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Ripening of salted cod

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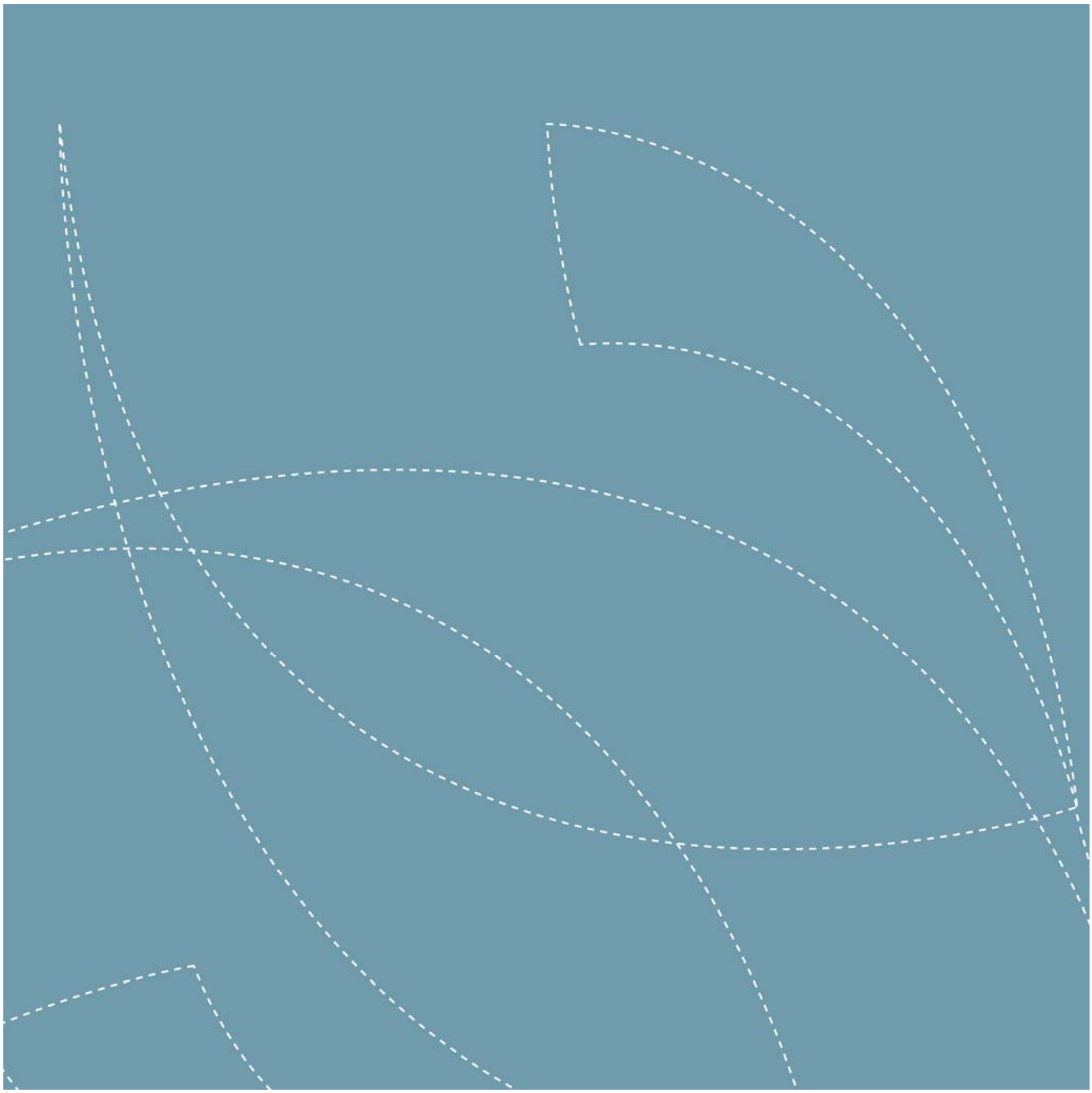

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1 Summary

Several projects have been carried out concerning methods for salting and of other steps in the processing for salted cod, but there is a lack of knowledge when it comes to explaining what ripening of cod is and who this is affected by the processing variables. Our wish is to increase yield to achieve higher profit in the salt fish industry, but at the same time it is very important to have better understanding of the how the processes affect the characteristics that define salt fish. The aim of this project has been to investigate processes that lead to ripening of salted cod especially in this relation to establishing new methods for measuring degree of ripening. The methods that have been tested are fluorescence spectroscopy, GC-sniff and proteolytic enzyme activity. Traditional chemical and sensorial methods have also been carried out as referent methods. The salt fish samples were produced industrially on Iceland, The Faeroe Islands and in Norway and effects of various production variables was studied in relation to degree of ripening.

2 Introduction

Market studies carried out in South European countries and in the Nordic countries the five recent years have revealed that young consumers are lacking knowledge concerning preparation of fish products. These consumers generally opinion is that dishes of fish tastes good when prepared by parents or grandparents, but in many cases, they do not know how to make the dishes themselves. The decline in consumption of fish is largest for fully preserved fish as salted, dried-salted (klipfish) and dried (stockfish) cod fish and is explained by the lack of product adaptation towards new/younger consumer. These products have to be prepared for 2- 10 days before consumption, and are therefore not adapted to younger generations, who prefer ready-to-use dishes.

Producers of salted fish, klipfish and stockfish must therefore start developing new products that are more consumer friendly, i.e. more easy and rapid to prepare. Two examples of products are ready-to-use products of desalted cod added marinades with various herbs/tastes or pickled products with long shelf life (antipasti). To achieve this goal, the producers, mainly located in the Nordic countries, need more knowledge concerning the factors that affect the product ripening. There is already a large amount of experience in the industry, but there has been carried out very few systematic studies of ripening or salted fish, klipfish or stockfish. A Nordic study on ripening of salted cod could therefore by the first step in the direction of more consumer adapted products of salted cod.

3 Theory and methods

Fish species as cod, saithe, haddock, torsk and ling are the main raw materials for production of salted cod and klipfish in the Nordic countries. All these species have a relative low fat content of 0-2 % fat. In spite of the low fat content, the fish muscle is highly exposed to rancidity when salt-cured and/or dried. This is due to the location of the fat, being situated in the muscle cell membranes. When large amounts of the water is removed from the muscle during the salt-curing or drying, the contact between the fat and several catalytic compounds increases as well as the oxygen supply. The membrane fat is mainly build up of phospholipids containing an especially high amount of poly unsaturated fatty acids that very easily can become rancid. A yellow-brown discolouration of the muscle surface is the most important negative result of rancidity for salt fish and klipfish. This is caused by a reaction between oxidized fat and components of the protein fraction.

A certain degree of rancidity is both necessary and desirable for sufficient ripening of the products. However, this process should be controlled to obtain a desirable degree of ripening based on consumer preferences. Today sensory evaluation is the most common method for determining the level of ripening for salt fish products. This method is both expensive and time consuming, and requires long extensive training for the panellists to obtain reproducible results. Therefore it is necessary to establish more rapid, cost efficient and objective methods for describing the degree for ripening in salted products.

Mainly the rancidity process can be divided in two types; the enzymatic and the non-enzymatic process.

A) Enzymatic rancidity can occur in fish, but the levels of lipolytic enzymes in the muscle are low in lean types of fish.

B) The non-enzymatic rancidity is auto catalytic and is most significant during storage of fish products.

4 Factors affecting the non-enzymatic fat oxidation

4.1.1 Frozen storage

Even if decreasing the temperature reduces the oxidation rate, rancidity will occur at frozen storage. Long periods of frozen storage of the raw material before the production of salted cod can result in highly oxidized muscle before the processing even begins.

4.1.2 Muscle damages during handling

When the muscle cell membrane becomes damaged, iron from the cytoplasm is released resulting in enhanced oxidation rate. Rough handling of the fish during capture can result in severe damages to the muscle cell membranes. In addition, if the fish also dies during capture, the elevated level of blood in the fish muscle after gutting will catalyse additional oxidation

4.1.3 Fat composition

The fat composition determined by the degree of unsaturated fatty acids and the amount of phospholipids has considerable significance for the oxidation. High levels of unsaturated fatty acids results in a muscle highly exposed for oxidation. The feed affects the fat composition, probably also having a season variation for wild fish.

4.1.4 Water phase pH

It has been shown that fatty acid oxidation is considerably restrained by pH-values above 6. Muscle pH in cod fish post death is affected by fish size, degree of maturation and feed intake prior to death. Normal post rigor pH for cod fish is in the range of 6,3-7,0.

4.1.5 Muscle water activity

Oxidation rate depends on the water activity (aW) in the muscle. Rancidity occurs most rapidly at aW 0,8 and most slowly at aW 0,2. During salt-curing the aW falls to 0,7-0,8 resulting in maximum rancidity. During freezing of the fish, the water activity also declines in the remaining water phase and the oxidation will increase.

4.1.6 External factors

The most important external factors are light (especially short waved), oxygen and temperature. High exposure to light, oxygen and temperatures above 0 °C all accelerates fat oxidation.

4.1.7 Pro oxidants and anti oxidants

Examples of pro oxidants that enhance oxidation in salted fish are the metal ions Cu^{+}/Cu^{2+} and Fe^{2+} as well as iron in haemoglobin and myoglobin. Anti oxidants that can inhibit the oxidation are, for example, the naturally occurring tocopherol, citric acid and ascorbic acid. These compounds bind to free radicals and therefore break the oxidation chain. Pro and anti oxidants are during processing of fish found in water, the salt and the process equipment that are in contact with the raw material throughout the processing.

4.2 Interactions between fat and protein during ripening of codfish

There has been carried out a limited number of studies aiming to reveal the protein degradation during ripening of fish muscle to fully ripened salted cod. The limited results show that some enzymes can be active in spite of high concentrations of salt. How the enzyme activity and the end products of it affect the ripening process and the sensory properties has, for the time being, not been documented. It is reasonable to assume some form of non-enzymatic oxidation of muscle proteins that can affect the degree of ripening. In addition, there is also a lack of established methods for detection of protein oxidation.

As the concentration of compounds produced by rancidity increase during salt ripening, they will most likely react with free amino acids located on the muscle proteins (eta lysine) forming yellow brownish macro molecules. These compounds entail not only undesired discolouration of the product, but also the development of undesired taste and smell. Due to the interactions between fat and proteins, the nutritional value of the product will be reduced because of the reduction of essential amino acids and fatty acids. In addition, the content of carcinogen compounds produced by the rancidity increases. Therefore, it is of great interest to be able to control the reactions occurring during salt ripening of salted fish.

4.3 Variables during production that can affect the ripening of salted fish

Properties of the fish muscle

- Rigor status when salted
- Muscle-pH when salted
- Haemoglobin level
- Freezing-Thawing before salting
- Chilled storage before salting

External factors

- Salt composition
- Salting method
- Brine composition
- Light
- Temperature
- Oxygen
- Storage period
- Water content/surface dryness

4.4 Methods for measurement of degree of ripening

The samples were collected from different processing places in Iceland, Norway and Faroe Islands. The sample identification used at Matís is shown in the Table 4.

4.4.1 Fluorescence spectroscopy

Fluorescence spectroscopy has proven to be an adequate rapid method for measurement of fat oxidation in poultry meat (Wold, 2000) and in diary products as cheese and sour cream (Wold *et al.,* 2005). Preliminary studies of fluorescence spectroscopy on klipfish made of saithe have shown a strong positive correlation between fluorescence spectra and degree of ripening of fish meat (Lauritzsen *et al*., 2005). This has also been shown for analysis of total amount of volatile nitrogen compounds (Sivertsen *et al.,* 2006). The traditional methods that

are most commonly used for these two analyses, TBARS and direct distillation, are both very time and cost consuming.

Measurement setup

Measurements of fluorescence were performed on all the 8 samples with the optical system shown in Figure 10. The excitation light source used was a 1mW UV led with a center wavelength of 375nm and a 3dB bandwidth of 24nm (#260019 Marl optosource), this gave approximately 2x more energy on the sample than was used in Sivertsen et. al. 2006 and a more even distributed light across the sample. The light was directed onto the sample at an angle of 50º and a distance of 50mm. The samples were placed into plastic sample cuvettes with a diameter 50 mm and depth of 10 mm exposing a flat circular surface for measurements. A sharp 420 nm long pass filter (#03FCG059 Melles Griot) was placed in front of the spectrometer to prevent reflected excitation light from overlapping the auto fluorescence light. The CCD detector (PIXIS 400B, Princeton Instruments Inc., Trenton, NJ) was mounted on a spectrograph (Acton SP-2150, Acton Research Group., Acton, MA), and cooled to -55ºC by a piezo cooling element. The entrance slit used in the spectrograph was 30 micro meter wide, and the grating used had 300 grooves/mm with a grating blaze at 500 nm. An exposure time of eight seconds were used per sample. Each sample was measured three times, were the sample was rotated 90 degrees between successive samples. This was done to remove some of the effects from uneven sample surface and directional illumination. The data recorded by the spectrometer is a spectra of 1340 16 bit values representing the raw counts registered by the spectrometer in the region 361.3-930.7nm. Since the cut-off filter removed all lights below 420nm, the spectra was cropped to the region 430-930nm. The black painted laboratory was kept at 18ºC and had a minimum of stray light, and the temperature of the samples was approximately 18ºC.

4.4.2 Measurement of colour

The colour of saltfish and klipfish is important for the price of the product. Measurements of colour have earlier been an adequate method for detection of degree of ripening for salt-ripened cod and saithe. Instruments like Minolta Chrometer and X-rite D22 can carry out these kinds of measurements in the L, a, b modus, where values of whiteness, red-green and yellow-blue colour are detected (Lauritzsen et al., 1999; Lauritzsen et al., 2004).

4.4.3 Sensory evaluation of salt-ripened and desalted products

The sensory properties studied were chosen based on the most important characteristics of the products from Iceland, Faroe Islands and Norway. An experienced panel of 8-10 persons can carry out describing tests, where each sensory property is given a value on the scale 0-3 for raw fish (Joensen *et al*., 2005) and on the scale 0-10 for cooked desalted products (Esaiassen *et al*., 2004; Joensen *et al.,* 2005; Magnusson *et al*., 2006). The sensory panel will in advance be trained to judge the selected sensory properties.

4.4.4 Total volatile nitrogen (TVN)

The amount of volatile nitrogen that is formed in fresh fish stored at chilled temperatures depends on the bacterial growth in the product. Volatile compounds like TMA, TMAO and DMAO will, among others, contribute to the amount of TVN. Chemical determination of TVN gives an indirect measure of the bacterial growth and is often used as a method for describing the product freshness. The freshness of the product can effect the further oxidation during the salt-curing process of saltfish or klipfish production. The content of total volatile nitrogen is measured by direct distillation adjusting pH by adding magnesium oxide (AOAC 920.03, 1990)

4.4.5 Thiobarbituric acid reactive substances (TBARS)

The level of oxidation can be determined chemically by measuring the content of thiobarbituric acid reactive substances (TBARS). This is the most commonly used method for determination of oxidation in food articles. Secondary oxidation products are extracted from the sample in tri chloride acetic acid that further forms are red coloured complex with tiobarbitursyre and finally measured spectrophotometerically (Dulavik *et al*., 1998).

