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THE QUALITY CHANGE IN SMOKED AND DRIED FRESH WATER SARDINE *(Rastrineobola argentea)* AND MARINE PELAGIC FISH (CAPELIN) AS INFLUENCED BY PROCESSING METHODS

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ABSTRACT

Lipid oxidation changes can result in production of repugnant flavour, destruction of valuable nutrients and even production of toxic compounds. The effects of different drying and smoking methods on lipid oxidation and microbial growth of fresh water sardine and marine pelagic fish (capelin) during processing and storage were investigated. The level of lipid oxidation resulting in formation of fluorescent compounds, free fatty acid and other primary, secondary and tertiary oxidation products was tested to assess the quality of sardine and capelin after processing and during storage. In addition to lipid oxidation measurement, microbial quality was analysed. The results showed that cold smoking of capelin accelerated lipid oxidation observed by increases in lipid hydroperoxides (PV) and free fatty acid (FFA) as well as in the development of fluorescence compounds (δFor and δFaq). Hot smoking was found to be good in destroying and limiting microbial growth in both capelin and sardine after processing and during storage. However, storage time had no effect on salt and water content; rather affected positively the microbial growth and lipid oxidation. For the same processing method, ungutted capelin influenced positively microbial growth and FFA after processing and during storage. This study also revealed high rate of lipid oxidation and microbial growth to sardine locally dried on rocks and sands, indicating poor quality of the product.

Keywords: Sardine, capelin, smoking, drying, storage condition, lipid oxidation, microbial growth, fluorescence intensity, TBARS, water activity, salt content.

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1 INTRODUCTION

Fish is a major source of food for humans providing significant portion of the protein intake in the diet of large proportion of the people, particularly so in developing countries. Fish is less tough and more digestible compared to beef, chicken and mutton. Moreover, has little or no religious rejection that gives it an advantage over pork and beef (Kumolu-Johnson and Ndimele 2011). One of the species used by majority of people in East Africa is the fresh water Lake Victoria sardine *Rastrineobola argentea* (Dagaa).

Rastrineobola argentea (Dagaa) is the fish species, which has remained abundant in Lake Victoria. It is also locally called *mukene* in Uganda, *omena* in Kenya. The species now supports a major artisanal fishery, ranking second to the Nile perch in Kenya and Tanzania (LVFO 2011). The Lake Victoria sardine constitutes over 38% of total fish landings from Lake Victoria in Tanzania. The demand for Dagaa has continued to rise over the last decade. The species provides a cheap source of fish proteins for both humans and domestic animals. This is becoming more important following the export drive for processed table fish species, the Nile perch and the tilapia (LVFO 2011). Although the species supports majority of artisanal fishery, little profit is realised. This is because the chemical composition, rough handling and storage results in contamination of fish flesh and easily spoiled. Fish will become unfit for human consumption for some hours after capture, unless it is subjected to some forms of processing or preservation (Clucas and Ward 1996).

The most common processing methods that have been used in Tanzania and other African countries for centuries are smoking and drying. Both techniques involve removal of water, resulting in inhibition of bacterial and enzymatic actions. Fish smoking in Tanzania is done in many different types of smoking equipment, including charcoal oven, in drum and altona kiln. In addition to these techniques that have been used for years, introduction of smoking house has created a chance of adapting a new method with added advantage of using less firewood, hence environmental friendly. Furthermore, the technique produces smoked fish that is relatively higher quality. Depending on the type of equipment used, wood chips or chunks, sawdust, pellets or whole logs are used for source of smoke and in most cases hard wood works fine for smoking fish (Ruiz *et al.* 1999).

In addition to smoking processing, open sun drying is also used for drying of sardine where water from the surface and inner part of fish evaporates and goes to the environment. The driving force of water vapour to enter the surroundings is the function of deficiency of water vapour. Since Tanzania is a tropical country with high average temperature, drying of sardine takes one to two days in which fish are spread on raised racks or sun dried on unhygienic sand, rocks and or grasses. Dagaa processed under these unhygienic premises are heavily contaminated, easily deteriorate and become un-favourable for human consumption. Provision of smoking techniques by the use of smoking house, with emphasis on fish quality assurance and storage information is important in improving the available processing method in both inland waters as well as along the coast of Indian Ocean. Generally there is an opportunity of selling the smoked fish to other market than those that buy sun dried lower quality fish through proper processing, packaging and transportation to interior area or export to Central Africa where it fetch better price. For the purpose of this study, the quality change with time for processed fish and fresh water sardine from Lake Victoria and Capelin (marine fish), which is related to Indian Ocean sardine, were used.

Capelin are small pelagic schooling fish, feeding on zooplankton. It is northern ocean fish commonly caught for their highly desired roe. Schools of capelin can be found throughout the northern areas of the Atlantic and Pacific oceans, and are fished commercially from waters off the coasts of Iceland, Norway and Canada. In addition to the value for human consumption of their eggs, capelin are also used in production of fish oil as well as fishmeal. Never the less they are also a major prey food for larger fish such as herring and cod. The fat content of capelin and other fish is unique in its quantities of long chain omega-3 fatty acids (LC omega-3s). The consumption of LC omega-3s derived from fish oil, either in processed fish or as encapsulated fish oil has been shown to help maintain health, especially cardiovascular health (Pike and Jackson 2010). Capelin were processed by cold and hot smoking technique in a smoking house and then taken for analysis.

Although both drying and smoking processes result in dehydration in the fish muscle then inhibiting bacterial growth and enzymatic action, but quality can deteriorate during storage due to lipid oxidation and microbial growth. Lipid oxidation is responsible for reduction in nutrient quality as well as changes in flavour. Microbial contamination could precipitate public health concern and economic loss in terms of fish spoilage (Kumolu-Johnson and Ndimele 2011).

The main objective of this project was to investigate the effects of different drying and smoking methods on lipid oxidation and microbial growth of fresh water sardine and capelin during processing and storage.

2 SMOKING AND DRYING OF FISH

2.1 Smoking of fish

In tropical countries, smoking has been practiced for a long time. Smoke preservation is successful because smoking kills the food poisoning and spoilage bacteria, or renders them harmless by altering the chemistry of the environment these spoilage organisms can grow in (Hilderbrand 2001). The reasons for smoking fish are varied but, as far as Tanzania is concerned, the process has proved relevant in prolonging shelf life and increasing protein availability to people throughout the year. Furthermore, it helps to reduce waste at times of bumper catches, enhance flavour and increase utilization in soups and sauces. Also, smoking fish helps in storing and making fish easy to pack, transport and market (Bligh *et al.* 1988; Mgawe and Mondoka 2008). According to Rawson and Sai (1966) smoking inhibits the action of microorganism and prolongs the shelf life because the heat extracts much of the water content of the fish. Therefore, smoke combines the preservation effects of drying and cooking.

Despite the fact that modem methods of fish preservation, such as freezing and refrigeration, are available to some extent, the demand still persists in Tanzania for the traditional flavour, taste and colour obtained by smoking. Before the introduction of the smoking house, five types of traditional smoking ovens were used. These include chorkor oven, the cylindrical (round) mud oven, the cylindrical metal or oil-drum oven, the rectangular mud oven and the rectangular/square metal oven. In all these methods the control of heat and smoke is difficult hence the fish processed results in an over dried, brittle and irregularly shaped product. Regulating the fire crudely controls smoke densities and temperatures and the process requires a high degree of supervision by an operator. Apart from drying, hot smoking partially cooks the product and also imparts smoky flavour.

Various research findings and recommendations have been made in Tanzania to improve processing techniques. The current methods used do not produce a product of constant good quality. Hence, there is a need of introducing the use of smoking and drying house for processing the catch of fishermen. This design has advantages over the traditional charcoal method of hot smoking used throughout Tanzania. It is much easier to regulate temperature and smoke concentration inside the kiln. Only small amount of firewood is consumed, the final product is less brittle and has an attractive yellowish-brown colour if the right smoke density is applied.

In this project, smoking process will be carried out by using a smoking house in which estimate of the amount of firewood used, temperature and the air speed inside the smoking chamber during pre-smoking process will be controlled.

2.2 Drying of fish

According to Arason (2003), drying means removal or extraction of water from a substance, usually by heating. Two things are of primary importance during drying i.e., the heat transfer that causes the evaporation of water and the mass transfer of the evaporated water through the substance and subsequently the removal of moisture away from the surface of the substance. In Tanzania, fish drying is done in different ways using sun energy. These include open sun drying where fish is spread on raised racks, on rocks and even on sand along the beach. In addition to open sun drying the use of solar panels is also common when relatively higher quality dried sardine is required. When raised racks or solar panel techniques are used, sardines are washed and in some cases fish is brined in a brine solution of 3% before drying. The addition of salt is important to enhance taste and slow down bacterial activities and other spoiling agents. Furthermore, it facilitates the drying process resulting in decreased drying time (Clucas and Ward 1996).

There are a number of reasons why a significant proportion of dried fish is spoiled by the time it reaches the markets. The fish may have been spoiled before drying. The drying process may not have been quick and, therefore, the fish took a long time to dry and quality deterioration occurs during the drying process. Weather conditions can affect the efficiency of sun drying. The dried fish may have been held in storage at the market for a prolonged period leading to quality deterioration during storage (Ward and Jeffries 2000). Furthermore, Owaga *et al*. (2009) reported that sun drying process leads to irregular and unpredictable quality as a result of slow drying. The harvesting and handling of the dagaa is a potential source of bacterial contamination due to lack of basic infrastructure such as the chilling and hygiene facilities at the landing, processing and marketing sites. The implications of reducing the amount of spoiled fish and proper processing is that, the trader in the market will receive a high price because the quality of the fish is good. If the quality of fish is good when the fish arrives in the market, then the processor should also gain from a higher price (Mgawe and Mondoka 2008).

Furthermore, Singh and Heldman (2009) stated that equilibrium condition that establishes a limit to the drying is one of the important parameters in food dehydration. Although the parameter represents an important portion of the gradient for moisture movement, water activity is important in analysis of storage stability for dry foods. Figure 1 explains the influence of water activity on rates of various deterioration reactions in foods. It shows the influence on browning reaction, autoxidation, enzymatic activity, free fatty acid formation and microorganism proliferation.

