
Energy	503	119
Fat	<0,5	kcal g
of which		
saturates	<0,1	g
monounsatured	<0,1	g
polyunsaturated	<0,1	g
Carbohydrates	0,5	g
of which		
sugars	<0,5	g
Protein	29	g
Salt	18,3	g

Nutritional information	Dried salted saithe (7/8)	
	Per 1	100g
Energy	472 kJ	111 kcal
Fat	0,5	g
of which		
saturates	0,1	g
monounsatured	0,2	g
polyunsaturated	0,2	g
Carbohydrates	0,6	g
of which		
sugars	-	g
Protein	26	g
Salt	18,6	g

Nutritional information	salte	ied d ling (/8)
	Per	100g
Energy	516 kJ	122 kcal
Fat	<0,5	g
of which		
saturates	<0,1	g
monounsatured	<0,1	g
polyunsaturated	<0,1	g
Carbohydrates	0,5	g
of which		
sugars	-	g
Protein	29	g
Salt	17,8	g

Nutritional information	Dried salted tusk (7/8)	
	Per	100g
Energy	504 kJ	119 kcal
Fat	<0,5	g
of which		
saturates	<0,1	g
monounsatured	<0,1	g
polyunsaturated	<0,1	g
Carbohydrates	<0,5	g
of which		
sugars	<0,5	g
Protein	29	g
Salt	19,0	g



Nutritional information	Desalted cod	
	Per 1	00g
Energy	284 kJ	67 kcal
Fat	<0,5	g
of which		
saturates	<0,1	g
monounsatured	<0,1	g
polyunsaturated	<0,1	g
Carbohydrates	<0,5	g
of which		
sugars	<0,5	g
Protein	15,8	g
Salt	2,1	g

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

Nutritional information	Desalted saithe	
	Per 1	00g
Energy	368 kJ	87 kcal
Fat	0,7	g
of which		
saturates	0,2	g
monounsatured	0,3	g
polyunsaturated	0,2	g
Carbohydrates	<0,5	g
of which		
sugars	<0,5	g
Protein	19,7	g
Salt	2,8	g

Nutritional information	Desalted tusk	
	Per 1	00g
Energy	386 kJ	91 kcal
Fat	<0,5	g
of which		
saturates	<0,1	g
monounsatured	<0,1	g
polyunsaturated	<0,1	g
Carbohydrates	<0,5	g
of which		
sugars	<0,5	g
Protein	21,1	g
Salt	3,2	g



Nutritional		:kfish orhua)
information	Per	100g
Energy	1294 kJ	305 Kcal
Fat	1,5	g
of which		
saturates	0,4	g
monounsatured	0,6	g
polyunsaturated	0,5	g
Carbohydrates	<0.5	g
of which		
sugars	<0.5	g
Protein	72,6	g
Salt	0,88	g

Nutritional	Dried cod heads	
information	Per 100g	
Energy	912 kJ	215 Kcal
Fat	2,7	g
of which		
saturates	0,8	g
monounsatured	1,2	g
polyunsaturated	0,7	g
Carbohydrates	0,7	g
of which		
sugars	-	g
Protein	46,9	g
Salt	3,5	g

Nutritional	Rehydrated Stockfish		
information	Per 100g		
Energy	339 kJ	79 kcal	
Fat	<0,5	g	
of which			
saturates	<0,1	g	
monounsatured	<0,1	g	
polyunsaturated	<0,1	g	
Carbohydrates	<0,5	g	
of which			
sugars	<0,5	g	
Protein	19,1	g	
Salt	0,09	g	

Nutritional information	Lutefisk	
	Per 100g	
Energy	<160 kJ	<40 kcal
Fat	0,04	g
of which		
saturates	0,01	g
monounsatured	0,02	g
polyunsaturated	0,01	g
Carbohydrates	0,2	g
of which		
sugars	-	g
Protein	5,1	g
Salt	0,24	g



Nutritional information	Stockfish Haddock	
	Per 1	00g
Energy	1270 kJ	299 kcal
Fat	0,9	g
of which		
saturates	0,3	g
monounsatured	0,2	g
polyunsaturated	0,3	g
Carbohydrates	<0,5	g
of which		
sugars	<0,5	g
Protein	72	g
Salt	1,6	g

Nutritional information	Stockfish Ling	
	Per 1	100g
Energy	1269	299 kcal
Fat	0,7	g
of which		
saturates	0,3	g
monounsatured	0,3	g
polyunsaturated	0,1	g
Carbohydrates	<0,5	g
of which		
sugars	<0,5	g
Protein	73	g
Salt	3,4	g

Nutritional information	Stockfish Saithe	
	Per 100g	
Energy	1293 kJ	305 kcal
Fat	1,3	g
of which		
saturates	0,9	g
monounsatured	0,3	g
polyunsaturated	0,1	g
Carbohydrates	<0,5	g
of which		
sugars	<0,5	g
Protein	73	g
Salt	1,3	g

Nutritional information	Stockfish Tusk	
	Per 100g	
Energy	1180 kJ	272 kcal
Fat	0,8	g
of which		
saturates	0,4	g
monounsatured	0,3	g
polyunsaturated	0,1	g
Carbohydrates	<0,5	g
of which		
sugars	<0,5	g
Protein	67	g
Salt	4,6	g