4.4.6 Muscle-pH after death

Muscle-pH is a very important parameter due to its effect on many properties of the fish muscle; oxidation, filet gaping, sour taste, consistency, juiciness and storage stability. Normally, muscle-pH in cod fish is in the range 6,3 to 7,0 depending on the feed status of the fish and degree of maturation. When the fish muscle is salted the pH normally decreases towards 6,0. Filet gaping and drip loss increases with a reduction in pH because the isoelectric point of fish muscle is pI~5.2 (Morrissey *et al.*, 1987). At the isoelectric point the protein net charge will be zero and the repulsive forces between the muscle fibres will be at the lowest level giving low water holding capacity (Offer *et al*., 1989). Additionally, it has been shown that low muscle-pH (6,38) entails increased yellow discolouration of salt ripened cod compared to high muscle-pH (6,95) (Lauritzsen *et al*, 1999; Lauritzsen and Olsen, 2004)

4.4.7 Proteolytic enzyme activity

In Stoknes et al. (2005) the activity of alkali and acid proteases in muscle tissue during salt-curing of cod at three different salting methods where investigated. The effect of raw material (fresh versus frozen) on proteolytic activity was also studied. The results showed that the activity of chymotrypsin, trypsine, collagenase and elastase was stimulated by increased salt concentration, but decreased later in the salt-curing process. Haemoglobin hydrolyzing activity decreased however with increased salt concentration in the muscle tissue.

The effect of proteolytic activity on the development of sensory characteristics has not been studied. Esaiassen et al. (2004) and Joensen et al. (2005) have carried out sensory studies of fresh and salt-cured cod, respectively, but the connection between sensory properties and enzyme activity was not investigated. There is a need for optimising the desirable effects of proteolytic activity during salt ripening of fish. This can be studied by carrying out control model trials where specific proteases are controlled (increased/decreased) and the effects on quality parameters as colour, texture, taste and smell are measured

4.4.8 Water, protein and salt in fish muscle

Water, proteins and salt content in the fish muscle determines to a large extent the product water activity. The water activity affects the storage stability concerning development of colour and the fat oxidation of the product. The water was calculated as the loss in weight during drying at 105°C for four hours (ISO 6496, 1999) /(*AOAC* 950.46, 1990). The protein content is measured as Kjeldahl protein (AOAC 981.10, 1990). Muscle salt concentration is measured by a standard method based on titration with silver nitrate (AOAC 937.09, 1990).

4.4.9 Rehydration

Four groups were used for training of the sensory panel, N1, N4, ISV1 and ISV4 where the rehydration was carried out in two steps, for 24 hours in the ratio 1:5 (weight of fish: weight of water), followed by rehydration for 4 days in the ratio 1:4. Due to high salt content of the fish, it was rehydrated for additional 24 hours, in the ratio of 1:1.5, before sensory analysis.

4.4.10 Rehydration yield

The rehydration yield was calculated from the weight changes during rehydration, defining the value before rehydration as 100%.

4.4.11 Sample preparation

Samples were collected from three pieces in each group and pooled together in a mixer (Braun Electronec, Type 4262, Kronberg, Germany) before analysis.

4.4.12 Water holding capacity

The water holding capacity (WHC) was determined by a centrifugation methods (Eide et al 1982). Approximately 2 g of the minced fish was weighed accurately and centrifuged (Heraeus Biofuge Stratos, Kendro Laboratory products, USA) at 210 x g, for 5 minutes at 0-5°C. The weight loss after centrifugation was divided by the water content of the sample and expressed as % WHC.

4.4.13 Sensory evaluation

Quantitative Descriptive Analysis (QDA), introduced by Stone and Sidel (1985), was used to assess cooked samples (MA07sky047-050) of eight sample groups of desalted cod (Table 1). Nine panellists all trained according to international standards (ISO 1993); including detection and recognition of tastes and odours, trained in the use of scales and in the development and use of descriptors participated in the sensory evaluation. The members of the panel were familiar with the QDA method and experienced in sensory analysis of cod. Three sessions were used for training prior to the sensory evaluation. The panel was trained in recognition of sensory characteristics of the samples and describing the intensity of each attribute for a given sample using an unstructured scale (from 0 to 100%). Most of the attributes were defined and described by the sensory panel during other projects (Magnússon et al 2006). The sensory attributes were 30 and are described in Table 1.

Samples weighing ca. 40 g were taken from the loin part of the fillets and placed in aluminium boxes coded with three-digit random numbers. The samples were cooked for 6 minutes in a pre-warmed oven (Convotherm Elektrogeräte GmbH, Eglfing, Germany) at 95-100°C with air circulation and steam, and then served to the panel. Each panellist evaluated duplicates of each sample in a random order in four sessions (four samples per session).

A computerized system (FIZZ, Version 2.0, 1994-2000, Biosystémes) was used for data recording.

4.4.14 Data analysis

QDA data was corrected for level effects (effects caused by level differences between assessors and replicates) by the method of Thybo and Martens (2000). Principal Component Analysis (PCA) on mean level corrected values of sensory attributes and samples was performed. Analysis of variance (ANOVA) was carried out on QDA data corrected for level effects in the statistical program NCSS 2000 (NCSS, Utah, USA). The program calculates multiple comparisons using Duncan's multiple comparison test. The significance level was set at 5%, if not stated elsewhere.

*Table 1 Sensory vocabulary for cooked samples of desalted cod (*Gadus morhua*)*

Sensory attribute	Description of attribute
Odour	
characteristic	Characteristic for desalted cod
sweet	Sweet odour
vanilla/boiled milk	Boiled milk, mushy
boiled potatoes	Odour reminds of boiled potatoes
sea	Fresh sea, seaweed
butter	Butter, margarine, popcorn
earthy	Earth, mushrooms, musty, timber, wet timber, dampness
table cloth	Reminds of a table cloth (damp cloth to clean kitchen table, left for 36 h)
sour	Sour odour, spoilage sour, acetic acid
TMA	TMA odour, reminds of dried salted fish, amine
sulphur	Sulphur, matchstick
Appearance	
light/dark colour	Left end: light, white colour. Right end: dark, yellowish, brownish, grey
homogenous/ heterogeneous	Left end: homogenous, even colour. Right end: discoloured, heterogeneous, stains
Flavour	
salt	Salt taste
sweet	Sweet flavour
sour taste	Sour taste, spoilage sour
ripening flavour	Charactieristic for desalted cod
sea	Metallic, sea, seaweed, algae
butter	Butter, margarine, popcorn
earthy	Earth, mushrooms, musty, timber, wet timber, dampness
TMA	TMA flavour, reminds of dried salted fish, amine
pungent	Pungent
frozen storage	Reminds of food which has soaked in refrigerator/freezing odour
Off-flavour/putrid	Intensity of off-flavour, putrid flavour
Texture	
flakiness	The fish portion slides into flakes when pressed with the fork
firm/soft	Left end: firm. Right end: soft. Evaluate how firm or soft the fish is during the first bite
dry/juicy	Left end: dry. Right end: Juicy. Evaluated after chewing several times: dry - pulls juice from the mouth
tough/tender	Left end: tough. Right end: tender. Evaluated after chewing several times
rubbery	Rubbery texture, chewing gum
foamy	Foamy, airy. Chew c.a. 5-6 times
clammy	Clammy texture, tannin (dry redwine)

4.5 Flavor Characterization by Headspace and GC

4.5.1 Sample preparation

Minced sample of salted cod were weighted (50 g) into a 100 mL vials Erlenmeyer flask. The samples were kept at -80°C and defrozen at 4°C over night before analyzed. Heptanoic acid ethyl ester was added as an internal standard to all samples by adding 0.5 mL of 10-mg/kg aqueous solution of the standard. Samples were kept at 25 °C for about 15 min before sample collection using HS-SPME.

4.5.2 Headspace solid phase microextraction (HS-SPME)

The SPME device and semi-polar fiber (polydimethylsiloxane/divinylbenzene [PDMS/DVB], 65µm) were purchased from Supelco (Bellefonte, Pa., U.S.A.). The fiber was conditioned before use in the GC injection port as recommended by the manufacturer. A blank analysis was performed to verify that no extraneous compounds were desorbed from the fiber. The SPME fiber was inserted through the spectrum of the sample vial and allowed to equilibrate with the headspace volatiles for 40 min. The fiber was then retracted into the barrel of the syringe and immediately inserted into the injector of the gas chromatograph. Duplicate analyses of each sample were done.

4.5.3 Gas chromatography-olfactometry

GC- sniff (or GCO) has earlier been used to characterise volatile compounds with smell during ripening of cod roe (Jonsdottir *et al*, 2004) and to characterise volatile compounds from chilled stored cod (Olafsdottir *et al*, 2005). The development of various degradation products during the chilled storage were described and quantified.

The volatile compounds on the SPME fibers were thermally desorbed for 2 min in the GC using splitless mode, with helium as the carrier gas at linear velocity of 22.9 cm/s. The volatiles were separated on a DB-5ms column (30 m × 0.25-mm inner dia × 0.25 µm, J&W Scientific, Folsom, Calif., U.S.A.). Measurements were performed on a GC (HP 5890, Hewlett-Packard, Palo Alto, Calif., U.S.A.). Helium was used as a carrier gas and the following temperature program was used: 50 °C for 7 min, 50 °C to 120 °C at 5 °C/min, and from 120 °C to 220 °C at 10 °C/min. The injector temperature was 250 °C and the detector temperature was 280 °C. The end of the column was split 1:1 between flame ionization detector (FID) and an ODO-1 olfactory detector outlet (SGE Intl. Pty. Ltd, Australia). Nitrogen, bubbled through water to add moisture, was used to drive the sample up to the sniffer. One person describing the odor sniffed the effluent two times. Intensity (quality and duration/retention times) of each odor was determined using an intensity from 0 to 5, 0 = not present; 5 = very strong. The assessor was trained in recognizing characteristic oxidatively derived odors by injecting into the GC-O, mixtures of standard compounds dissolved in ether and sniffing the effluent. GC- sniff (or GCO) has earlier been used to characterize volatile compounds with smell during ripening of cod roe (Jonsdottir *et al*, 2004) and to characterize volatile compounds from chilled stored cod (Olafsdottir *et al*, 2005).

4.5.4 Gas chromatography-mass spectrometry

The salted cod samples were prepared in the same way as for the GC-O measurements except that the volatile compounds were collected for 45 min at 100 ml/mL using Gilian LFS-113D Air sampler on 250 mg Tenax 60/80 (Alltech, Il, USA) in stainless steel tubes (Perkin-Elmer, Buchinghamshire, UK) for the combined ATD 400 and GC-MS measurements. Volatile compounds were thermally desorbed (ATD 400, Perkin-Elmer, Buchinghamshire, UK) from the Tenax tubes and separated with the same type of column and the same conditions as for the GC-O measurements. The mass detector ion range was 35-300 m/z. These measurements were done for identification of the volatiles.

4.5.5 Identification and quantification of the volatile compounds

Identification of the volatiles was done by matching retention indices (RI), calculated according to Van den Dool and Kratz (1963) based on ethyl esters (i.e., RI of ethyl pentanoate is 500) and verified by the database Flavornet (Acree and Arn, 2004), and mass spectra of samples with authentic standards (Sigma-Aldrich Chemical Co., St. Louis, Mo., U.S.A.). Tentative identifications were based on the MS library data in the HP GCD ChemStation software (Hewlett Packard). Semi-quantitative estimation of concentration of

components was done by calculating the peak area ratio (PAR), that is, the ratio between the total ion count of each peak and internal standard.

4.5.6 Statistical analysis

Multivariate analysis was performed by the Unscrambler 9.7 software package (CAMO AS, Trondheim, Norway). The main variance in the data set of GC-O values and GC-MS mean PAR values was performed was studied using principal component analysis (PCA). All the data were mean centered and scaled to equal variance prior to PCA. Cross validation was used in the validation method.

5 Results

5.1 Production of salt-cured cod

A total of 4 different samples of salt-cured cod were produced in Grindavik, Iceland (Thorbørn Fisk and Visir) Thorshavn, Faroe Island (Føroyar Fiskavirking) and in Vannøy, Norway (GPG AS). Samples were sent to Fiskeriforskning after 1 and 4 months of storage. Whole pieces of fish were cut out for sensory analysis and the rest homogenised before frozen storage at – 80 C. When all samples had been received and prepared, some were sent to Matis, Reykjavik, Iceland and to Møreforsking, Ålesund, Norway for analysis.

5.1.1 Føroyar Fiskavirking hf., Thorshavn, Faroe Islands

At Føroyar Fiskavirking fresh cod raw material was used for the production of splitted saltfish. The method used for salting was injection with brine followed by pickle salting. During the storage the salt-cured samples were stored in cardboard boxes with no light entering the boxes. Samples (5 fish) were shipped to Tromsø after 1 and 4 months of storage.

5.1.2 Visir hf., Grindavik, Iceland

At Visir the raw material used was fresh cod stored on ice for 5 days before processing. The fish were splitted before being injected with brine followed by 2 days in brine. After the brine salting the fish were dry salted at 14 °C. The salt used was of the type “Bahamas”. Further storage of salt-cured cod was carried out in light sealed cardboard boxes at 0-5 °C. The average weight of the 5 fish stored for 1 month was 3,13 kg after salt-curing and the average weight for the fish stored for 4 months was 3,17 kg.

5.1.3 Thorbjörn Fiskanes hf., Grindavik, Iceland

The raw material used at Thorbjörn Fiskanes was also fresh cod stored for approximately 5 days on ice before filleting and further salting with Tunis salt (Zaris 2007 WB). The fish were injected with brine, then stored for 1 day in brine and finally dry salted 12 °C. the storage of salt-cured fillets was done in light sealed cardboard boxes at 0-5 °C. The average weight for 1 and 4 months old salted fillets were respectively 1,03 kg and 0,90 kg.

5.1.4 GPG-Norge AS

At GPG the raw material used was fresh fish that was splitted before picklesalting at 2-4 °C. The further storage of the salt-cured fish was at -2 - + 2 °C in sealed boxes (no light).

5.2 Analysis at Fiskeriforskning, Tromsø

5.2.1 TBARS

The level of TBARS in the 8 samples of salt-cured cod is shown in Figure 1.