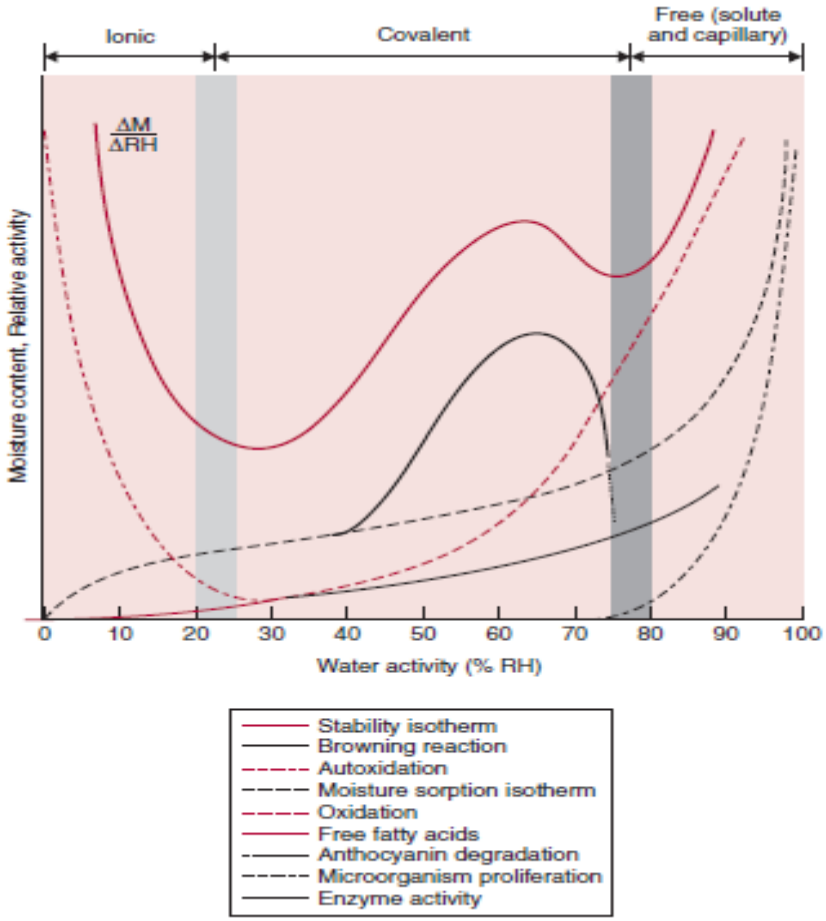


Figure 1: Influence of water activity on rates of various deterioration reactions in foods (Singh and Heldman 2009).

Analysing the quality level of sundried dagaa makes data on the quality of sun dried dagaa availability to both processors and consumers. This will help in market targeting for the processors. For example it can facilitate the selling of the products in supermarkets where the quality and safety assurance is important.

2.3 Quality Changes during Drying and Smoking

2.3.1 Lipid Oxidation

Lipid oxidation can occur at different stages of the production chain such as during processing and storage causing quality deterioration including off-odour and off-flavour. Moreover, it can cause loss in essential fatty acids, vitamins, consumer acceptability and economic loss (Azhar and Nasa 2006). Lipid oxidation progress depends on different factors, such as the amount of lipid present, the degree of unsaturated fatty acids in the muscle, salt composition and storage conditions of products (Nguyen, *et al*. 2012). It results from the chemical deterioration of fats whereas other deteriorative reactions such as microbial or enzymes attacks can largely controlled by lowering the temperature, this is not particularly helpful in preventing oxidation since low threshold are involved (Azhar and Nasa 2006).

Several methods have been used for measuring primary (hydroperoxides) and secondary (TBARS) oxidation products in foods for determining the degree of quality changes.

(Rosenthal and Kanner 1992). Also hydrolysis may influence the formation of oxidation products and affect the fish quality (Miyashita and Takagi 1986). However, primary and secondary oxidation products may react with biological amino constituents (proteins, peptides, free amino acids and phospholipids) to produce interaction fluorescence compounds. Analysis of these interaction products by fluorescence detection is becoming complementary method to TBARS and PV measurements (Aubourg *et al*. 1997).

The total lipid content of biological samples is an important quantity used in many biochemical, physiological, and nutritional studies. Thus, reliable methods for the quantitative extraction of lipids from tissues are of critical importance. Natural lipids generally comprise mixtures of nonpolar components such as glycerides (primarily triacylglycerol) and cholesterol, as well as some free fatty acids and more polar lipids (Shahidi and Zhong 2005). Isolation, or extraction, of lipid from tissues is performed with the use of various organic solvents. In principle, the solvent or solvent mixture used must be adequately polar to remove lipids from their association with cell membranes and tissue constituents but also not so polar that the solvent does not readily dissolve all triacylglycerol and other nonpolar lipids.

The formation of hydroperoxides as primary lipid oxidation products may break down to a variety of non-volatile and volatile secondary products that are determined by peroxide value measurement (Shahidi and Zhong 2005) The formation rate of hydroperoxides outweighs their rate of decomposition during the initial stage of oxidation, and this becomes reversed at later stages. Therefore, the peroxide value (PV) is an indicator of the initial stages of oxidative change (Shahidi and Wanasundara 1997). However, one can assess whether a lipid is in the growth or decay portion of the hydroperoxide concentration by monitoring the amount of hydroperoxides as a function of time. The PV represents the total hydroperoxide content and is one of the most common quality indicators of fats and oils during production and storage (Ruíz *et al*. 2001). A number of methods have been developed for determination of PV, among which the iodometric titration, ferric ion complex measurement spectrophotometry, and infrared spectroscopy are most frequently used (Shahidi and Wanasundara 1997).

The peroxides, which are primary oxidation products of fat and oil, are transitionary intermediates that decompose into various secondary products. Measurement of secondary oxidation products as an index of lipid oxidation is more appropriate because secondary products of oxidation are generally flavour active while primary oxidation products are flavourless and colourless (Martinsdottir *et al*. 1997). Secondary oxidation products include aldehydes, ketones, hydrocarbons and alcohols that can be assessed by measuring thiobarbituric acid reactive substances (TBARS) and fluorescence.

TBARS test is the most widely used method to detect the oxidation of lipids in foods due to its simplicity and accuracy, although its detail mechanism upon each species is rather uncertain (Hu and Zhong 2010). The red pigment produced by the reaction of thiobarbituric acid (TBA) directly with oxidised foods or their distillates has been identified to be a condensation product of one molecule of malondialdehyde (MDA) with two molecules of TBA. As MDA is believed to be derived from some decomposition product of the oxidised unsaturated fatty acids, spectrophotometric measurement of the TBA–MDA complex gives what is thought to be a quantitative measure of fat oxidation (Youn-Ju and Yoon 2013).

In addition of the TBARS method of assessing the secondary oxidation products, analysis of interaction compounds produced by primary and secondary oxidation products reacting with biological amino constituents (proteins, peptides, free amino acids and phospholipids) by

means of their fluorescent properties is a rapid and useful way of assessing lipid changes. Such measurements have been conducted at a single excitation/emission wavelength maximum and are considered complementary to other analyses (Miyagawa *et al*. 1991). The fluorescence properties of processed fish samples have been measured at different excitation/emission maxima (Aubourg *et al*. 1997). From a qualitative view, a fluorescence shift towards higher wavelength maxima was reported as a result of increasing lipid oxidation products with time and temperature of processing. The fluorescence ratio between two maxima (F3/F1, where F3 is 393/463 nm and F1 is 327/415 nm) showed an interesting correlation with fish quality (Aubourg *et al*. 1997).

Lipid hydrolysis is another chemical reaction that affects the quality of fish during storage. Fat hydrolysis breaks down the acyl groups of triglycerides and produces free fatty acid (Adawiyah *et al.* 2012). This reaction has negative effect on the quality of food and produces off-flavour. Lipid hydrolysis activity is determined by measuring the free fatty acid (FFA) level in the total extracted lipid (Bligh *et al.* 1988). Hydrolysis reactions are strongly influenced by the presence of water in the system. Water acts as a controller of diffusion process, dissolution of solute, proteins and enzymes activation. Water also acts as the reactants and co-substrate (Adawiyah *et al.* 2012). Therefore, it is important to study the effect of water activity on fat hydrolysis in a fish.

2.3.2 Microbial Growth

Undesirable microorganisms may contaminate fish and fishery products during handling process or through raw material, personnel and mobile equipment such as motor vehicles. Furthermore, they can access through leakage and openings in buildings or through pests and some pathogens that may even become established in the handling premises and form niches where they can survive for long periods of time (Prakash *et al*. 2011). Many of these microorganisms occur naturally in aquatic and general environments, and may be transmitted into fish before capture, during and after processing leading to quality loss (Lyhs 2009).

Many pathogenic bacteria are naturally present in aquatic environments, such as *Clostridium botulinum* type E, pathogenic Vibrio spp and *Listeria monocytogenes*. Other microorganisms are of the animal/human reservoir such as Salmonella, Shigella, Escherichia coli and enteric virus (Gibson 1992). Despite various methods of fish processing such as smoking and drying the type and population of microbes are also dependent on fish type, the quality of fish during processing and post-smoking storage conditions (Nyarko *et al*. 2011). The distribution of microbial flora in smoked and dried fish products varies largely, depending on the quality of fish at the time of processing, the smoking temperature and duration, the salt content, and the drying time (Nickelson *et al*. 2001).

Water activity (aw) and water content plays an important role for microbial growth and subsequent spoilage of the processed fish. Water activity is a measure of the free or "unbound" water present in food products. It is a critical factor affecting the shelf life of foods. It is a better indicator than total moisture to predict the stability of food with respect to physical properties, rates of detrimental reactions, and microbial growth (Lupin 1986; Hilderbrand 2001). Several factors can influence growth and the rate of multiplication of organism in food products. These include temperature, pH and water activity. Out of all these, water activity is the most important factor. Most bacteria do not grow at water activity below 0.91, and most moulds cease to grow at water activity below 0.80. By measuring the water activity of foods, it is possible to predict which microorganisms will or will not be potential sources of spoilage.

Many traditional as well as recent food preservation processes attempt to control spoilage by lowering the availability of water to microorganisms (Troller, and Christian 1978). Reducing the amount of free or unbound water can also minimize other undesirable chemical reactions that occur in foods during storage. The processes used to reduce the amount of free water in foods include drying and smoking, which result in preservation of food.

Spoilage of fish can also be caused by the presence of both total and faecal coliform. The analysis of these microbes is important to indicate the level of contamination and improper sewage treatment before discharging to the water bodies. There are two main sources of coliforms which are human and animal waste (faecal in origin) septic systems, sewage, animal yards or within the environment ("vegetative")- soil, vegetation, sediment, insects (Rompré, *et al*. 2002). Coliforms are "indicator" organisms associated with bacteriologically polluted water, their presence in finished fish product is indicative of contamination and may be associated with disease causing organisms (Rompré, *et al*. 2002). Total coliform and faecal coliform are analysed whereas the total coliform indicates the presence of all coliform group bacteria, both vegetative and faecal. Faecal Coliform consists of various genera and species of coliforms that are specifically associated with human or animal waste including E. coli. Faecal coliform testing can help pinpoint the source of pollution in water where fish are harvested.

Fungal contaminations are also common problem and it adversely affects the quality of smoked fishes. The presence of these fungi in smoked and sun dried fish is acquiring importance in view of the safety and quality of fish (Prakash *et al.* 2011). Thus, there is always a possibility that these microorganisms may be passed on to the raw material during handling and processing. In general, when a healthy fish is caught, the flesh is sterile with immune system that prevents bacteria to proliferate easily. Whereas after death the fish's immune system collapses allowing easily access of microorganisms into the fish flesh (Gibson 1992).