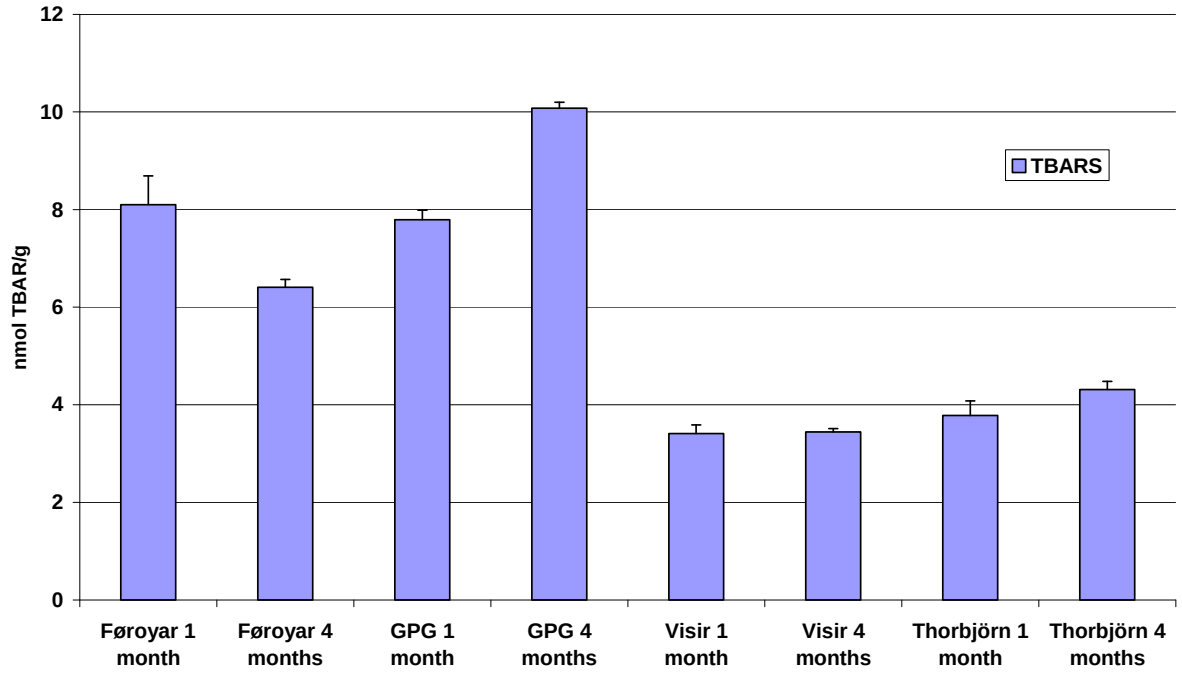


Figure 1 Level of TBARS in 8 samples (pooled samples of 5 fish each) of salt-cured cod stored for 1 or 4 months. Standard deviation for parallels is shown

In 3 of 4 groups the level of TBARS rises slightly from 1 to 4 months storage. The 2 Icelandic products have similar and lowest values at around 4 nmol TBARS/g fish muscle. Føroyar have the second highest levels, but the level dropped from 8,1 nmol TBARS/g at 1 month to 6,4 nmol TBARS/g after 4 months of storage. The highest levels were found in fish produced at GPG with levels just below 8 after 1 month and slightly above 10 nmol TBARS/g after 4 months.

5.2.2 Instrumental yellow (b*-value)

The results from the instrumental measurement of colour are shown in Figure 2.

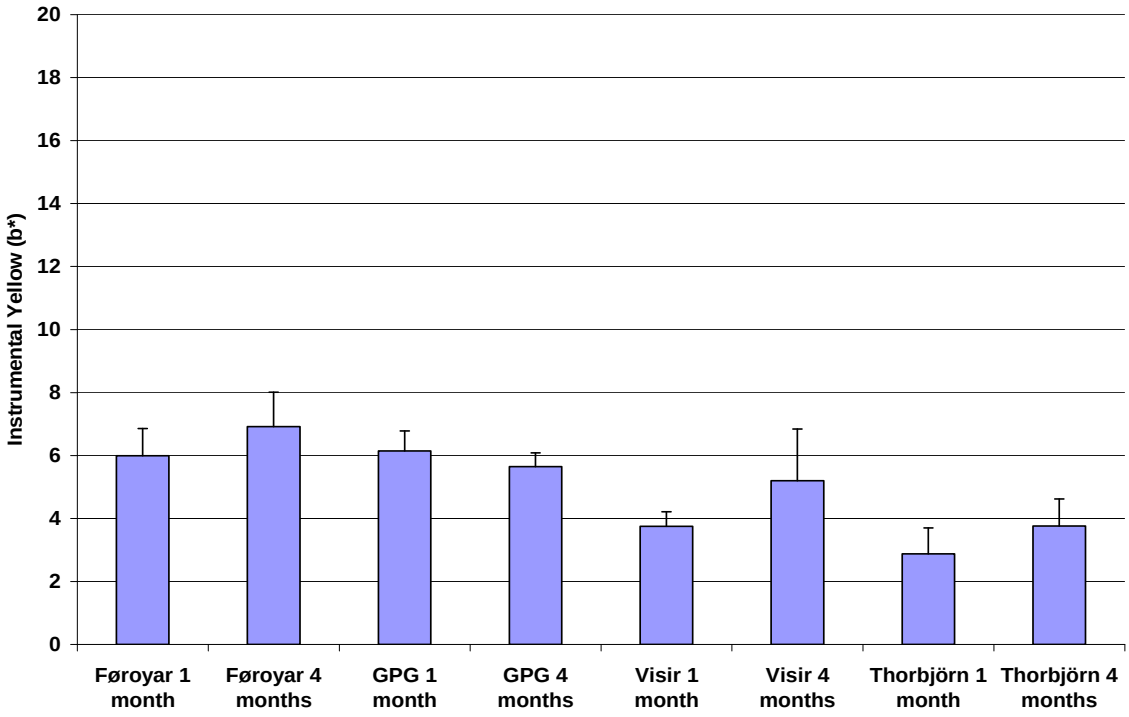


Figure 2 Instrumental yellow (b-value) in 8 samples (pooled samples of 5 fish each) of salt-cured cod stored for 1 or 4 months. Standard deviation for parallels is shown*

In 3 out of 4 groups the b*-value increased slightly from 1 to 4 months of storage, but the levels were relatively low for all samples. The lowest values of yellow (least yellow) were found in fish produced at Thorbjörn (3-4) while the highest values were measured in fish from Føroyar (6-7).

5.2.3 pH-value

The pH-value in the fish muscle was measured in duplicate for all 8 samples and the results are shown in Figure 3.

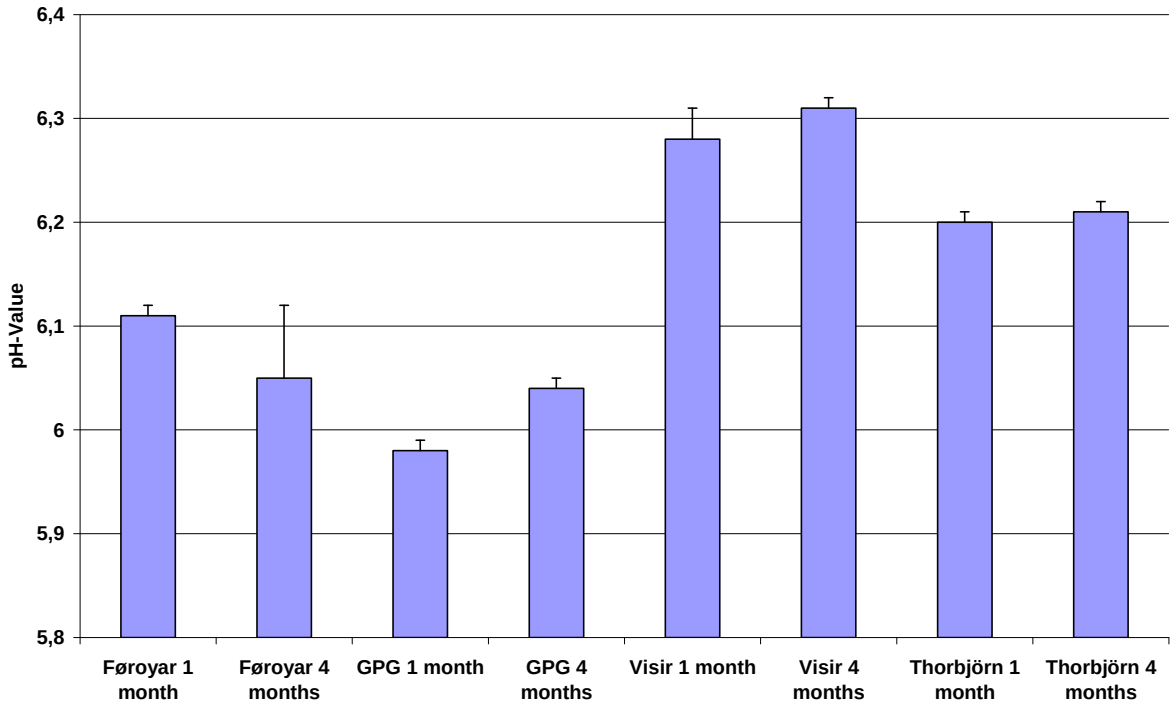


Figure 3 pH-value in fish muscle of 8 samples (pooled samples of 5 fish each) of salt-cured cod stored at 1 or 4 months. Standard deviation for parallels is shown

Also in these analyses 3 out of 4 groups increased in pH from 1 to 4 months. Highest pH was measured in fish from Visir (6,28-6,31) followed by Thorbjörn (6,2), Føroyar (6,05-6,11) and GPG (5,98-6,04).

5.2.4 TVN analysis

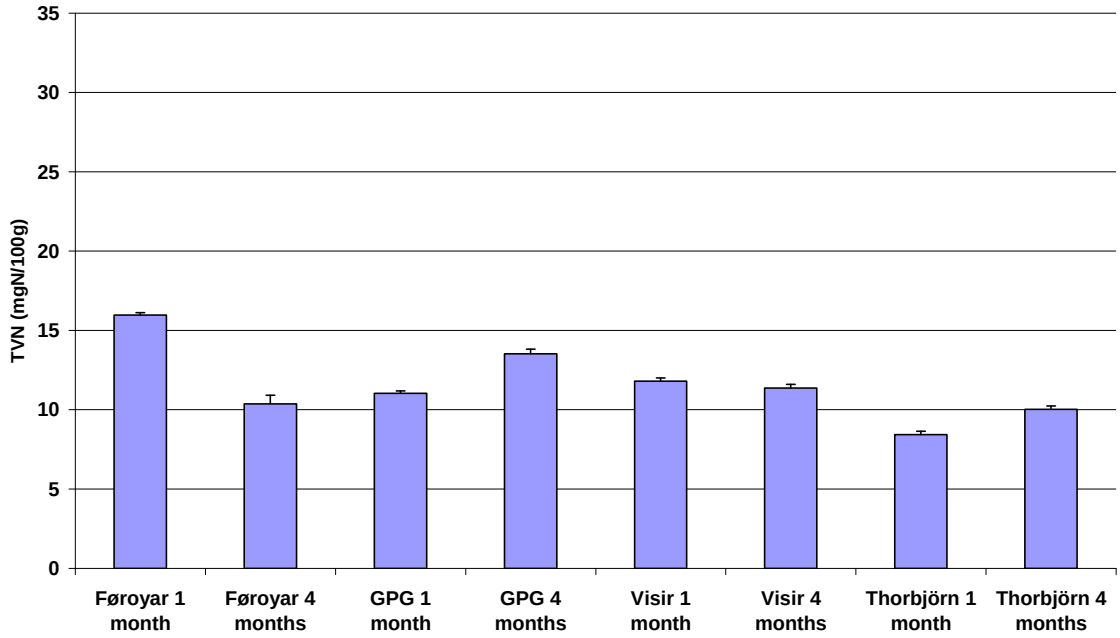


Figure 4 TVN-values in 8 samples (pooled samples of 5 fish each) of salt-cured cod stored for 1 or 4 months. Standard deviation for parallels is shown

As the results show in Figure 4 the TVN-values vary from 8,4 to almost 16 mgN/100g fish muscle. Salt-cured samples from Thorbjörn have the lowest values (8,4-10) and Føroyar the highest at 10-16 mgN/100 g fish muscle.

5.2.5 Protein content

The protein content was measured as Kjeldahl protein (AOAC 981.10, 1990) and is shown in Figure 5.

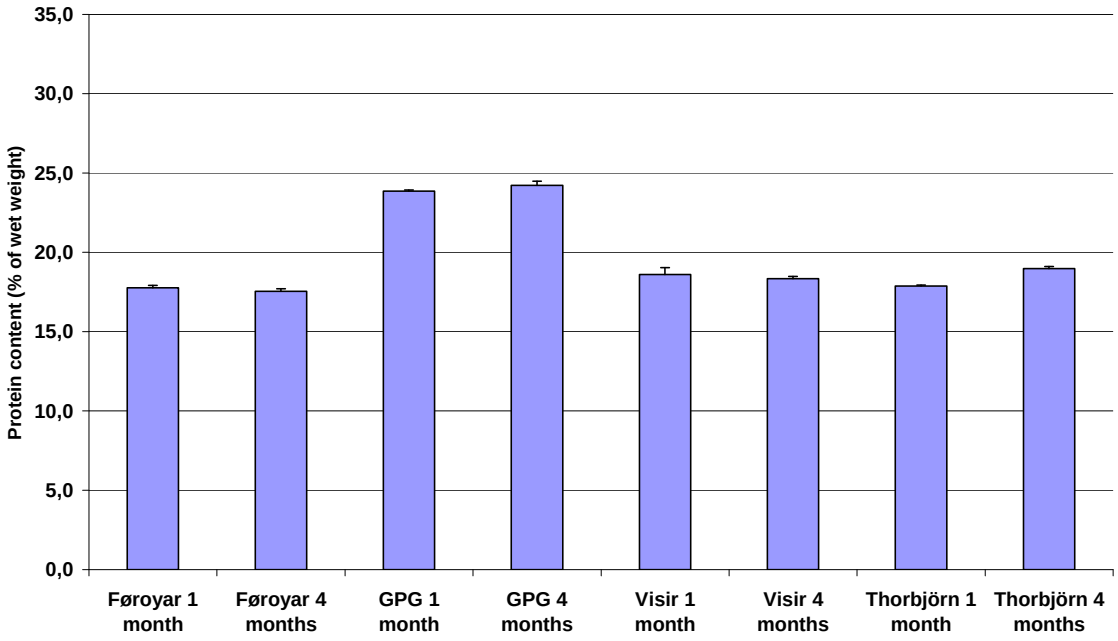


Figure 5 Protein content in 8 samples (pooled samples of 5 fish each) of salt-cured cod stored for 1 or 4 months. Standard deviation for parallels is shown.

The salt-cured cod produced at GPG had the highest protein content of about 24 %. The other products had a protein content of 17-18 %. There was no systematic change during the storage time, but the protein content was relatively stable for all samples.

5.2.6 Water content

The water content was determined by drying the product to a constant weight at 105°C As shown in Figure 6, the highest water content is found in samples produced at Føroyar (59-61 %). The lowest water content is in products from GPG (55 %). The two Icelandic products have a water content of approximately 57-59 %. The water content was stable during the storage for every group.