Determination of microbiological quality of such processed fishes from different processing techniques is very important for guarding consumer's health and hygiene. In Tanzania, smoked and sun dried fishes are popular in the local markets and some are exported to neighbouring countries. But, sometimes the export of sun dried and smoked fish products are hindered due to their poor quality and absence of quality information.

3 MATERIALS AND METHOD

3.1 Experimental design

Fresh water sardine (Dagaa) harvested from Lake Victoria in Tanzania, was washed on board without any other treatments and was purchased from local fishermen. Thereafter, the dagaa was iced and transported to a processing area where proper washing and salting was done. A 5% salt concentration solution was used for salting. The salted sardine was then allowed to drain on racks (dripping) and divided into three portions depending on the processing method to follow thereafter. The first portion was dried on raised racks using direct open sun drying at ambient temperature of between 28-35 °C. The second portion was dried in a solar drying panel with internal temperature of between 37-42 °C. The third portion was hot smoked at a temperature of 70 °C in a smoking house. In addition two samples of dried sardine that had been locally dried on rock and beach sand were bought from the area where drying was taking place. All the samples were packed in polyethylene bags, each weighing 100 g and transported

to Iceland where analysis for lipid oxidation and microbial level was done. The experimental design is presented schematically in Figure 2.

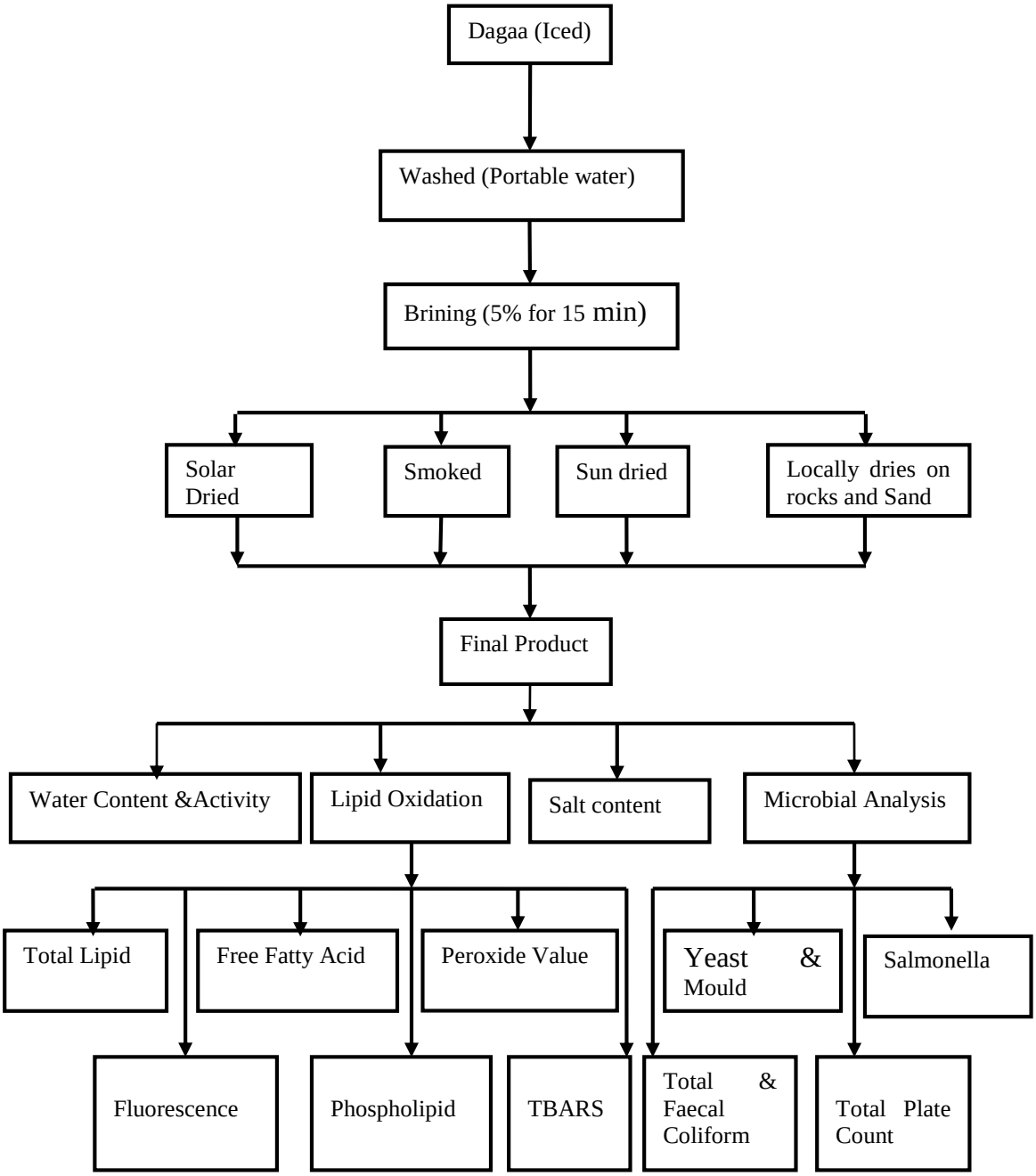


Figure 2*:* Experimental design on the determination of the effect of processing methods on the quality of sardine.

Capelin (*Mallotus villosus)* was caught in December, 2012 by trawler fishing vessel of Samherji Company and then frozen on board. The frozen capelin was transported to Matis where the experiments were carried out. The fish was thawed at temperature of 2 °C for 18 hours and properly washed using clean tap water before dividing into two portions which were treated differently. The first portion containing 12 kg was gutted and then brined by immersing in 5% salt concentration solution while the second portion of 10 kg was not gutted but brined using the same salt concentration of 5%. After brining, the brined capelin was further divided

into two parts that were either cold or hot smoked, ending up with four capelin treatments. In addition the control of air speed during pre-drying and smoking temperature was done. The experimental design is presented schematically in Figure 3.

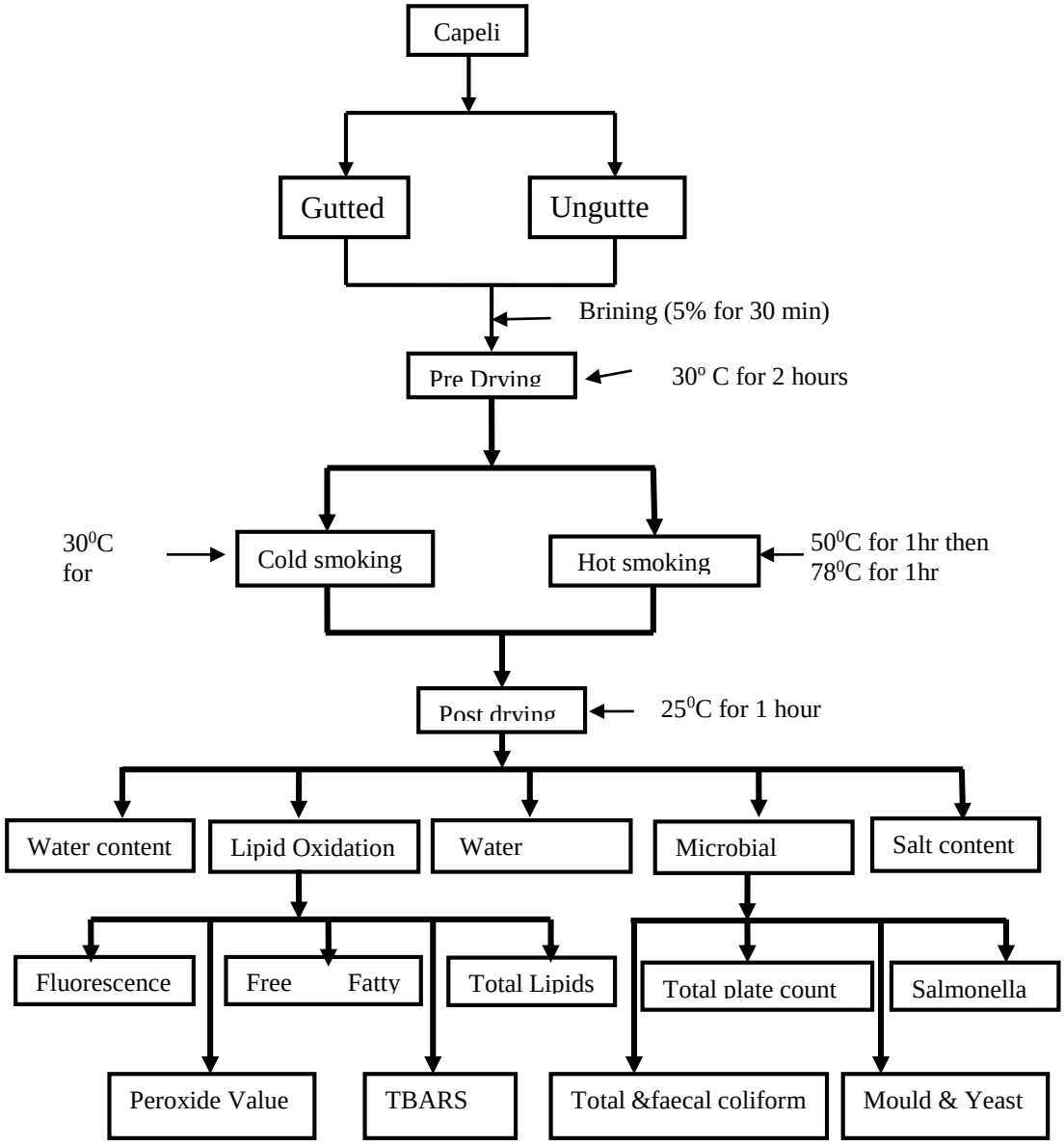


Figure 3*:* Experimental design on the effect of cold and hot smoking on quality of capelin

The quality changes of smoked and dried sardine stored at 30 °C and relative humidity of 78% was done. Also capelin due to its higher fat content was kept at 2 °C during storage time. The samples were taken out to determine lipid oxidation and microbial growth at zero storage time, after two, four and six weeks of storage. In addition, the quality of capelin raw materials was analysed. Figure 4 summarises the experimental design for both capelin and sardine during storage time.