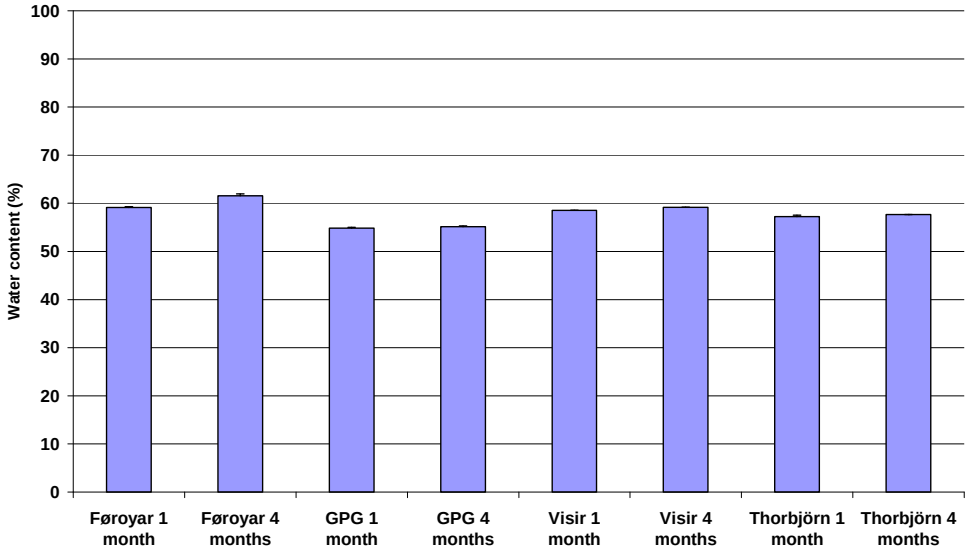


Figure 6 Water content in 8 samples (pooled samples of 5 fish each) of salt-cured cod stored for 1 or 4 months. Standard deviation for parallels is shown

5.2.7 Cathepsine D activity

Cathepsin D is one of the most important lysosomal proteases in fish muscle (Bonete *et al.*, 1984; Gildberg, 1988). It is an aspartic acid- proteinase that is effectively inhibited by the peptide Pepstatin A. As the name indicates, it will also inhibit pepsin. In pure fish muscle however, it is mainly the cathepsine D activity that is inhibited. Therefore the cathepsine D activity is defined as the activity that is inhibited by Pepstatin A (Gildberg, 1987).

After thawing of the 8 samples overnight at 10°C, 40 g of each sample was homogenised in 120 ml cold water (30 sec., Waring blender) and then centrifuged (10 min, 2000 x g). Chathepsine D activity was measured as amount TCA soluble protein material (in TYR eq.) after incubation in 2 % haemoglobin for 1 hour at pH 3 and 25°C.

To achieve a picture of total protein degradation during storage of the salt-cured cod, autolysis was also measured. This was determined by measuring the amount of liberated protein material (tyrosine equivalents /gram muscle) soluble in 5 % TCA. Table 2 shows cathepsin D activity and autolysis in the 8 sample of salt-cured cod.

Table 2 Cathepsin D activities in 8 samples of salt-cured cod measured as µmol tyrosine equivalents degraded haemoglobin per gram and hour at pH 3 and 25 °C. Autolysis measured as µmol tyrosine equivalents liberated low molecular muscle protein per gram muscle.

Sample	Føroyar 1 month	Føroyar 4 months	GPG 1 month	GPG 4 months	Visir 1 month	Visir 4 months	Thorbj. Fisk. 1 month	Thorbj. Fisk. 4 months
Cathepsine D	0.0667	0.2833	1.5498	0.0833	1.1958	0.8726	0.3717	0.7434
Autolysis	3.1506	2.6172	2.7005	2.7422	2.1573	1.8085	1.8085	1.9545

No conformity was found between high cathepsin D activity prior to the frozen storage and autolysis before frozen storage. The two samples with highest levels of autolysis (Føroyar 1 month and GPG 4 months) have the lowest cathepsin D activity. One explanation to these results could be a degradation of muscle proteins that also has entailed degradation of muscle enzymes. Highest cathepsin D activity was measured in the sample from GPG stored for 1 month and Visir stored for 1 month (1.5 og 1.2 µmol Tyr eq. per gram and hour).

5.2.8 Removal of salts before analysis of enzyme activity (Møreforsking, Ålesund)

When analyzing the enzyme activity in fish extracts it was found that the salt concentrations had detrimental effects on the performed enzyme activity assays. Tests were performed that showed a declining or stagnating trend in the enzyme activity when the salt concentration was 10% or higher (Figure 7). In addition, the high concentration of salts in the samples lead to precipitation, and accurate measurements of the enzyme activity were not possible to perform. Methods to remove the salts, either completely or partially were therefore pursued. The salts did not have to be completely cleared, but adequate to not precipitate.

Sigma recommended a small gel filtration column, but this method was evaluated to be too time consuming. Marianne Løfsgaard recommended Solid Phase Extraction (SPE), and Per Helland meant that we could use C-18 SPE columns, already available at MFAA.

C18-SPE

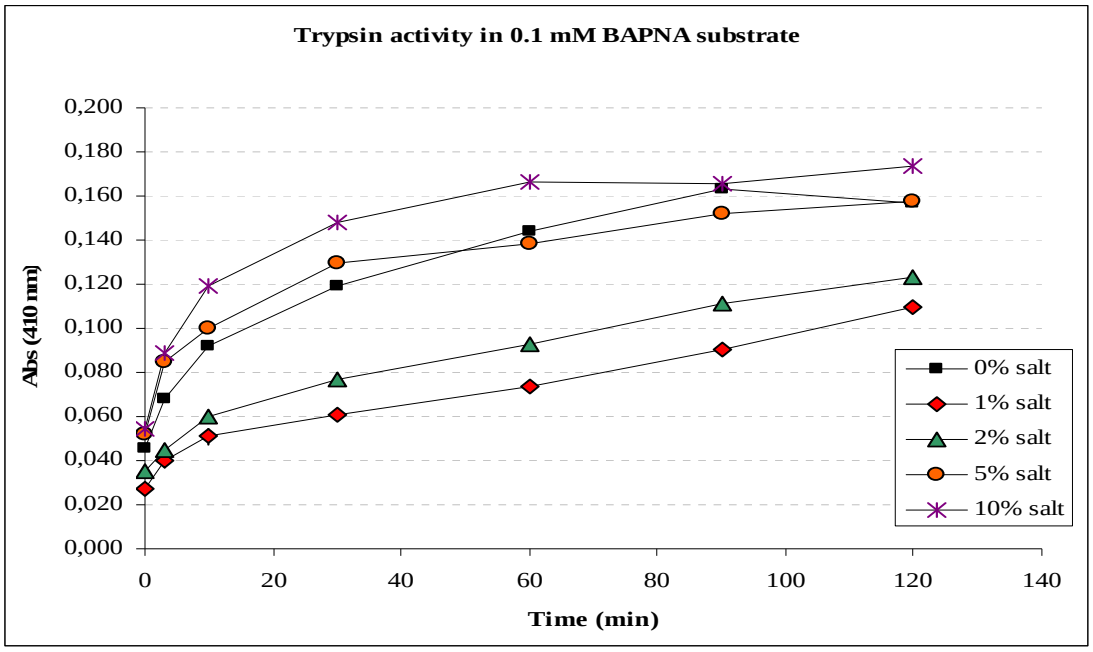
Salt-cured fish was homogenized and filtrated as described earlier. The C-18 columns were mounted on a vacuum manifold. 1 ml 100% methanol was passed through the column and subsequently 4 ml 0.01% Trifluoric acid (TFA) for calibration of the column. 500 µl sample was then added onto the column and slowly passed through to allow the proteins to bind to the column, and the salts pass through. 5 ml 0.01% TFA was used to wash the column and the samples were eluted with 500 µl 60% ethanol. The elute was stored at -20°C or 4°C until further use (four days).

The activity of Trypsin was measured on the elutes, using two parallels each from the elutes stored at -20°C and 4°C. Unfortunately, the enzyme activity in these samples was nearly undetectable (Figure 8) and we concluded that this method removes the proteins as well as the salts. In the SPE C-18 column washing fractions there were apparent crystals, indicating that a great amount of salts had been removed from the samples. However, it is possible that methanol is not the correct solute for performing enzyme activity measurements. We therefore sought other methods for the salt removal.

ZEBA™ DESALT COLUMNS

We found a matrix column called ZEBA™ Desalt Columns, and ordered a5 piece trial package. We followed the enclosed protocol, starting with a centrifugation to remove the column buffer. 1 ml of the fish extract sample was added followed by centrifugation, after which, the proteins were supposed to be in the flow through fraction and the salts should have stayed in the column material. The samples were analyzed for total protein directly after this procedure, but unfortunately the total protein was barely measurable in comparison to the standard curve (Figure 9).

A



B

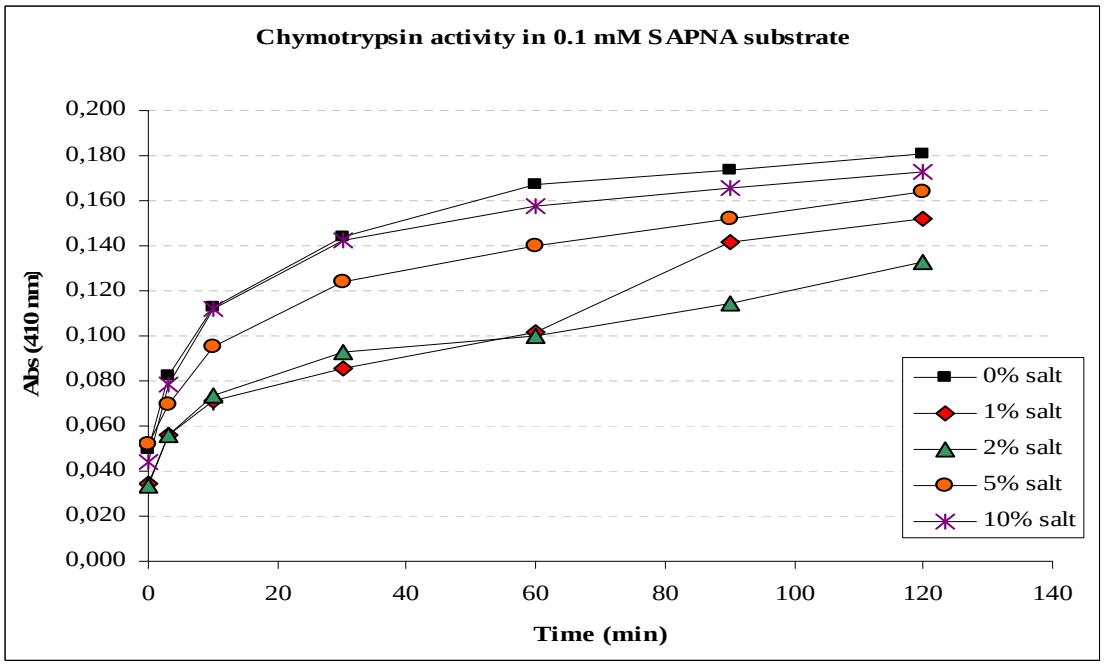
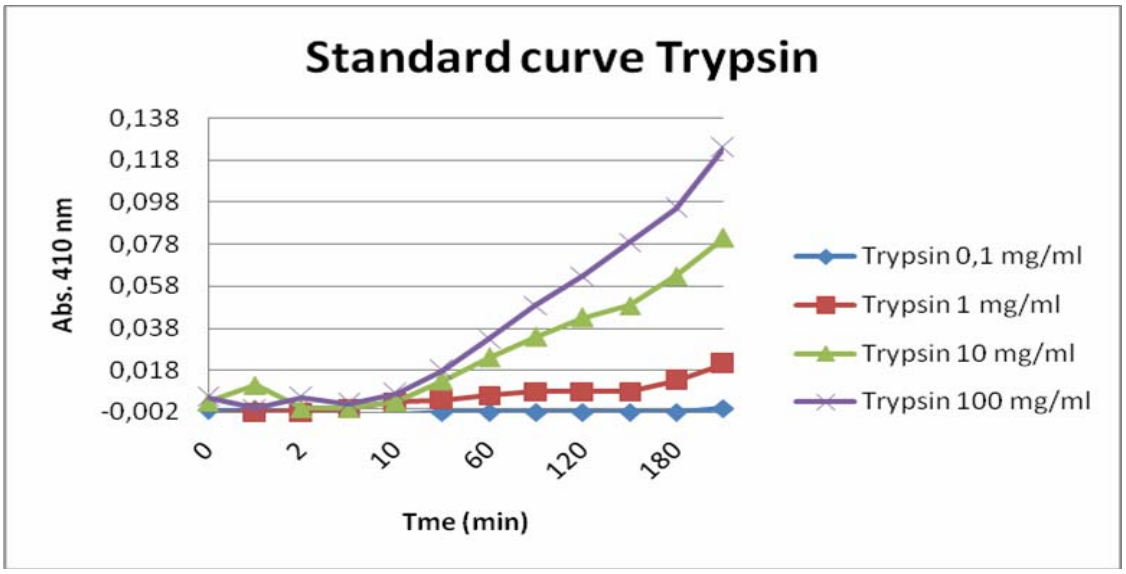


Figure 7 Test of the effects of salt concentration on the enzyme activity

The graphs shows the enzyme activity measured overtime (min) for Trypsin (A) for Chymotrypsin (B) using different concentrations of salt. The activiy decreases (A) or stagnates (B) when the concentration reaches 10 %.

A



B

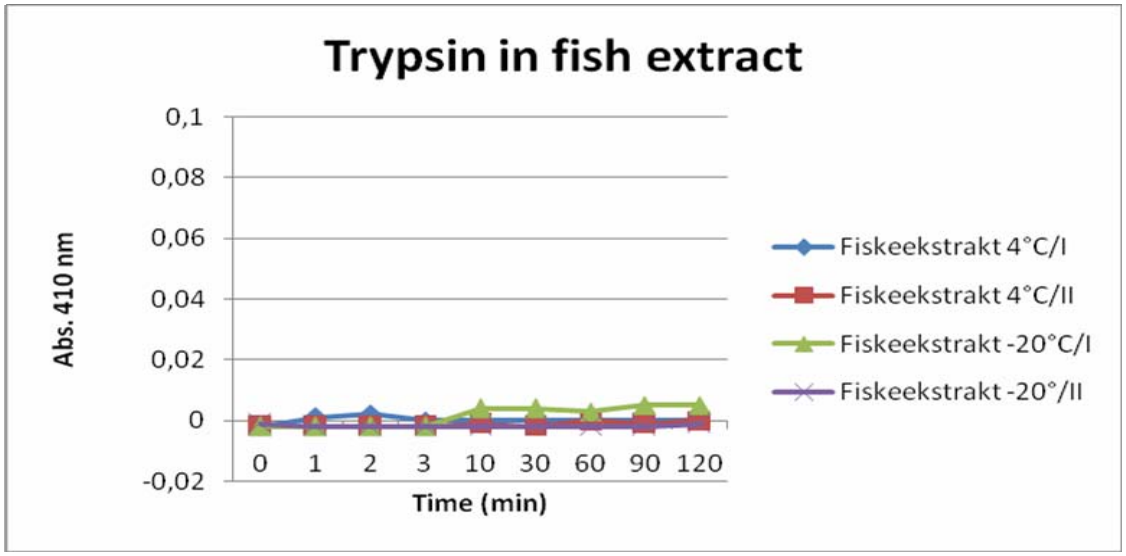
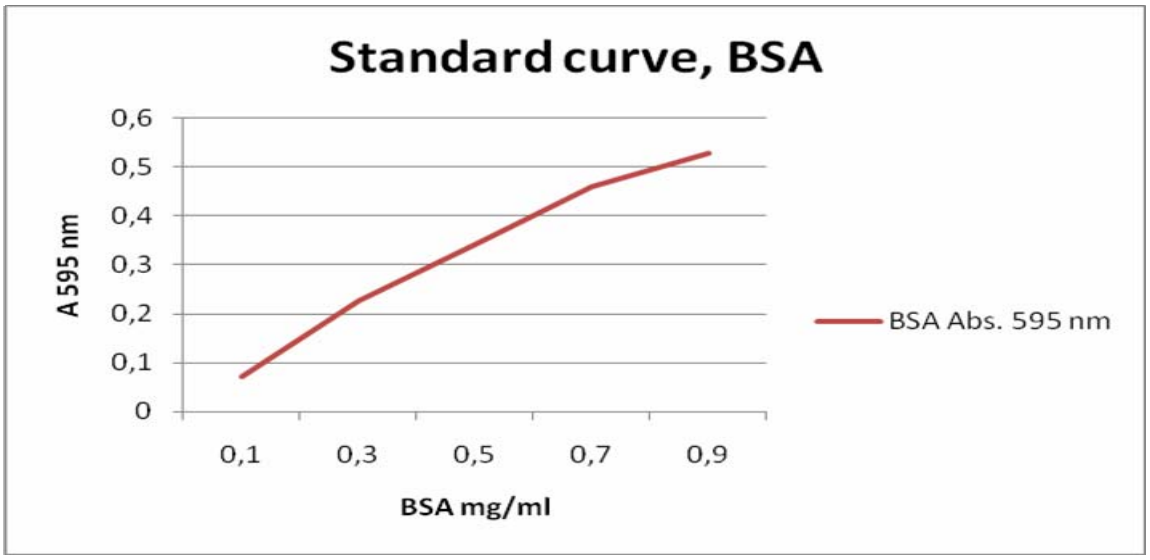


Figure 8 Trypsin activity in C-18 column salt purified fish extracts

The graphs shows standard curve for Trypsin (A) and the activity of Trypsin in fish extracts, salt purified with C-18 SPE column and stored at either 4 °C or -20 °C, over time (min).The Trypsin activity is lower than 0,1 mg/ml.