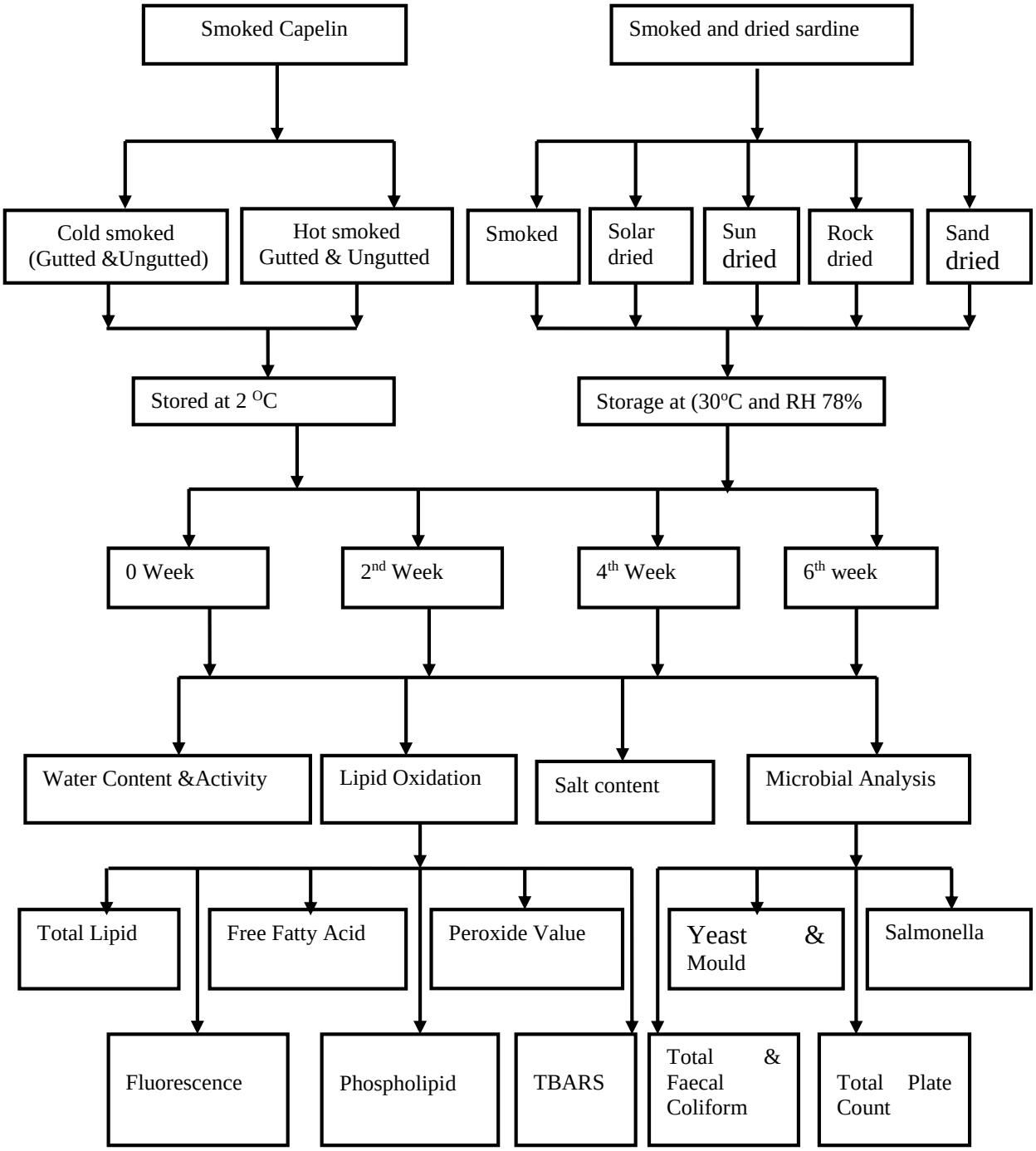


Figure 4: Experimental design on the effect of storage time and conditions on quality of sardine and capelin.

3.2 Analytical Methods

3.2.1 Water content measurement

Water content was determined as the weight loss during drying at 103 °C ± 1°C for 4 hours according to the ISO 6496 (1990) method.

3.2.2 Water activity Measurement

The Novasina AW-Center (AWC503 RS-C, Axair AG, Switzerland) was used to measure water activity. The equipment can measure three samples simultaneously in a temperature controlled heating chamber. The instrument used sensors that had previously been calibrated with saturated salt solutions at six humidity reference points: RH =33%, 53%, 75% and 90%, offering a measuring range of a_w from 0.06 to 1.00. The sensors in the measuring chamber indirectly registered the humidity change in the conductivity of a hygroscopic electrolyte. Measurements were done at 25 °C ± 0.2 °C. The samples were placed in a clean and dry plastic sample cup. The analysis was done in duplicate.

3.2.3 Salt content measurement

Salt content of all the samples was determined by titration according to AOAC (1995). Soluble chloride was extracted from the samples with water containing nitric acid. The chloride content of the solution was titrated with silver nitrate and the end point was determined potentiometrically.

3.2.4 Lipid Analysis

Total Lipids

Lipids of the fish samples were extracted according to the Bligh and Dyer (1959) method. The lipid content was determined gravimetrically and the results expressed as g lipid/100 g of sample. The rest of the extracts used for determination of free fatty acid content, phospholipids and for fluorescence measurements

Free fatty acid determination (FFA)

Free fatty acid content was determined by the method of Bernardez, e*t al*. (2005), based on complex formation with cupric acetate-pyridine, followed by absorbance reading at 715 nm (UV-1800 spectrophotometer, Shimadzu, Kyoto, Japan). Results were expressed as g FFA/100 g lipids.

Thiobarbituric acid-reactive substances (TBARS) determination

Thiobarbituric acid-reactive substances were measured using the method of Lemon (1975) with modifications. A 2.0 g sample homogenised with 10 ml of 7.5% trichloroacetic acid (TCA) using an Ultra-Turrax homogeniser (Kika Labortechnik, T25 basic, Staufen, Germany) at 2000 rpm for 10 s. The homogenate was then centrifuged at 5000 x *g* for 20 min at 4 °C (Tj-25 Centrifuge, Rotor TS-5.1-500, Beckman Coulter, California, USA). A mixture of 0.5 ml of supernatant and 0.5 ml of 0.02 M thiobarbituric acid solution was heated in a water bath at 95 °C for 40 min. The heated samples were cooled down on ice and absorbance was read at 530 nm (Sunrise Microplate Reader, Tecan Austria GmbH, A-5082 Grödig, Austria). The results were expressed as µmol malondialdehyde per kg sample (µmol MDA/kg) and calculated using a standard curve prepared from 1,1,3,3-tetraethoxypropane (TEP).

Lipid hydroperoxides determination (PV)

Lipid hydroperoxides were determined by the ferric thiocyanate method (Santha and Decker 1994). The results were expressed as µmol lipid hydroperoxides per gram sample.

Phospholipid determination

Phospholipid content of the fish muscle was determined on the lipid extraction (Bligh and Dyer 1959) by using a spectrophotometric method (Stewart 1980), based on complex formation of phospholipid with ammonium ferrothiocyanate, followed by absorbance reading at 488 nm (UV-1800 spectrophotometer, Shimadzu, Kyoto, Japan). The results were expressed as percentage of total lipid content and calculated using a standard curve prepared from phosphatidylcholine.

Analysis of fluorescence

The excitation and emission of the lipid extraction (Bligh and Dyer, 1959) was determined using Perkin–Elmer LS 50B fluorescence spectrometer (Perkin-Elmer, Massachusetts 02451, USA). The fluorescence shift (δF) was calculated as the ratio between the relative fluorescence intensity at excitation/emission maxima 393/463 nm and 327/415 nm. The fluorescence shift (δF) was determined in both aqueous methanol phase (δFaq) and organic-chloroform phase (δFor) (Aubourg *et al*. 1997)

3.2.5 Microbial determination

Determination of total plate counts

The conventional "pour plate" method was used on Plate Count Agar. Incubation temperature was 37 °C and incubation time was 72 h). At the end of the incubation period, the petri plates containing between 30 and 300 colonies were selected. Plates with more than 300 colonies cannot be counted and were designated too many to count (TMTC). Plates with fewer than 30 colonies were designated too few to count (TFTC). Colonies on each plate were counted using a colony counter and results were recorded.

Determination of total and faecal coliforms

The total and faecal coliform was determined by the most probable number (MPN) method. Preenrichment is in LST broth (37 °C for 24/48 h) and confirmation tests were done in BGLB broth for total coliforms (37 °C for 48 h) and in EC broth for faecal coliforms (44 °C for 24 h).

Analysis of Salmonella

The procedure involves first enrichment in BPW broth (37 °C for 18h). Second enrichment was in Rappaport-Vassiliadis (RV) broth (41.5 °C for 24h) and tetrathionate broth (41.5 °C for 24 h). From these broths, streak onto two solid media: XLD and BG (37 °C for 24 h). Typical colonies (2-4) were inoculated into TSI-agar and LI agar (37 °C for 24 h). Confirmation was done by testing for flagellar (- H-) and somatic (- O-) antigens.

Determination of yeasts and moulds

The isolation medium used was Dichloran Rose-Bengal Chloramphenicol Agar (DRCB Agar). Fish samples (20 g) were homogenised with 180 mL of sterile alkaline water in a laboratory

homogenizer and serial dilutions were prepared. Then Surface plating techniques were used, where 0.1 mL of each dilution was spread with a bent sterile glass rod on duplicate plates of pre-poured and dried standard plate count. Plates were incubated at 22 °C for 120 h. Colonies were counted and expressed as $\log_{10}$ CFU/g of fish sample

3.3. Statistical analysis

The data sets obtained were analysed by General Linear Modelling (GLM) to investigate the main effects of different drying and smoking methods on the indicators of lipid oxidation and microbial growth in fresh water sardine and capelin during processing and storage. ANOVA and Duncan's Multiple-Comparison Test were used for comparisons of means using the NCSS 2000 software (NCSS, Kaysville, Utah, USA). Significance of differences was defined at the 5% level ($p < 0.05$).

A multivariate analysis on weighted principal component (PCA) was performed on all data obtained using Unscrambler® (Version 10.2, CAMO ASA, Trondheim, Norway). PCA was performed to detect the most important factors of variability and to describe the relationship between variables and observations. All data were mean centred and scaled to equal unit variance prior to PCA. Full cross-validation with uncertainty test was performed in all validation models.

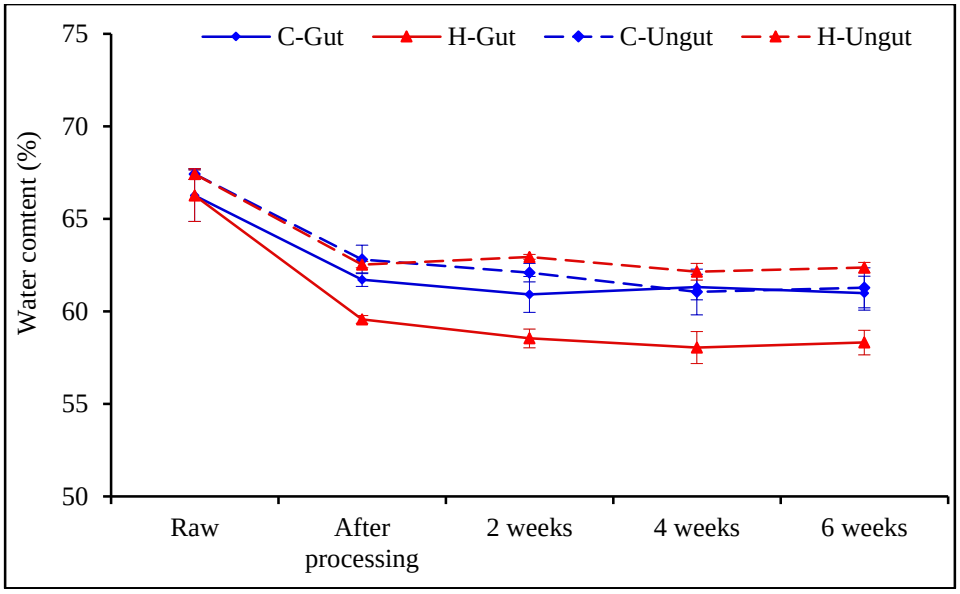
4 RESULTS

4.1 Water content

Generally, the water content of capelin (gutted and ungutted) decreased significantly ($p<0.05$) after smoking (cold and hot smoking) and then remained rather stable during the storage period (Figure 5a). The water content of ungutted samples was higher than that of gutted samples. Moreover, the water content of hot smoked-gutted capelin samples was significantly ($p<0.05$) lower compared to that of cold smoked-gutted samples. Whereas, the water content of cold and hot smoked ungutted samples was similar.