A



B

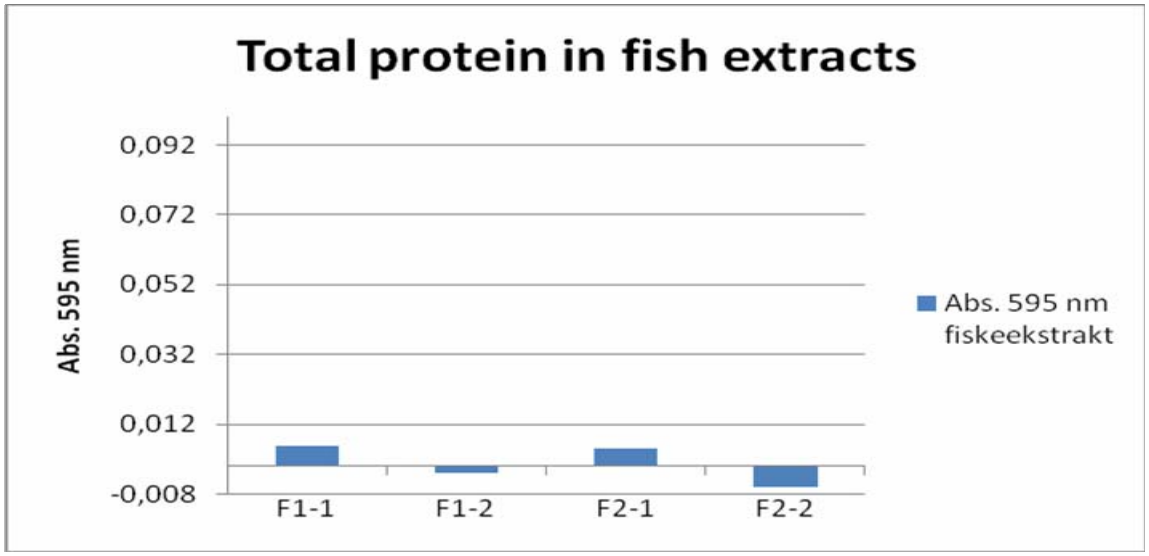


Figure 9 Total protein in Zeba™ Desalt Column salt purified fishextracts

The graphs shows standard curve based on BSA protein standard (A) and totalt protein in wto measurements of two parallelsof fish extrakt (B). Total protein is barely measurable.

Further on it is desirable to investigate for the total protein content in the samples before and after the column separation. This can be achieved by applying a small amount of the sample on a standard SDS PAGE protein gel, with subsequent staining by Coomassie Brilliant Blue. Any loss of protein will be clearly visible. There was no time to perform this test before the end of the project, but the equipment is readily available at Aalesund University College.

So, for the time being we must conclude that we were not able to adequately decrease that salt concentrations of the fish extracts, and therefore the enzyme activity could not be measured.

5.2.9 Fluorescence spectroscopy measurements

The sample identification codes for the fluorescence analysis are found in Table 3. The fluorescence spectra for the eight samples are shown in Figure 11. We see that most of the variation happens in the region 430 – 600nm, and more specific at 470nm.

Table 3 Explanation of sample codes

Sample	Føroyar 1 month	Føroyar 4 months	GPG 1 month	GPG 4 months	Visir 1 month	Visir 4 months	Thorbj. Fisk. 1 month	Thorbj. Fisk. 4 months
Sample code	S1	S2	S3	S4	S5	S6	S7	S8

The fluorescence at 470nm is plotted in Figure 12. We see that sample one and two (s_1 and s_2) has the highest fluorescence at 470nm of all the samples. This indicates that the production method used for these particular samples gives the most oxidized product. We also see from Figure 11 that the samples stored for one month (s1, s3, s5 and s7) always has a lower fluorescence than the samples from the same producer stored for four months (s2, s4, s6 and s8).

Principal components analysis was performed on the data to reveal any potential grouping of the data. Pc1 vs. Pc3 (Figure 13a) shows that the samples are grouped with respect to storage time. The only samples that seemed to group together in the PC space with respect to producer were s1 and s2. These are seen as the blue samples in the Pc2 vs. Pc4 plot in Figure 13b.

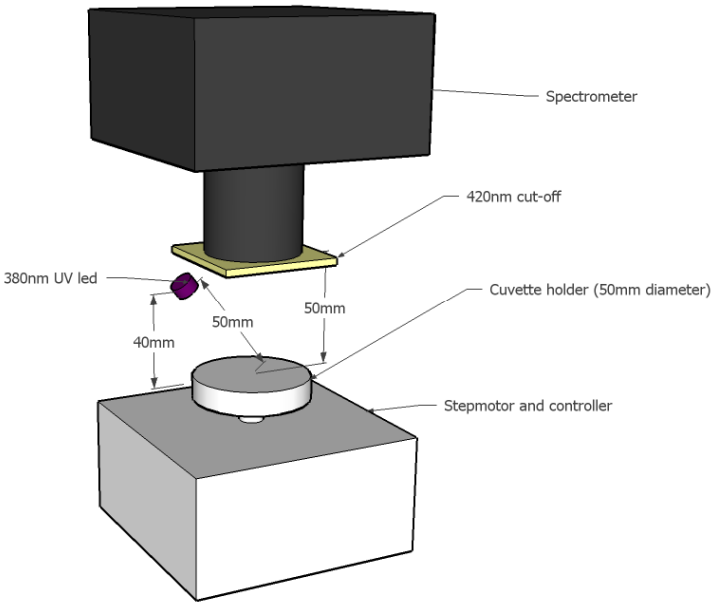


Figure 10 Fluorescence spectroscopy setup

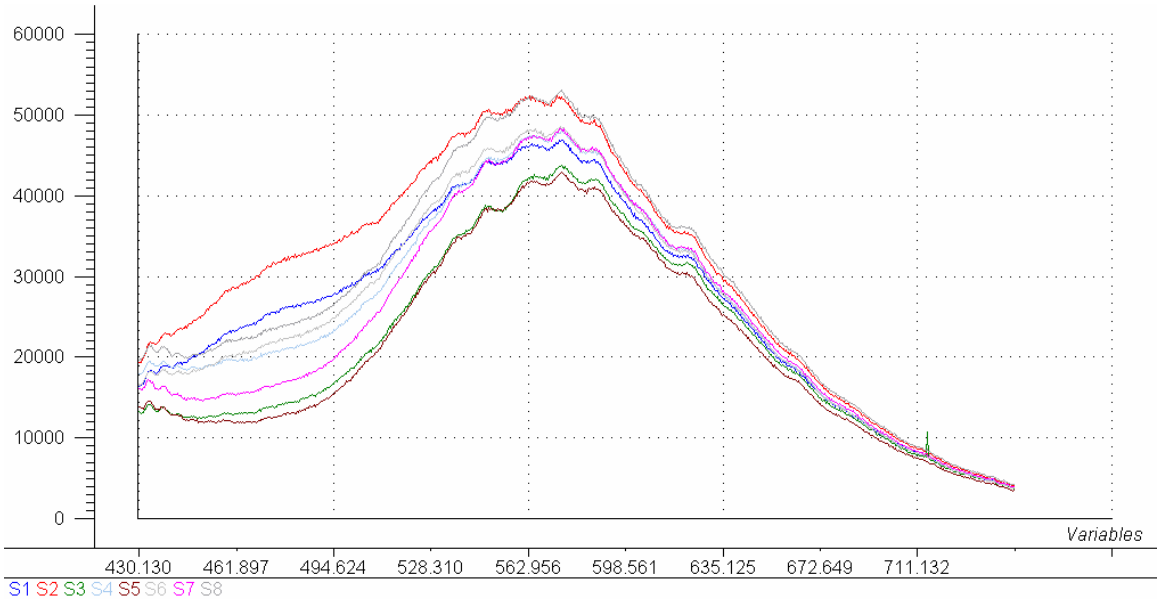
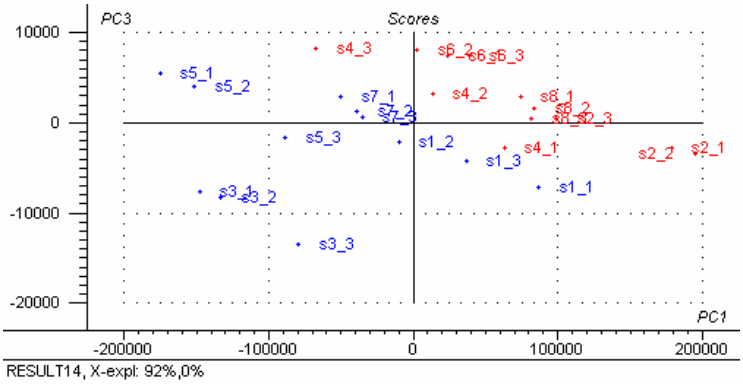
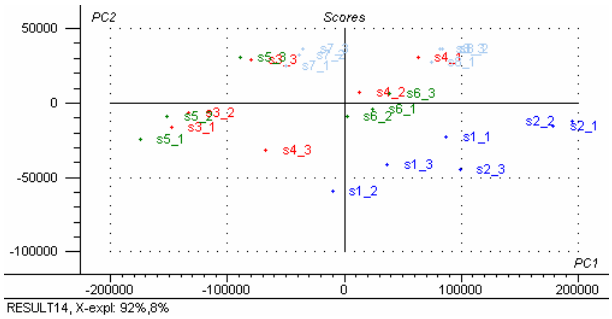


Figure 11 Fluorescence spectra of the eight samples. Raw counts as a function wavelength in nm

Figure 12 Fluorescence at 470nm for the three measurements, at 0, 90 and 180 degree rotation, as a function of the eight different samples



(a)



(b)

Figure 13 PCA of the fluorescence spectra. (a) Pc1 vs. Pc3 where the red samples represent samples stored for four months, and blue represents samples stored for 1 month. (b) Pc1 vs. Pc2 where red, blue, light blue and green represent samples made by the four different producers respectively

5.3 Results from Matís, Iceland

The sample identification used at Matís is shown in Table 4.

Table 4 Sample origin and ripening time

Sample name	Origin	Ripening time (months) prior to desalting
F1	Faroe Islands	1
F4	Faroe Islands	4
IST1	Iceland, Thorbjorn	1
IST4	Iceland, Thorbjorn	4
ISV1	Iceland, Visir	1
ISV4	Iceland, Visir	4
N1	Norway, GPG	1
N4	Norway, GPG	4

5.3.1 Water and salt content

The water content before desalting was lowest in samples from Norway (54-54.7%) but highest in the samples from Faroe Islands (58.3-61.5%). After rehydration the water content was in the range of 82.1-85.9%, still lowest in the samples from Norway. The salt content was in the range of 19.3-21.8% before rehydration but 1.0-1.4% after rehydration of 144 hours. The results indicate effects of injection, i.e. the only samples that where not injected were from Norway, these had lower water content than other groups and slightly lower salt content. In the samples from Faroe Islands, higher value was obtained after longer storage which might indicate that the fish had been stored at a moist place, i.e. the relativy humidity in the environment has been higher then water activity in the fish.

Table 5 Water and salt conent of salted and desalted samples (N=Norway; IsV=Iceland-Visir hf; IsT=Iceland – Thorbjorn hf, F=Faroe Islands, 1=1 month, 4=4 months of ripening)

	Water (%)		Salt (%)	
	Before rehydration	After rehydration	Before rehydration	After rehydration
F1	57,1	84,9	21,8	1,4
F4	61,5	85,6	19,5	1,2
IST1	57,5	85,3	21,1	1,0
IST4	58,3	83,6	21,1	1,1
ISV1	58,6	85,9	20,8	1,1
ISV4	58,4	85,3	21,0	1,4
N1	54,0	82,1	19,3	1,3
N4	54,7	82,6	20,0	1,0

The samples used for training of the sensory panel contained about 2% salt, (see appendix) but then the water was only replace once with fresh water (rehydration time 120 hours) instead of two times for 144 hours rehydration time. The optimum salt content in rehydrated products in Southern Europe is 0.8-1.1%, where traditional dishes have been quiet different from what is known in the Nordic countries. In Iceland the traditional way to cook heavy salted cod was to boil it in water, where the salt content is higher since the fish is furter desalted during cooking.

5.3.2 Water holding capacity

The water holding capacity of heavy salted samples was in the range of 65-79%, lowest in the Icelandic samples from Vísir hf. It seemed to be slightly higher after longer ripening time.

The water was lower than after rehydration due to the high salt concentration and denaturation of proteins in the heavy salted samples. The salt content decreased during rehydration and the water binding properties of the muscle increased at the same time. After rehydration, it was in the range of 90.8-99.4%, the results indicated negative effects of longer ripening time in the samples from Norway and Faroe Islands.

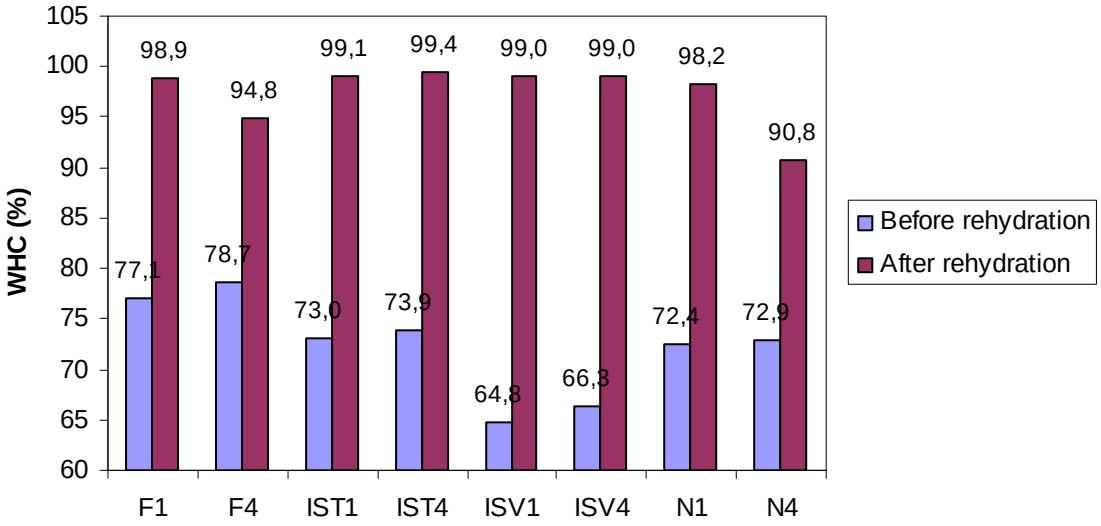


Figure 14 Water holding capacity of heavy salted cod, before and after rehydartion. (N=Norway; IsV=Iceland-Visir hf; IsT=Iceland – Thorbjorn hf, F=Faroe Islands, 1=1 month, 4=4 months of ripening)

5.3.3 Rehydration yield

The rehydration yield after the first 24 hours was in the ranges of 16-22%, after 120 hours 125-136% and after 140 hours 121-143% (Figure 15). The highest values were obtained for samples from Norway but the lowest for samples from Faroe Islands.

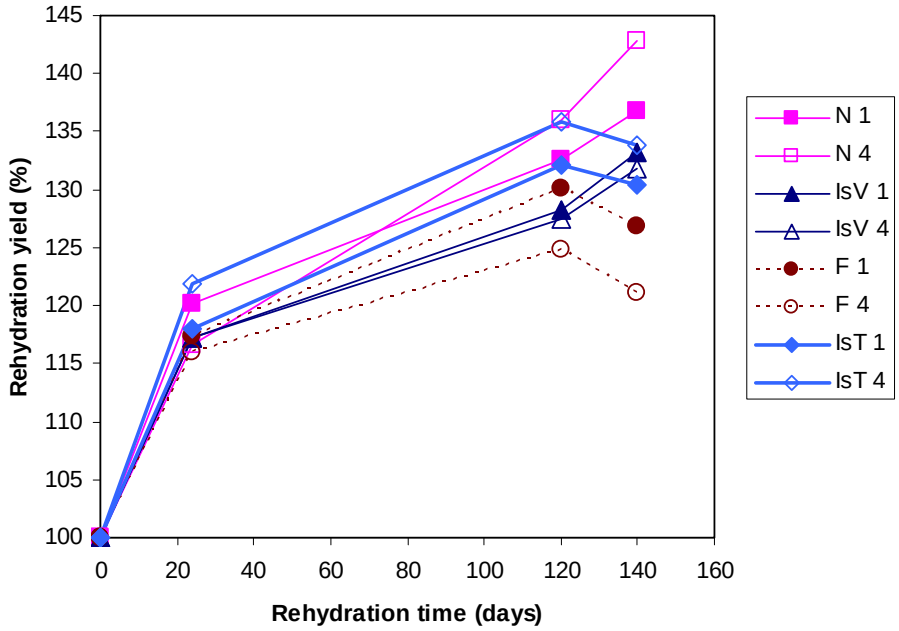


Figure 15 Rehydration yield of heavy salted cod, water changes were carried out after 24 and 120 hours. (N=Norway; IsV=Iceland-Visir hf; IsT=Iceland – Thorbjorn hf, F=Faroe Islands, 1=1 month, 4=4 months of ripening)

It is known that the condition of the raw material and the salting methods affects weight changes during rehydration. Higher curing or salting yield normally leads to lower rehydration yield. Injection increases the curing yield compared to using only brine or pickle salting as the intial step of the curing process. Only the fish from Norway was not injected which has probably resulted in lower curing yield and lower water content after curing as was observed compared to other groups

The samples from Faroe Islands had a much stronger curing odor as raw and had a darker apperance than from other producers. The same was found in the sensory analysis after cooking. This might indicate that the raw material or temperature during storage may have differed from others producers, resulting in reduced water binding properties. Theses were the only samples were ripening time had negative effects on the weight increase during rehydration. The water content tended to be higher in these samples which might indicate higher curing yield of the fish and therefore lower rehydration yield.

5.3.4 Sensory analysis

Eight samples of desalted cod were evaluated with sensory evaluation to observe if sample origin (processing place) or ripening time influenced sensory characteristics.

OBS: Comparison of samples that where water was changed1x (2% salt - training) or 2x (1% salt) can be seen in the appendix .

Figure 16 illustrates how the different samples were described by the sensory attributes. The first two principal components (PC1 and PC2) show the main structured information in the data and explain 80% (67% and 13 % respectively) of the sensory variation between the samples.

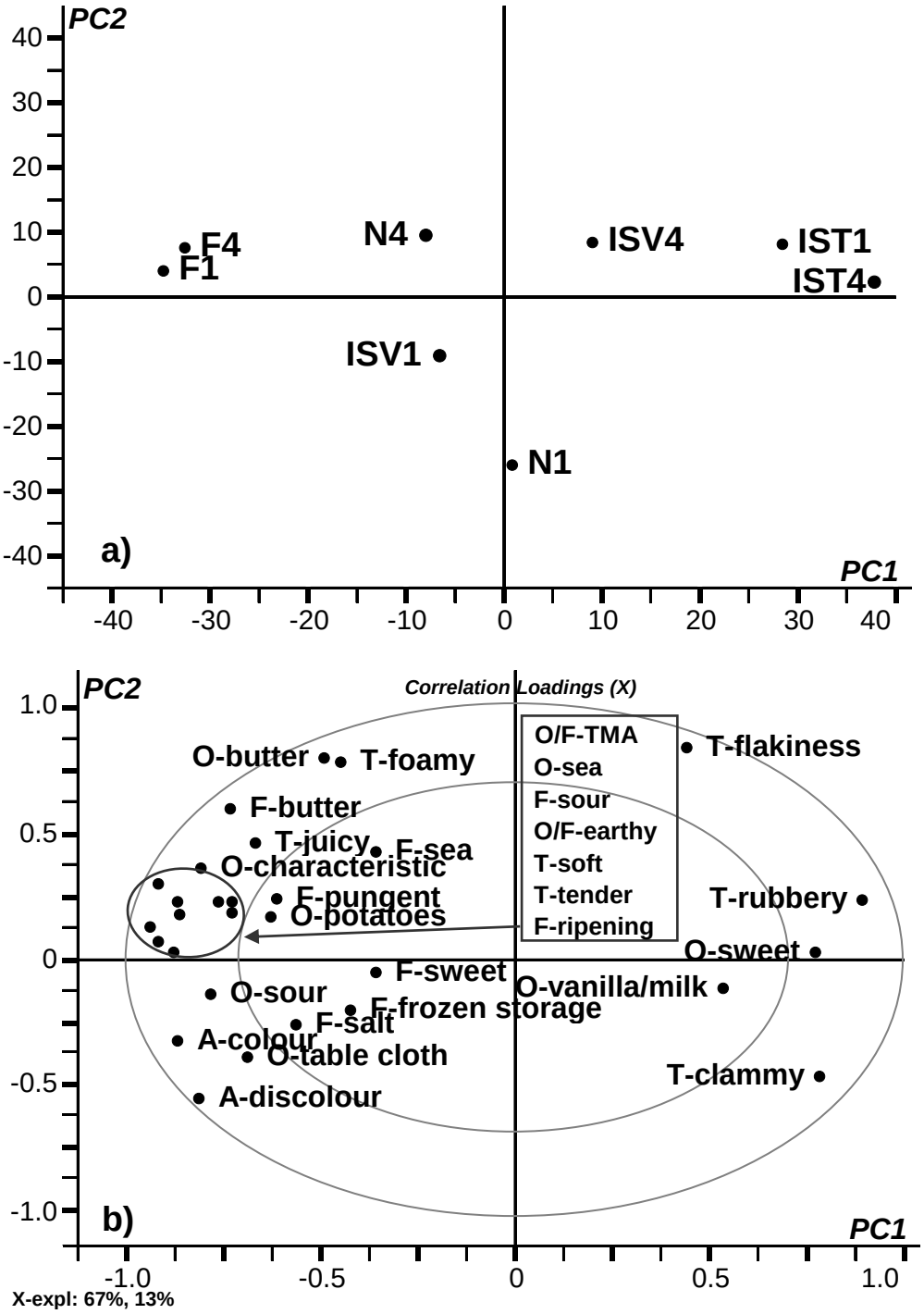


Figure 16 PCA describing sensory quality of the products as evaluated by a trained sensory panel. a) Scores; b) correlation loadings. PC1 vs PC2 (X-expl.: 67%, 13%). Ellipses mark the 50% and 100% explained variance limits. O = odour; A = appearance; F = flavour; T = texture

The predominant difference between the samples was due to texture, mainly rubbery, to the right, tender and soft, to the left in Figure 16. The samples were also very different with regard to ripening flavour and odour characteristic for desalted cod, which more described samples to the left in Figure 16. These samples were also described with attributes characteristic for extended storage time, such as sour and TMA odours and flavours. However, the intensity of these attributes was low. Samples to the left in Figure 16 were more characterised with dark colour and discoloured appearance compared to samples to the right.

The sample groups were different with regard to most sensory attributes (Table 6). The origin of the samples influenced the sensory characteristics of the samples more than the ripening time prior to desalting. The samples F1 and F4 are both located to the left side of Figure 16, and were characterised by dark colour and discoloured appearance, characteristic, earthy, table cloth, sour and TMA odours, ripening, earthy and TMA flavours, soft, tender and juicy texture. The samples IST1 and IST4 are both located to the right side of Figure 16, and were very different sensory characteristics than F1 and F4, - the whitest and most homogenous colour, more vanilla/milk odour, but less characteristic, earthy, table cloth, sour and TMA odours, less ripening flavour, more rubbery texture, but less soft and tender texture.

Ripening time appeared to play a slightly bigger role in N1 and N4 samples, as N4 had more characteristic odour, and flakier texture compared to N1.

Table 6 Average sensory scores (QDA scale 0-100%) for the 8 products. Different letters show significant differences within a line

Sensory attribute		F1	F4	IST1	IST4	ISV1	ISV4	N1	N4
Appearance									
colour	***	32 ab	39 a	13 cd	19 bc	27 ab	22 bc	31 ab	28 ab
discolour	***	34 ab	37 a	19 cd	20 bc	35 ab	24	40 a	27
Odour									
characteristic	***	48 a	50 a	28 cd	32 bc	43	45 ab	39 bc	52 a
sweet		26	30	30	33	33	32	33	32
vanilla/milk	***	28	26 ab	31 ab	35 a	34	29 bc	33	24 cd
potatoes		29	28	23	22	25	23	28	26
sea		24	27	19	19	22	26	27	30
butter	ms	17	21	16	14	21	23	20	26
earthy	***	14 ab	15 a	7 d	8 bc	11	12	14	16 ab
table cloth	**	14 a	14 a	6 b	10	11	9	15	14
sour	***	11 ab	13 a	5 cd	6 bc	7 bc	9	13 ab	11
TMA	***	13 a	12 ab	4 cd	4 cd	7 bc	9	8 bc	11
sulphur	*	5	6 a	2 b	3	3	5	5	5
Flavour									
salt	***	52 ab	50 ab	43 bc	32 d	50 ab	60 a	54 ab	49 ab
sweet		28	29	29	27	36	33	35	34
ripening	***	51 a	46 ab	24 d	26 d	37 bc	43 bc	41 bc	46 ab
sea	*	23	28	25	19	27	30	28	32
butter	**	22	25 a	17	16 b	28	29	27	31
earthy	***	13	17 a	8 b	8 b	11 b	9 b	12 b	15
sour	**	14	14	9	9	12	11	11	10
TMA	***	13 a	13 a	5 b	3 b	8 b	7 b	9	12
pungent	ms	14	15	12	5	9	14	13	18
frozen storage		7	10	6	7	8	8	11	11
Texture									
flakiness	***	53	58 a	60 a	64 a	56	66 a	46 b	62 a
soft	***	62 a	54 ab	42 bc	29 ce	55 ab	44 bc	45 cd	61 a
juicy	*	61	62 a	54	49	60	57	54 b	55
tender	***	60 a	56 a	39 bc	29 cd	53 ab	42 bc	38 bc	42 bc
rubbery	***	17 de	16 de	45 ab	47 a	24 cd	43 ab	29 cd	36 bc
foamy		35	37	33	34	38	42	34	42
clammy	**	17 b	17 b	32 a	29	26	30	33	30

ms (marginal significance, p = 0,05-0,10); * (p < 0,05); ** (p < 0,01); * (p < 0,001)**

The appearance of the samples was very different (Table 6). The IST1 and IST4 samples were very white, but other samples were rather or very dark (F4). The IST samples also had very homogenous colour, but F4 and N1 had very discoloured appearance.

Odour characteristic for desalted cod was the single most characteristic odour for the samples, with the exception of IST samples, though this sensory attribute was obvious for IST, but just to the same degree as sweet, vanilla/milk, potatoes, and sea odours. Sweet, vanilla/milk, potatoes and sea odours were obvious for all the samples. Butter odour was discernible of most samples, but only hints of butter odour were detected of F1, IST1 and IST4. Hints of earthy, table cloth, sour and TMA odours were detected of all samples, but sulphur odour was not detected of any of the samples.

Salty flavour dominated the flavours of F, ISV and N groups, with average scores close to or above 50. Ripening flavour was very characteristic for all samples with the exception of IST samples, though ripening flavour was obvious for IST samples. Sweet, sea and butter flavours were obvious of most samples, with the exception of IST samples which had slightly less sea and butter flavours. Earthy, sour and TMA flavours were not detected in IST samples, but hints of earthy, sour, TMA and pungent flavours were detected of most other samples. Frozen storage flavour was not detected in any of the samples.

The texture characteristics of the samples were very different. Generally, the samples were very flaky, but F samples were the most soft, juicy and tender, while IST4 was the least soft and tender. The F samples were not rubbery, but IST, ISV4 and N4 were very rubbery. All samples were foamy, and all had a clammy texture with the exception of F samples.

Summary

The samples were very different with regard to sensory characteristics. The different sensory characteristics appeared to mostly depend on origin, and samples from the Faroe Islands were very different from the Icelandic Thorbjorn samples, as the samples from the Faroe Islands had more characteristic odour for desalted cod and ripening flavour, soft, tender and juicy texture but also hints of spoilage indicators, while the Icelandic Thorbjorn samples had much lower intensity of characteristic odour of desalted cod and ripening flavour, more rubbery texture and no hints of spoilage indicators. The N and ISV were not as different, but ripening time appeared to influence the sensory characteristics somewhat for N samples and result in more characteristic odour.