As the same pattern of changes in water content during storage of capelin, the water content of all dried sardine samples was rather stable during the storage time (Figure 5b). The water content of sun and solar dried samples was similar and significantly ($p<0.05$) lower than that of the other samples. The highest water content was obtained in the rock and sand dried sardine samples.

(a)



(b)

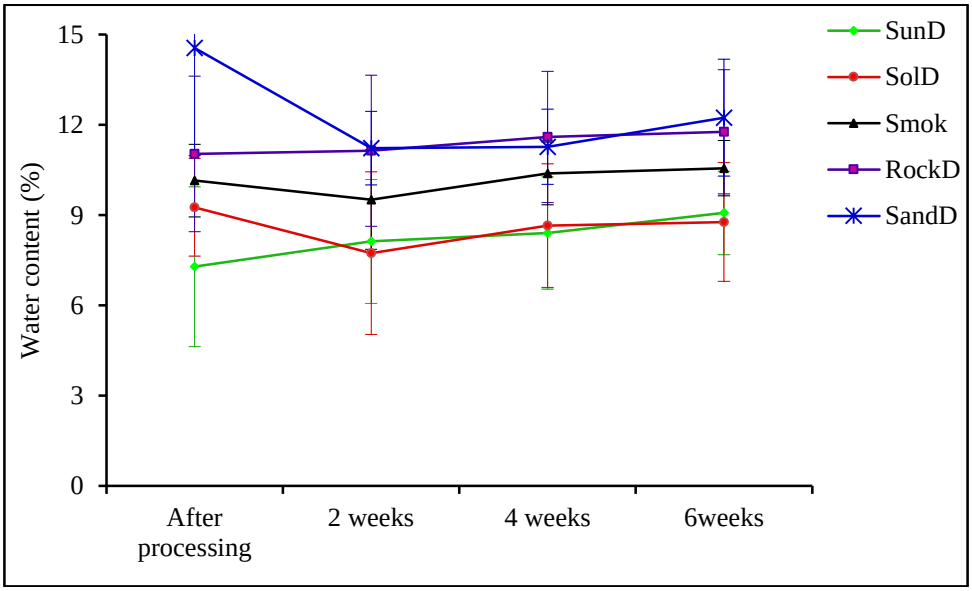


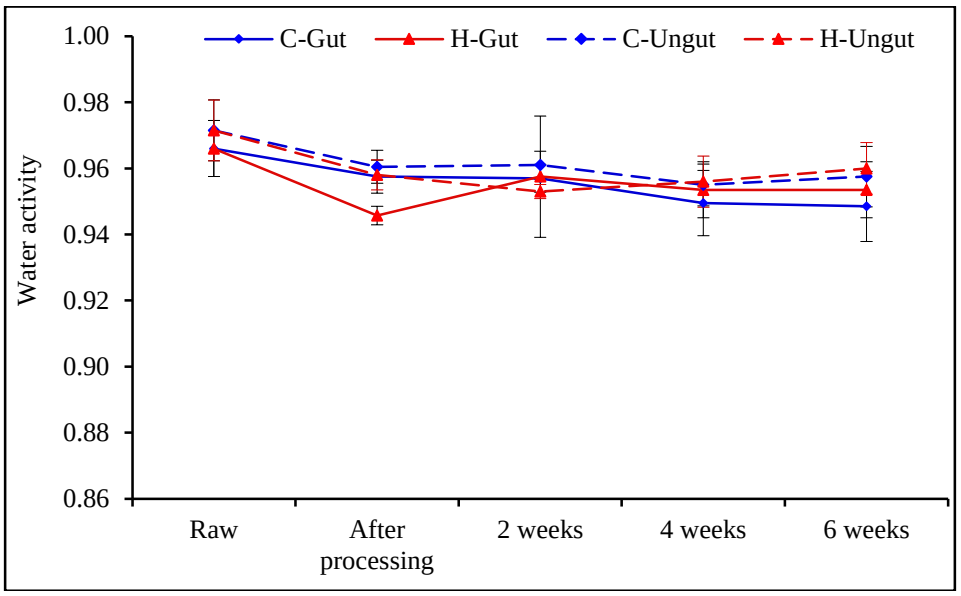
Figure 5: The change in water content of capelin (a) and sardine (b) as influenced by processing method and storage for six weeks.

4.2 Water Activity

In accordance with changes in water content during processing and storage, the water activity of all capelin and sardine samples decreased after processing and was rather stable throughout the storage period (Figure 6a and 6b, respectively). No significant ($p>0.05$) differences in water activity value were found between different treatments of capelin.

A significant difference in water activity was observed among the different treatments of sardine. The sun and solar dried sardine samples had the same water activity value and significantly ($p<0.05$) lower than that of other samples. Water activity of rock and sand dried sardine samples was the highest.

(a)



(b)

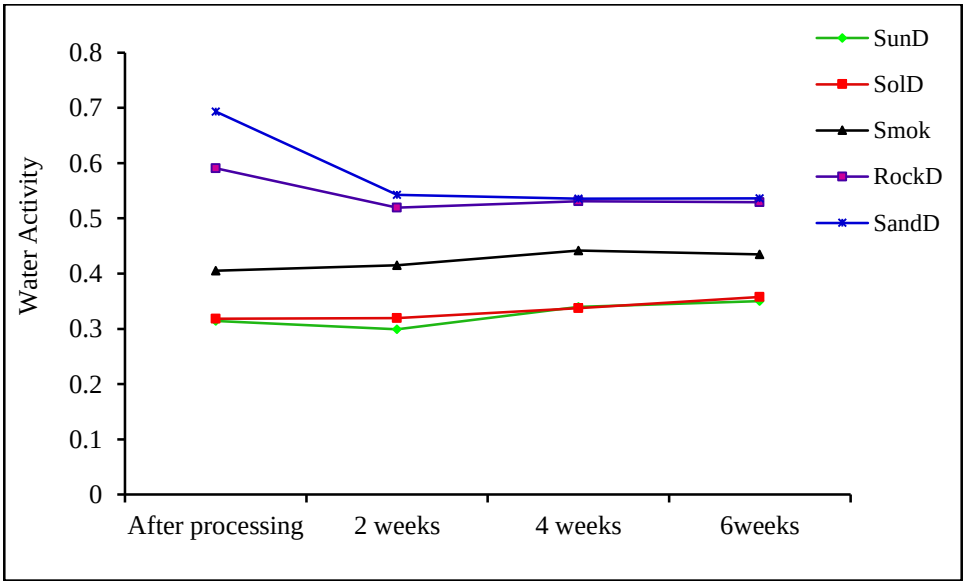


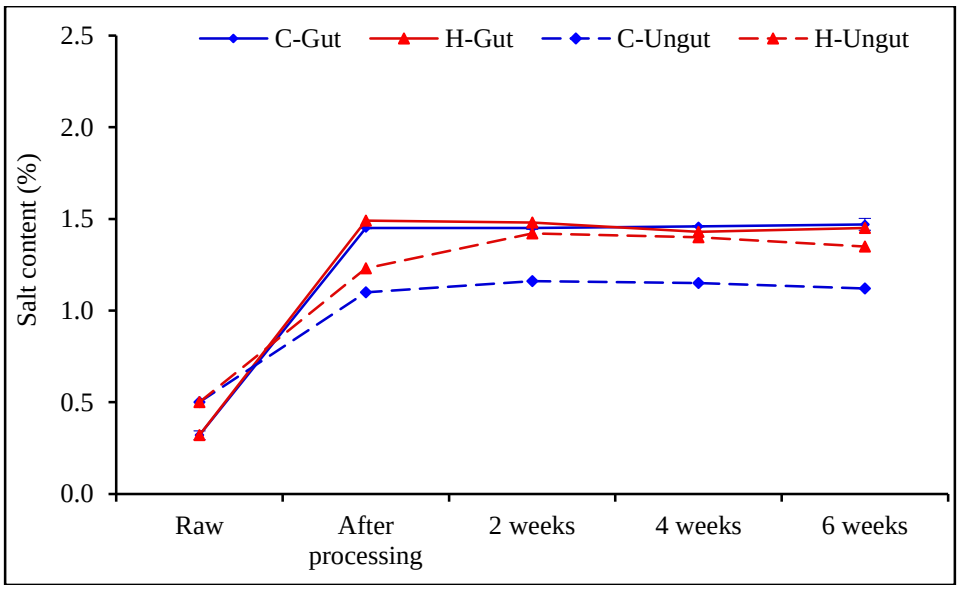
Figure 6*:* The change in water activity of capelin (a) and sardine (b) as they differ by processing methods and storage time.

4.3 Salt Content

The pattern of changes in salt content was inversely related to the changes in water content. The salt content of all capelin samples significantly ($p<0.05$) increased after processing (i.e. brining and smoking steps) and then remained rather stable during the storage time (Figure 7a). In general, the salt content of gutted capelin was higher than that of ungutted fish. The salt content of hot smoked capelin was higher than that of cold smoked capelin. However, no significant ($p>0.05$) differences in salt content were found between the treatments throughout the processing and storage period.

The salt content of all sardine samples remained stable during storage for 6 weeks (Figure 7b). The salt content in sun dried sardine was highest, followed by solar dried, then smoked and least value was for rock and sand dried samples since the fish were not brined before drying.