5.3.5 Detection and characterization of volatile compounds

Various volatile compounds have been detected in ripened products like dry cured ham as a consequence of protein and fat degradation, i.e. proteolysis and lipolysis. The formation of the flavor is due to complex combination of enzymatic or chemical reactions like lipid oxidation, Maillard reactions, and Strecker degradations (Toldrá, 1998; Toldrá and Flores, 1999; Toldrá and others, 2000). Similar processes have been reported in ripened seafood products like in sugar salted ripened roe products (Jónsdóttir *et al,* 2004) and ripened anchovy (Triqui and Reineccius, 1995; Triqui and Guth, 1997). Information about the volatile compounds in salt-cured cod is however lacking. In this study the key aroma compounds were characterized using static headspace sampling technique. Because of their low level, it can be difficult to detect these volatile compounds using gas chromatography-mass spectrometry (GC-MS). However, by using a gas chromatography-olfactometry (GC-O) technique, it is possible to detect compounds that have very low odour thresholds.

Characteristic odors and key volatile compounds in the salt-cured cod samples are shown in Figure 17 and Table 7, respectively, to demonstrate which odors are most dominating in the aroma profile. The odor descriptions are listed in Table 7 according to retention times with

corresponding compounds identified by GC–MS, but some of the components detected by GC–O were not identified. It must be pointed out that the most abundant compounds quantified by GC–MS do not necessarily contribute to the most intense odors as can be explained by different odor thresholds.

Rancid, potato like and boiled potato like odors contributed by heptanal and *cis*-4-heptenal were the most potent odors, especially in samples after 4 months of storage (Figure 17 and Table 7). Methional, formed via Strecker degradation of methionine and eluting at a similar time as *cis*-4-heptenal and heptanal, could also be responsible for the boiled potato like odor although not detectable with the static headspace sampling method. Heptanal and *cis*-4-heptenal were detected in low concentration by GC-MS but because of their low odor threshold (3 ppb and 0.04 ppb, respectively), they can have high sensory impact as seen by the GC-O results (Figure 17). The odor of *cis*-4-heptenal, which is derived from lipid oxidation of n-3 unsaturated fatty acids (PUFA), has been described as boiled potato like odor. In fact, this aldehyde does not exhibit a fishy-type aroma by itself, but it rather participates in the expression of the overall fishy odor. Its odor has been described both as cardboardy, paint-like (Hardy and others, 1979) as well as boiled potato-like (Josephson and Lindsay, 1987a; 1987b).

Malty like odor contributed by 3-methyl butanal was one of the most potent odor in many samples. This compound is probably originating from the amino acid leucine. It has an odor threshold of 0.06 ppm and have therefore moderate flavor impact. This compound has been suggested as an indicator for the ripening process of ripened anchovy (Triqui and Zouine, 1999).

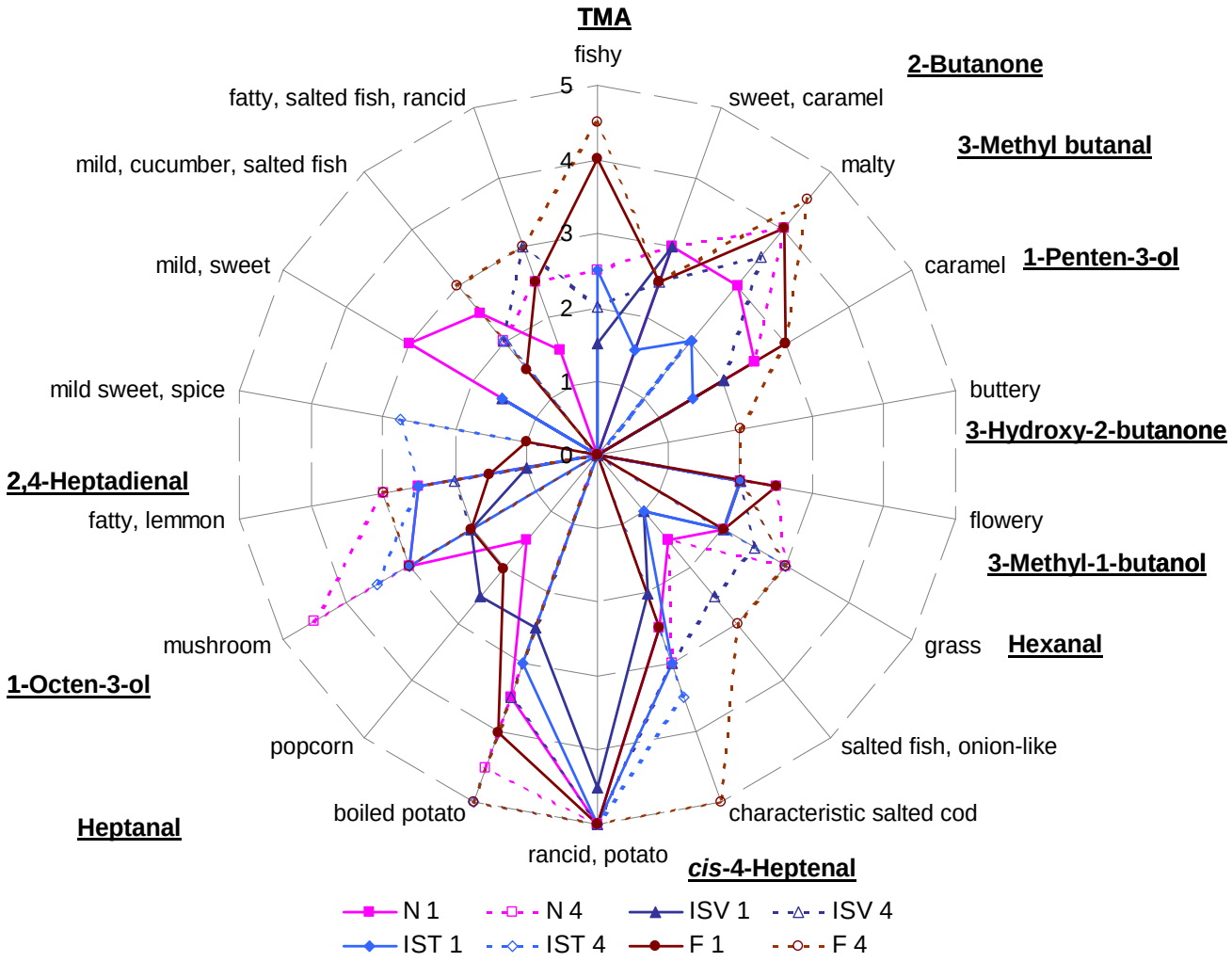


Figure 17 GC–O odor evaluation of volatile compounds detected in salt-cured cod after 1 and 4 months of storage. (ISV=Iceland-Visir hf; IST=Iceland – Thorbjorn hf, N=Norway; F=Faroe Islands, 1=1 month, 4=4 months of ripening). Odors also listed in Table 1 according to retention time

TMA is a potent odorant with a characteristic fishy, dried fish, ammonia-like odor. Additionally, TMA has been noted for intensifying fishiness by a synergistic action with certain volatile unsaturated aldehydes derived from autoxidation of polyunsaturated fatty acids (Karahadian and Lindsay, 1989). TMA was highest in the F samples, especially in F-4, evaluated both with GC-MS and GC-O, which is in accordance with the sensory results. TMA is though difficult to quantify with GC-MS because of its volatility.

An unknown compound, giving a characteristic salted cod like odor was identified in all the samples, especially in the samples stored for 4 months. According to the retention index this can possibly be a C6 lipid derived compound, e.g. hexanol, 2-hexenol, 2-hexenal or 3-hexenol. Because of its contribution to the overall aroma profile it would be interesting to identify this compound.

Other key volatile compounds in the salt-cured cod are derived form lipid oxidation, e.g. hexanal, 1-octen-3-ol, 2,4-heptadienal, 1-penten-3-ol, and 2-butanone. The grass odor is contributed by hexanal, mushroom odor is caused by 1-octen-3-ol and fatty, lemon like odor

explained by 2,4-heptadienal, caramel like odor by 1-penten-3-ol and sweet, caramel like odor by 2-butanone.

Acetoin (3-hydroxy-2-butanone), giving a buttery like odor, is a lipid oxidation product or it can be derived from amino acids. This compound was only indentified in the F samples. It was characteristic for the spoilage of chilled cod fillets packed in styrofoam boxes and was attributed to the growth of *Photobacterium phosphoreum* (Olafsdottir *et al*, 2005). The flowery like odor was contributed by 3-methyl-1-butanol that is derived from the amino acid leucine. Compounds like acetoin, 3-methyl butanal and 3-methyl-1-butanol can act as substrates for Maillard reactions (Toldrá, 1998) and therefore influence the flavor of salt-cured cod.

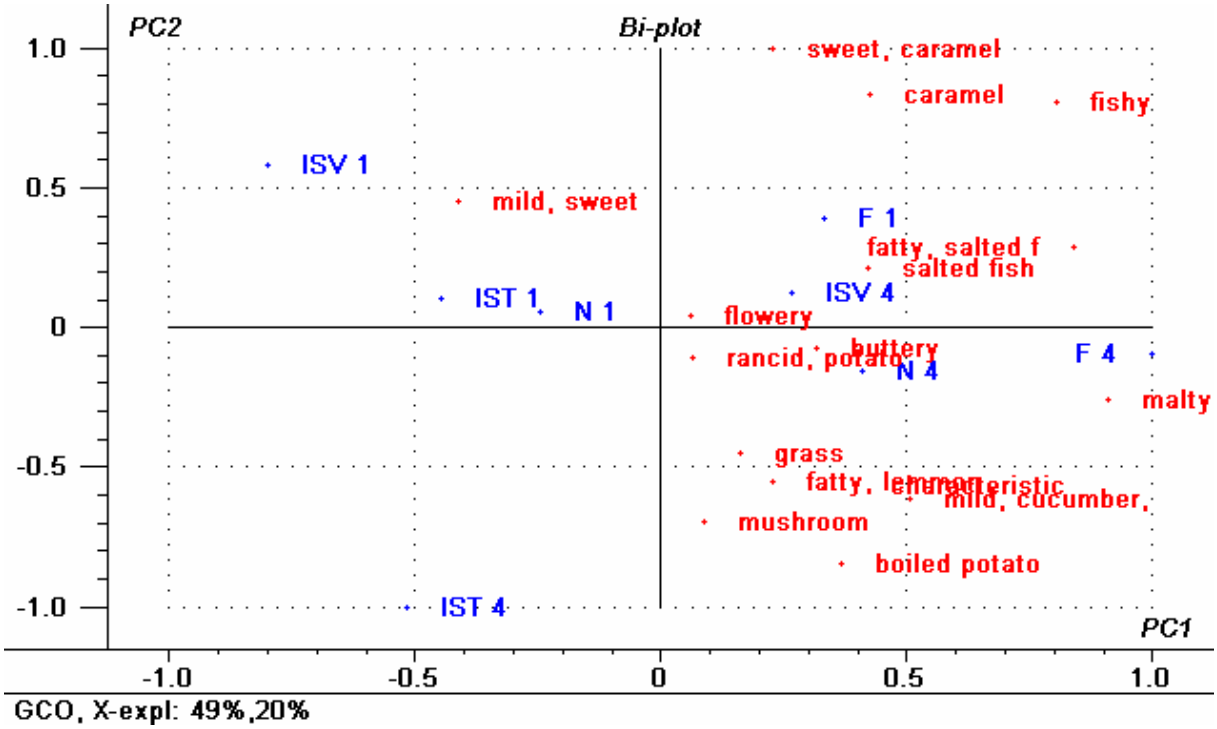


Figure 18 PCA biplot of GC–O odor evaluation of volatile compounds detected in salt-cured cod after 1 and 4 months of storage. (ISV=Iceland-Visir hf; IST=Iceland – Thorbjorn hf, N=Norway; F=Faroe Islands, 1=1 month, 4=4 months of ripening)

The main variance in the GC-O data set was studied by PCA (Figure 18). The F samples, N4 and ISV4, located to the right on the biplot, were characterized by high fishy odor (TMA), malty (3-methyl butanal), salted fish (unknown), mild cucumber (unknown), potato (*cis*-4-heptenal and heptanal), and caramel (1-penten-3-ol) like odors and to some extend odors like buttery (acetoin), grass (hexanal), fatty (2,4-heptadienal) and mushroom (1-octen-3-ol). The sensory analysis revealed that ripening flavor was very characteristic for all samples with the exception of IST samples. The GC-O analysis indicates that combinations of these volatile compounds are responisble for this characteristic ripening flavor. The Faroe Islands samples had also a hint of spoilage indicators, possibly derived from TMA. The IST samples had much lower intensity of characteristic ripening flavor as described by the sensory panel. This is in accordance with the GC-O results as can be seen in Figure 18 were the IST samples are located on the left side of the biplot.

Table 7 Volatile compounds of salt-cured cod after 1 and 4 months of storage. (ISV=Iceland-Visir hf; IST=Iceland–Thorbjorn hf, N=Norway; F=Faroe Islands, 1=1 month, 4=4 months of storage). Odor evaluation by GC–O and quantification by GC–MS expressed as mean peak area ratio stdv)

Compound	RI DB 5msa	ID means[b]	Odor description (GC O)c	ISV 1	ISV 4	IST 1	IST 4	N 1	N 4	F 1	F 4
Ethanol	<165	MS	n.d.	90.7	87.3	56.2		27.0	75.0		
TMA	<165	MS, 1, 2	Fishy		55.1					104	201
Acetic acid	194		n.d.		6.7			20.2		49.2	30.8
2-Butanone	211	MS, 1, 2	Sweet, caramel	275	64.9	27.2	116	26.0	94.9		56.4
3-Methyl-butanal	255	MS, 1, 2	Malty	14.0	103		29.1	51.9	213	448	1619
1-Penten-3-ol	265	MS, 1, 2	Caramel	42.8	127	37.3	186	55.2	157	46.8	105
3-Hydroxy-2-butanone	277	MS, 1, 2	Buttery							7.4	18.5
3-Methyl-1-butanol	325	MS, 2	Flowery	4.1	79.8	10.4	18.2	3.0	9.3	14.7	42.3
2,3-Butanediol	379	MS, 1, 2	Buttery							3.4	8.7
Hexanal	397	MS, 1, 2	Grass	6.9	11.1	6.3	32.6	10.4	22.9	10.5	25.8
Unknown	451	2	Stockfish, mushroom, moldy								
Unknown	469	2	Mushroom								
3-Methyl-butanoic acid	485	MS	n.d.		2.7	1.6	42.8		26.0	2.8	49.4
cis- 4-Heptenal	497	MS, 1, 2	Potato		3.5					2.9	19.1
Heptanal	500	MS, 1, 2	Boiled potato								5.5
Unknown	527	2	Popcorn								
Dimethyl trisulfide	559	MS, 1	n.d.		2.2			0.6			
Unknown	572	2	Caramel, sweet								
Phenol	574	MS	n.d.	1.5		1.7		2.3		1.0	1.3
1-Octen-3-ol	577	MS, 1, 2	Mushroom		1.8	1.8	41.8			2.2	5.7
6-Methyl-5-hepten-2-one	590	MS	Mild flower	6.3	8.4	9.0		9.2	13.7	8.3	26.2
Decane	600	MS	n.d.	3.7	6.8	8.6	16.5	4.2	24.6	29.1	27.6
2,4-heptadienal	612	1,2	Soap								
D-Limonene	634	MS	n.d.	1.5		1.2		2.3		2.2	4.3
Unknown	645	2	Flower								
Unknown	684	2	Mild, sweet								
Undecane	703	MS	n.d.	11.5	5.1	6.4	8.4	3.4		12.6	11.1
2-Nonen-1-ol	707	MS	n.d.		7.7						19.2
Unknown	756	2	Mild, sweet								
Unknown	784	2	Mild, cucumber/salted fish								
Decanal	809	MS, 1	n.d.	3.2	3.6	5.4	7.9	4.2	4.5	3.0	7.8
Unknown	840	2	Fatty, salted fish								

[a]Calculated ethyl ester retention index on DB 5 ms capillary column.

[b]Identification means: MS = mass spectra 1 = authentic standards; 2 = odour identification.

[c]n.d. = odour not detected by GC–O.

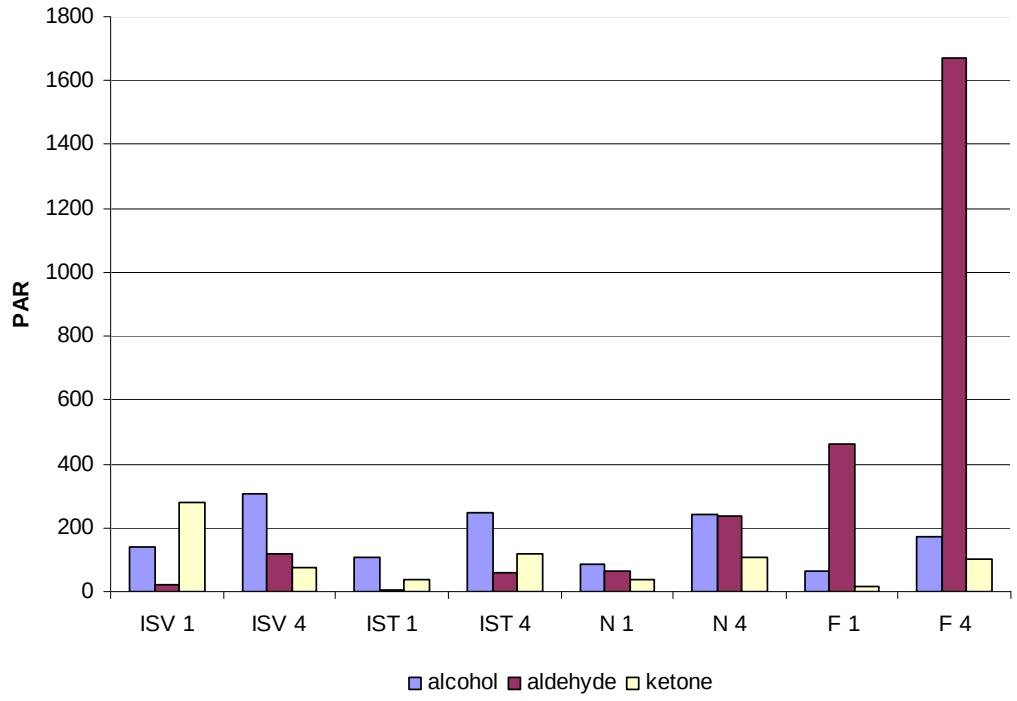


Figure 19 *Sum of alcohols, aldehydes and ketones identified by GC-MS (mean peak area ratio, PAR) detected in salt-cured cod after 1 and 4 months of storage. (ISV=Iceland-Visir hf; IST=Iceland – Thorbjorn hf, N=Norway; F=Faroe Islands, 1=1 month, 4=4 months of ripening)*

The main classes of compounds (alcohols, aldehydes and ketones) based on the sum of the PAR for respective compounds in each class are shown in Figure 19. The amount of aldehydes increased during storage in all products and was much higher in the F samples compared to the other samples. The F samples had more characteristic odor and ripening flavor but also hints of spoilage indicators, as mentioned before. It must be kept in mind that many aldehydes (e.g. *cis*-4-heptenal) and ketones have very low odor threshold. Therefore, they have a great impact on the overall flavor although they do not influence the total amount of aldehydes. Similar trend was seen for alcohols, i.e. increased amount during storage and the amount of ketones did also increase during storage except in ISV samples.

Summary

The GC-O and GC-MS results show that oxidatively derived compounds (e.g. *cis*-4-heptenal and heptanal) in a combination with protein degradation compounds (e.g. 3-methyl butanal) are the key aroma compounds in salt-cured cod. These results indicate that a certain degree of lipid oxidation is both necessary and desirable for the development of characteristic aroma of salt-cured cod.

6 Discussion

In this study, salt-cured samples from 4 different producers located on Iceland, the Faeroe Islands and Norway have been analysed. The aim was to describe the characteristics of the samples and investigate the degree of ripening during storage (1 and 4 months) by carrying out a wide range of analysis on both salt-cured and desalted salt-cured samples.

Lipid oxidation studied by both TBARS and measuring yellow colour showed increasing values during storage, but the differences found in 3 of 4 samples were smaller than the difference between the 4 samples. Other degradation indicator analysis as pH and TMA showed similar trends, the sample origin had more influence than the storage time.

The protein and water content was only to a small extent affected by storage time. Norwegian samples were separated from the other samples by having a higher protein and lower water content, reflecting the salting method which not included injection of brine.

Measurements of enzyme activity of cathepsine D and autolysis gave no systematic results concerning neither origin nor storage time. The analyses of trypsine activity were not able to be carried out due to problems with removing salts without loss of proteins from the samples.

For fluorescence analysis, the Faroe samples gave the highest levels, indicating highest degree of oxidation. These samples also gave the highest scores in yellow colour and TBARS. At 470 nm all 4 salt-cured products had higher fluorescence scores for samples stored 4 months than for 1 month (Figure 11 and Figure 12). The results indicate that fluorescence spectroscopy can be a promising method for measuring storage/ripening of salt-cured cod. However, also in this analysis, the difference between origins (where the samples had been produced) was larger than for the storage time.

Water and salt content of salt-cured sample were relatively stable during the storage period. The exception was the Faeroe sample, gaining 4,3 % water and loosing 3,3 % NaCl during storage, indicating that the fish were stored at humid conditions.

In the sensory analysis of desalted fish, samples from the Faeroe Islands were separated from the other samples by having a dark colour, discoloured appearance, characteristic (salt-cured) odour, with some off-odours and flavours, but with soft and tender texture. Samples from Thorbjorn on Iceland were also separated from the others by having a white and homogenous colour, more vanilla/milk and less characteristic odours, but with less soft and tender texture. Also in the sensory analysis, the origin of the samples played a more important role in sensory characteristics than storage time. Increased ripened/characteristic odour with longer storage time was only found for the Norwegian sample.

7 Conclusions

In this work a wide range of different analysis have been carried out in order to characterise ripening of salt-cured cod as well as detect effects of storage time on degree of ripening.

The findings show that the origin of the salt-cured samples influences the product characteristics more than the storage time, suggesting that ripening of cod can be affected by adapting a desirable production of salt-cured cod rather than ripening by storage of salt-cured cod.

Parameters that are likely to affect the ripening are type and quality of the raw material, salting methods applied and storage conditions during curing and further storage. Since we for some of the products have limited information concerning how the different samples have been produced, it is difficult to identify the production parameters involving ripening. It is of great importance to identify these parameters in order to adapt the production to the desired degrees of ripening of the salt-cured products.

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9 Appendix

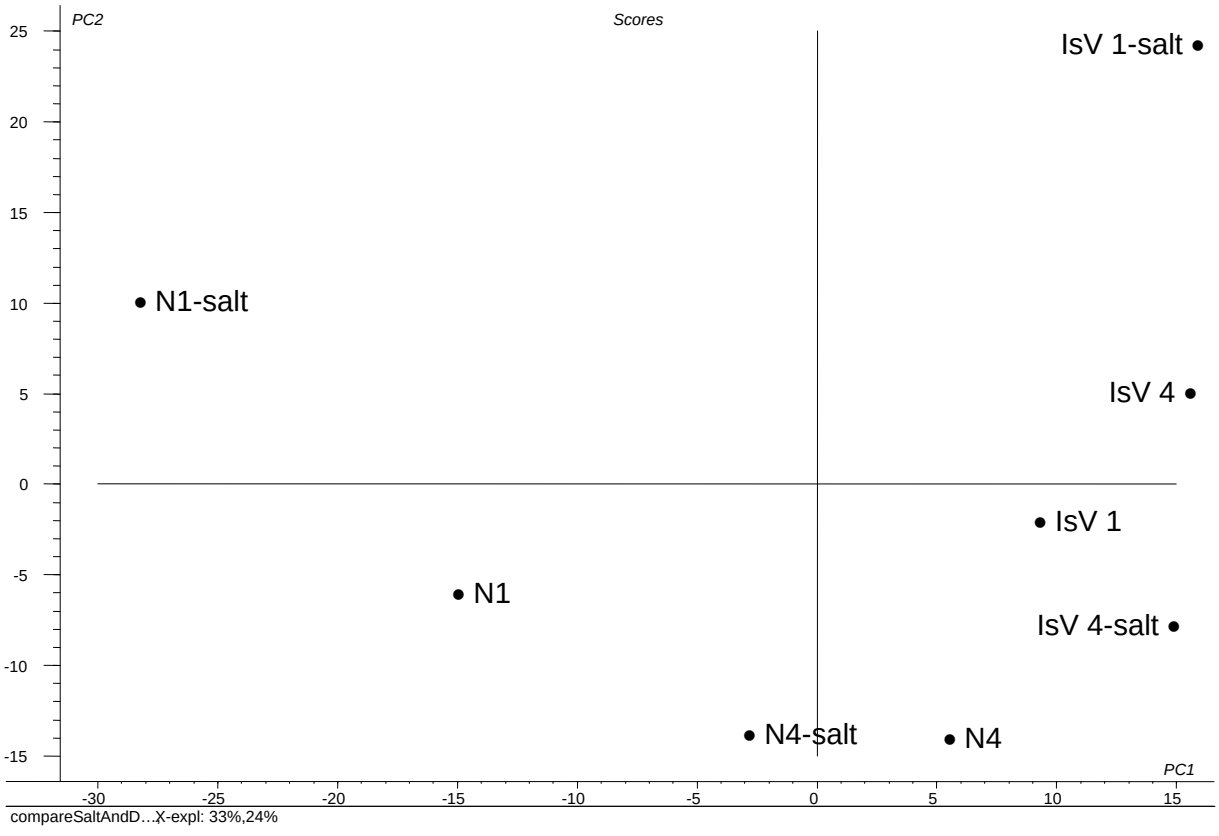
9.1 Chemical content and water holding capacity

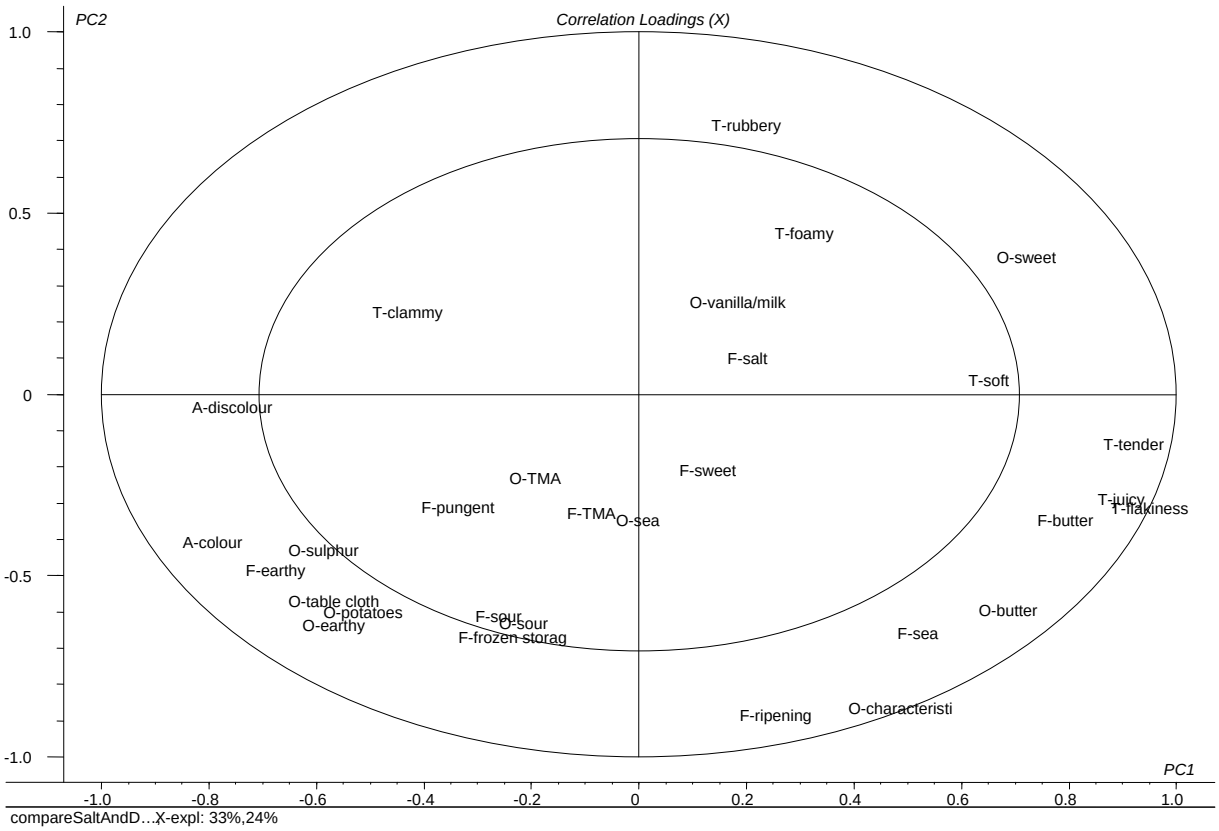
Results from analysis of the samples used for training of the sensory panel.

	Water (%)	Salt (%)	WHC (%)
F1-train	84,2	2,2	99,1
F4-train	84,2	2,0	98,7
IST1-train	84,5	2,1	99,4
IST4-train	83,9	1,8	99,4
ISV1-train	84,7	2,4	97,8
ISV4-train	84,6	2,1	97,3
N1-train	82,2	2,0	98,4
N4-train	82,4	1,9	-

9.2 Comparison of groups after 120 hours desalting and 144 hours desalting:

The results for the the training (120 hours rehydration = salt) and the sensory analysis (144 hours rehydration) were comparable. However the difference between samples was more significant after 120 hours than 144 hours. The reason for the adding time and water changes to the process was the high salt content (~2%) which was believed to interrupt evaluation of other factors. Due to the thickness of splitted fish, it takes longer time to rehydrate such products compared to fillets, even though the fish was cut into pieces like in this trial.




Correlation Loadings (X)
PC2
PC1
1.0
0.5
0
-0.5
-1.0
-1.0
-0.8
-0.6
-0.4
-0.2
0
0.2
0.4
0.6
0.8
1.0
T-rubbery
T-foamy
O-sweet
T-clammy
O-vanilla/milk
F-salt
T-soft
A-discolour
T-tender
O-TMA
F-sweet
T-juicy
T-flakiness
F-pungent
F-TMA
O-sea
F-butter
A-colour
O-sulphur
F-earthy
O-table cloth
O-potatoes
O-earthy
F-sour
O-sour
F-frozen storag
O-butter
F-sea
F-ripening
O-characteristi
compareSaltAndD…X-expl: 33%,24%

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