(a)



(b)

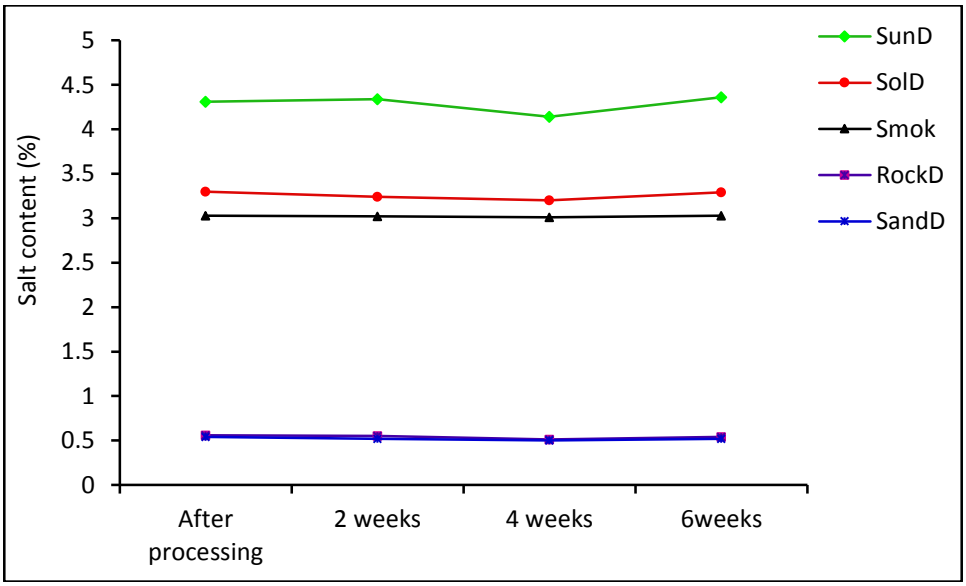


Figure 7*:* The change in salt content of capelin (a) and sardine (b) as influenced by processing methods and storage time.

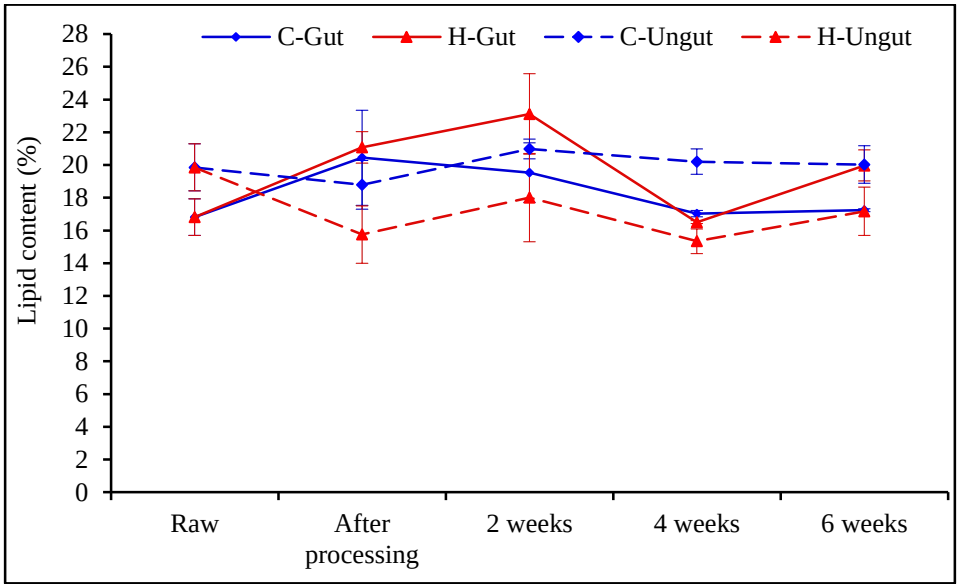
4.4 Total lipid content.

Generally, the lipid content of cold smoked capelin (gutted and ungutted) samples was rather stable during processing and storage, different from hot smoked where there was an increase in lipid content of gutted sample and decrease in ungutted capelin after storage for 2 weeks

(Figure 8a). However, both cold and hot smoked capelin was observed to have stable lipid content after week 4 to subsequent storage, up to 6 weeks.

The lipid content of all sardine samples was slightly decreased during the 6 weeks of storage (Figure 8b). The lowest lipid content was obtained in rock and sand dried sardine, but no significant ($p>0.05$) differences in lipid content were found between the treatments.

(a)



(b)

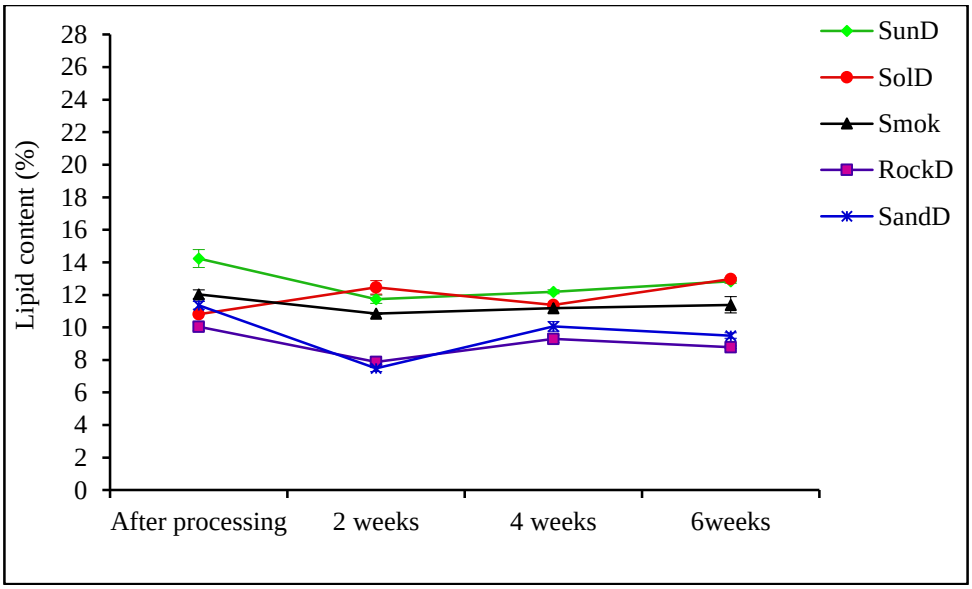


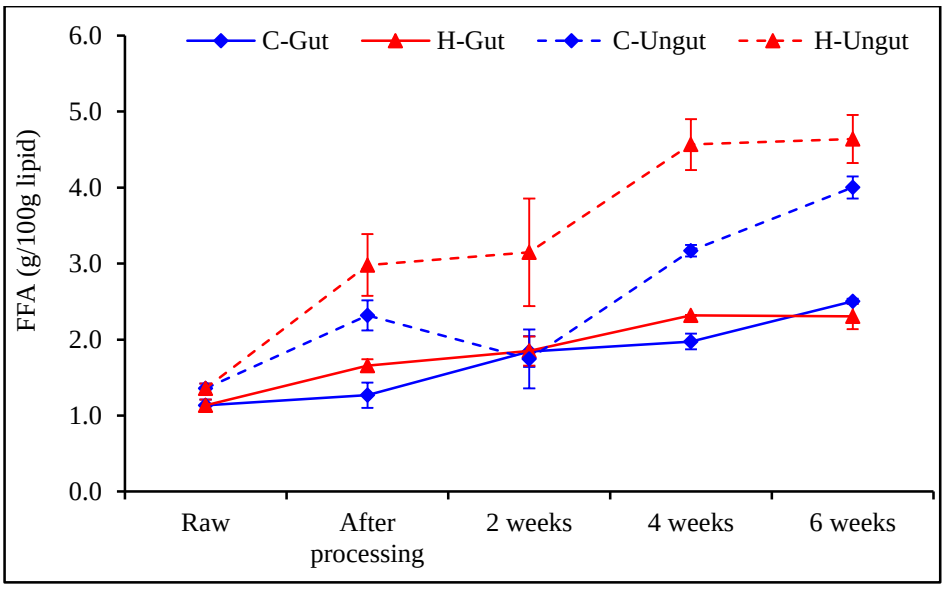
Figure 8*:* The change in total lipid of capelin (a) and sardine (b) as for different processing methods and storage time.

4.5 Changes in Free Fatty Acid (FFA) content.

In general, the FFA content of all capelin samples increased significantly ($p<0.05$) throughout the processing and storage time (Figure 9a). The FFA content of hot smoked capelin was higher than that of cold smoked capelin. Differences were also noted between the gutted and ungutted capelin where ungutted samples had higher values of FFA content compared to that of the gutted samples. Similarly, the FFA content of all dried sardine samples increased during

storage for 6 weeks (Figure 9b). The FFA content of sun and solar dried sardine samples was significantly ($p<0.05$) lower than that of rock and sand dried sardine samples. Furthermore, the smoked sardine samples had lowest FFA value.

(a)



(b)

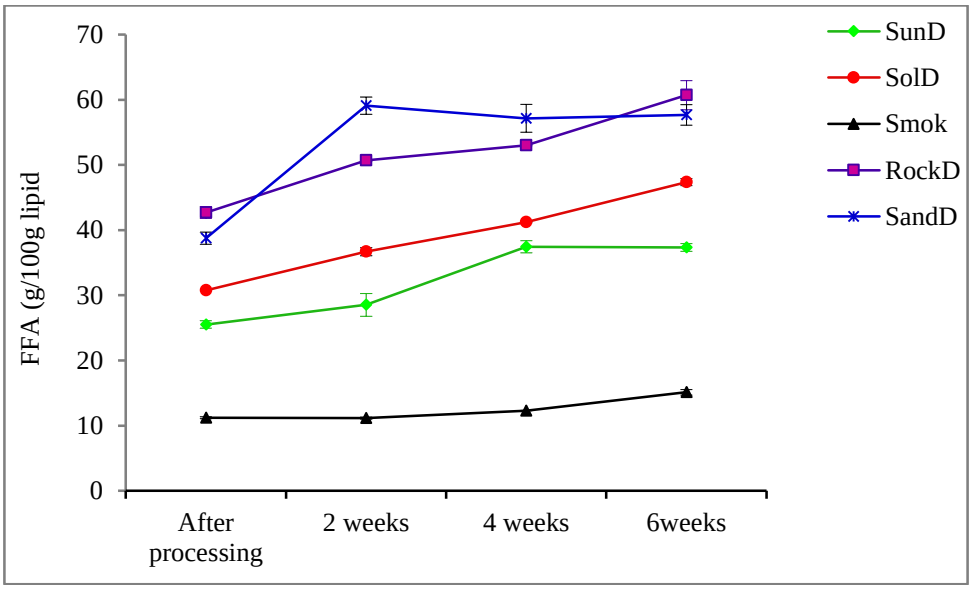


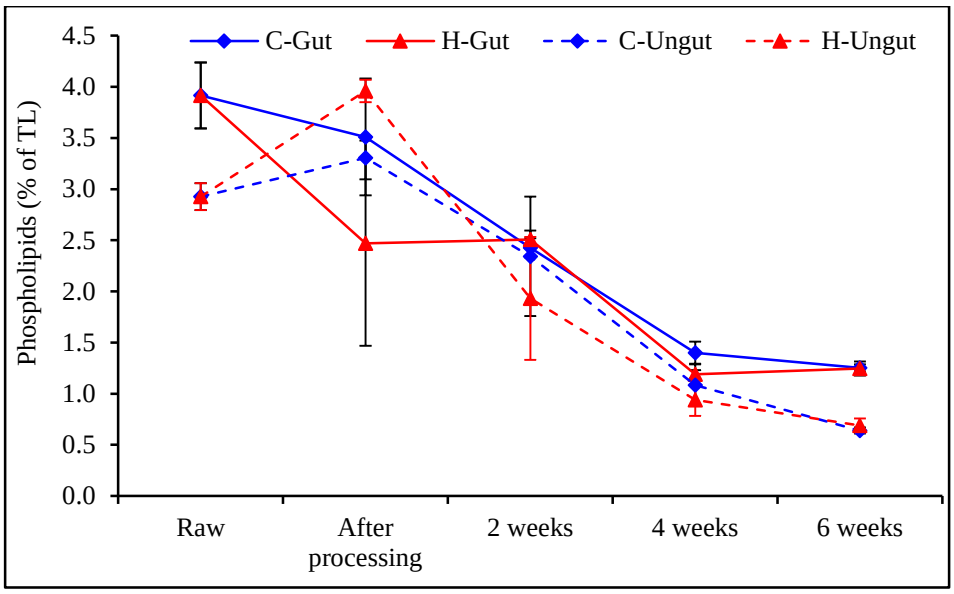
Figure 9*:* The change in free fatty acid of capelin (a) and sardine (b) for different processing method and storage time.

4.6 Change in Phospholipid Content

The phospholipid content of gutted capelin (cold and hot smoked) decreased after processing, whereas an increase in phospholipid content was observed in ungutted samples (cold and hot smoked). During the storage period, the phospholipid content of all capelin samples significantly ($p<0.05$) decreased (Figure 10a). The phospholipid content of cold and hot gutted smoked capelin was higher than that of cold and hot smoked ungutted samples. Furthermore, for the last two weeks of storage there was no change in phospholipid content of both cold and hot smoked gutted capelin. At the same time there was a steady decrease for the ungutted

towards the end of storage for week 6. A significant ($p<0.05$) difference in phospholipid content was noted for different treatment of sardine (Figure 10b). The smoked sardine contained higher phospholipid value than that of other treatments throughout the storage time. Lowest values were noted for the sardine locally dried on rocks and sand.

(a)



(b)

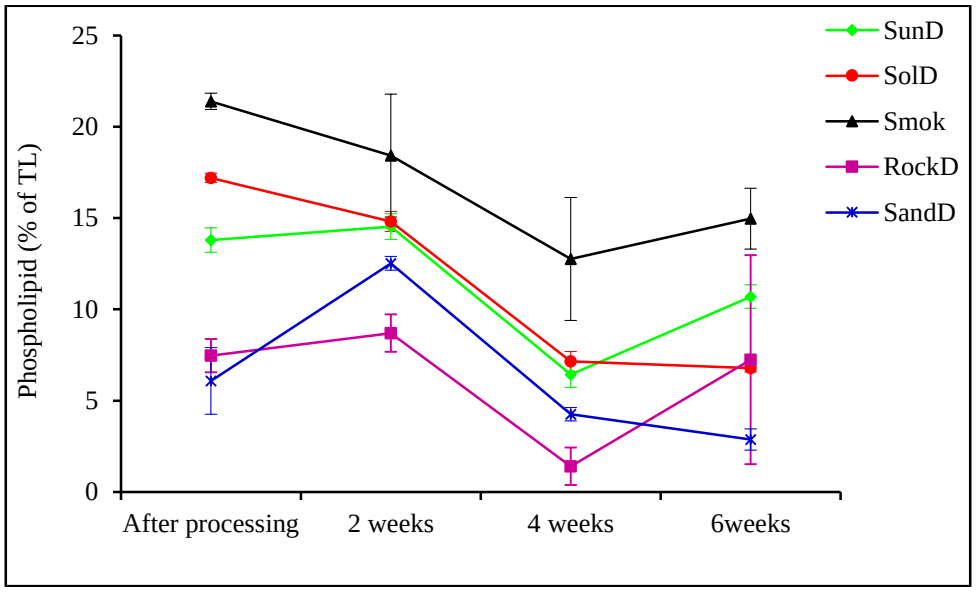


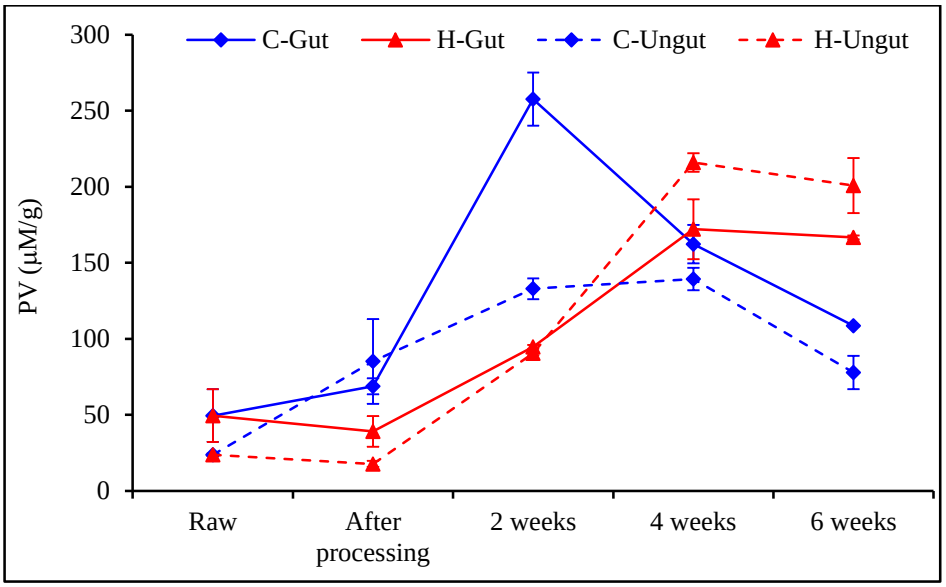
Figure 10: The change in phospholipids in capelin (a) and sardine (b) as influenced by processing method and storage time.

4.7 Changes in peroxide value (PV)

The peroxide value differed between the gutted and ungutted capelin. A general trend of increased PV was observed in all capelin samples after processing and during the first 4 weeks of storage (Figure 11a). However, a decrease in PV content of all capelin samples was found during the last 2 weeks of storage. Moreover, at the end of storage time the cold smoked capelin (gutted and ungutted) had significantly ($p<0.05$) lower PV content compared to that of hot smoked samples. The PV content for fresh water sardine processed by sun drying, rock drying and sand drying was constant after two weeks of storage (Figure 11b). The PV content of all

samples increased from week 2 to week 4 of storage time and rather remained stable during the last 2 weeks of storage.

(a)



(b)

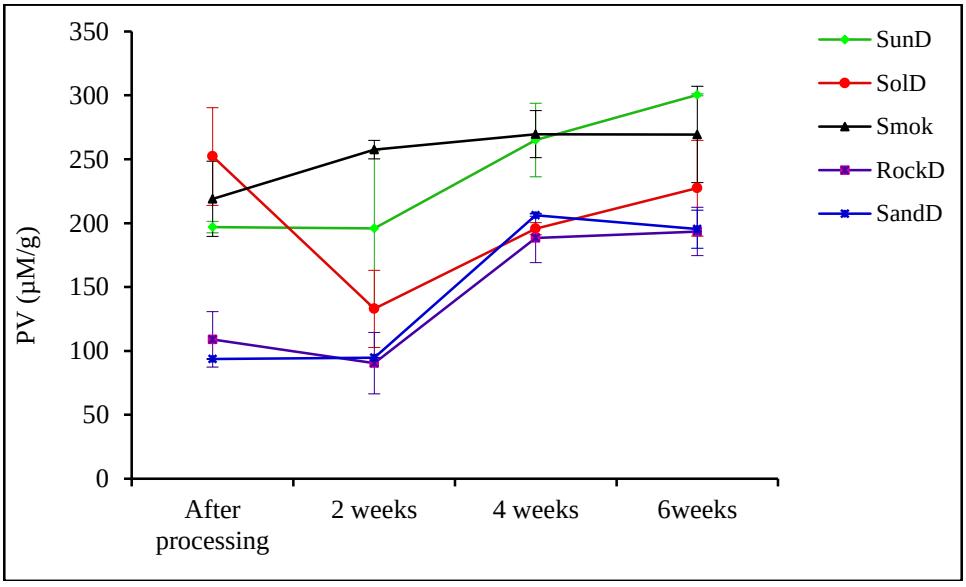


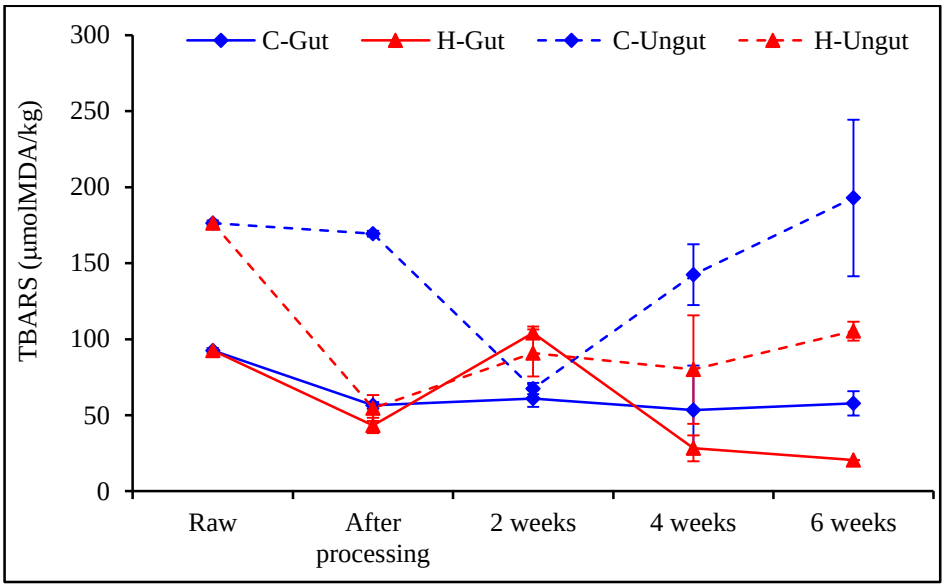
Figure 11: The change in peroxide value in capelin (a) and sardine (b) as influenced by processing method and storage conditions.

4.8 Thiobarbituric acid-reactive substances (TBARS)

The result for TBARS content of capelin (Figure 12a), showed a decrease after processing. After two weeks of storage, the TBARS content increased for the hot smoked capelin, whereas further decrease occurred in cold smoked capelin. During the last 4 weeks of storage, the TBARS content of cold smoked gutted and hot smoked ungutted samples was rather stable. The TBARS content of cold smoked ungutted capelin increased significantly ($p<0.05$), but a significant ($p<0.05$) decrease was observed in hot smoked gutted samples (Figure 12a). The TBARS for sardine varied greatly with the processing method (Figure 12b).

Generally, the TBARS content of all samples decreased during the first 4 weeks of storage, except an increase was found in the sun dried sardine samples after 2 weeks of storage. During the last 2 weeks of storage time, the TBARS content of all samples was rather stable. The sun dried, sand dried and rock dried samples contained higher TBARS values than that of smoked and solar dried samples (Figure 12b).

(a)



(b)

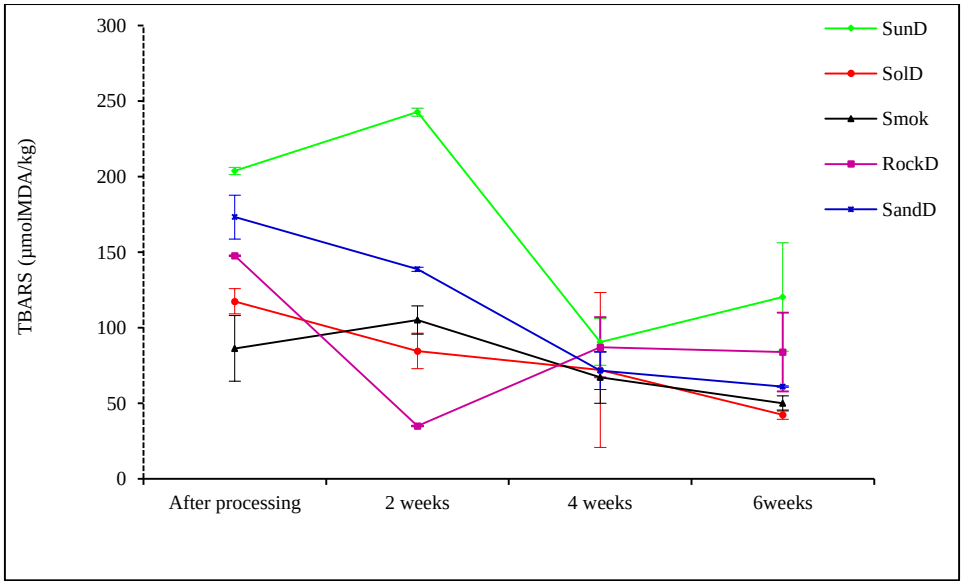
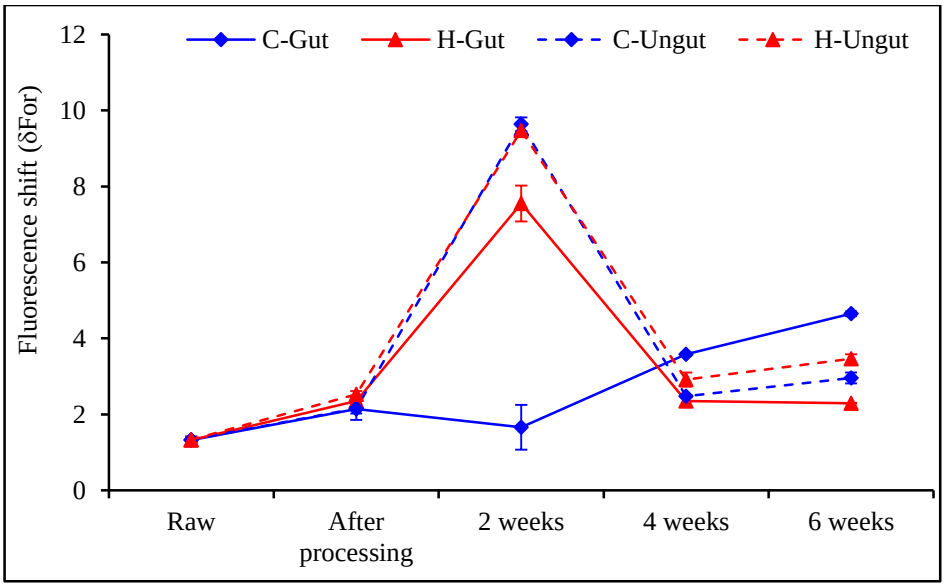


Figure 12: The change in TBARS content of capelin (a) and sardine (b) as influenced by processing method and storage time.

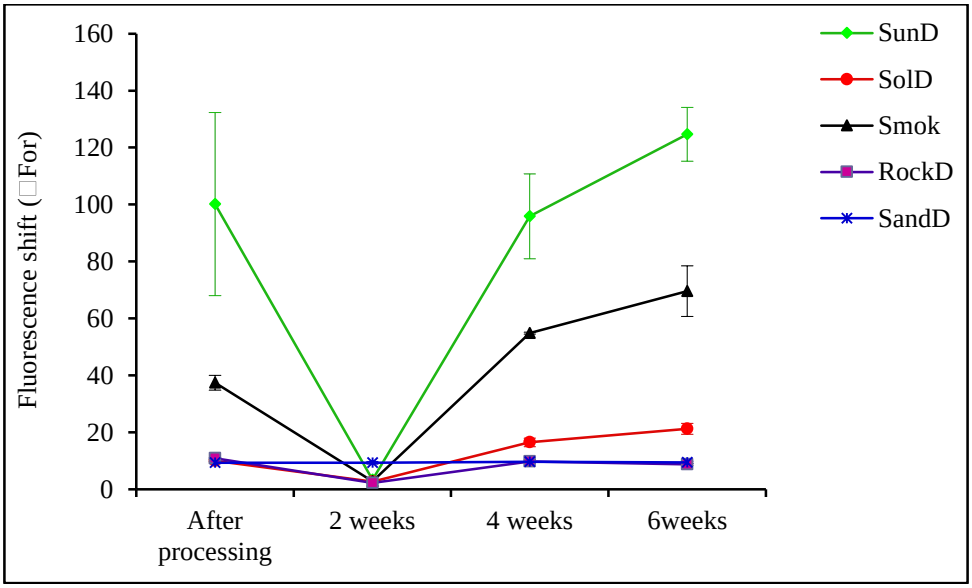
4.9 Fluorescence measurements.

The results of fluorescence shift, measured in the both organic and aqueous phases from the Bligh and Dyer lipid extraction (δFor and δFaq) of different capelin samples, showed a general progressive increase in both δFor and δFaq values for the first 2 weeks of storage time. At the fourth week sampling, there was a noted decrease in fluorescence shift and remained constant toward the end of the experiment (Figure 13a and 13c, respectively). Exceptional patterns were shown by sardine samples where δFor and δFaq decreased during the first two weeks of storage and then increased during the subsequent storage period (Figure 13b and 13d, respectively). Also, the sardine samples that were locally dried on the rock and beach sand showed a constant value of throughout the storage.

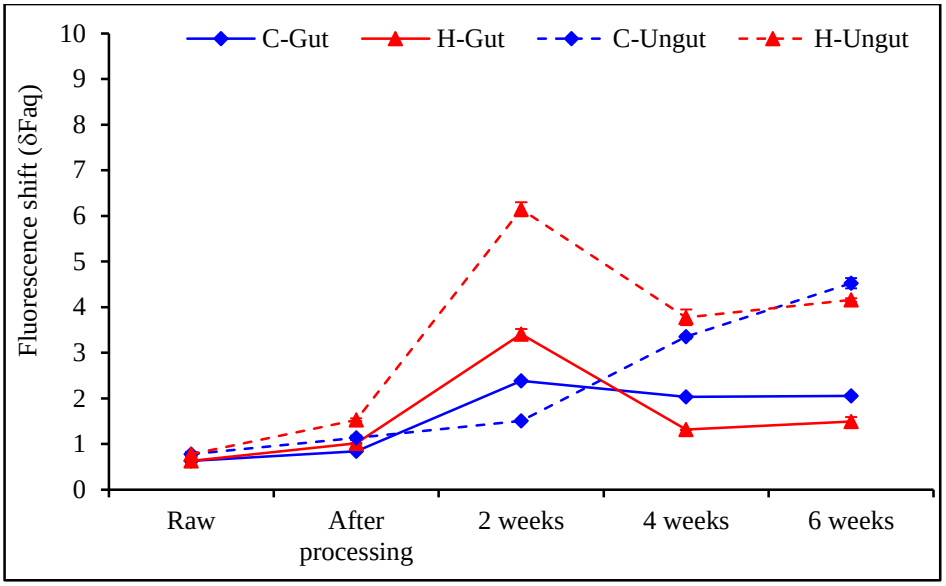
(a)



(b)



(c)



(d)

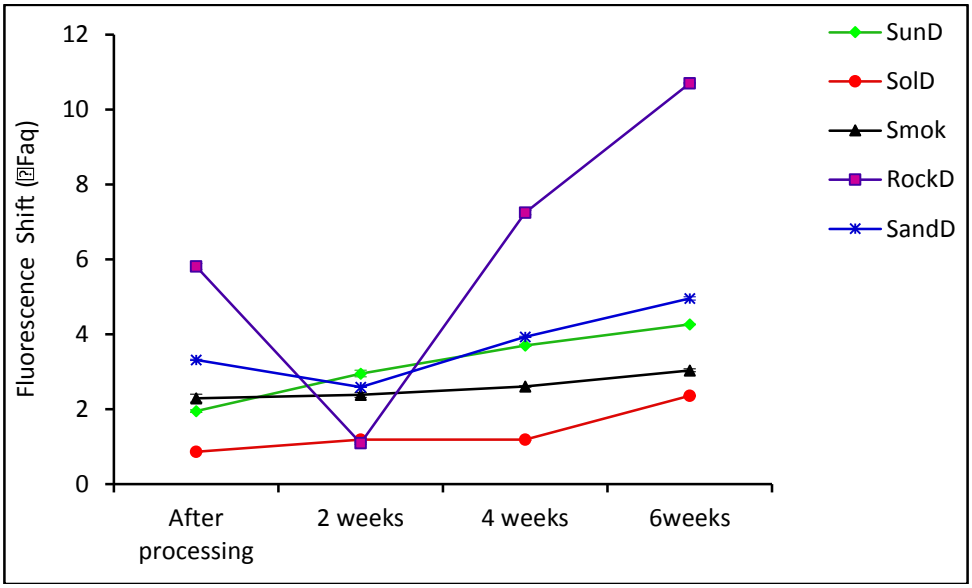


Figure 13: The change in fluorescence shift of organic phase in capelin (a) organic phase in sardine (b), aqueous phase in capelin (c) and aqueous phase of sardine (d) as influenced by the processing method and storage conditions.

4.10 Principal component analysis

Principal component analysis (PCA) was carried out in order to gain an overview of the similarities and differences among the variables. The bi-plot of scores and loadings from the first two components (PC1 and PC2) of capelin can be seen in Figure 14. The first two components explained 61% of the total variation between the samples. The PC1 represented 42% of the total variation, and mainly described the changes during the processing and storage of samples. Lipid hydrolysis (FFA), lipid oxidation product (PV) and level of interaction compounds (δF_{aq}) between lipid oxidation products and amino constituents increased with time. Fluorescence intensity of aqueous phase (δF_{aq}) was significantly correlated with FFA and PV values. The PC2 accounted for 19 % of the total variation between the samples, mainly due

to the different smoking methods (cold and hot smoking) and different sample treatments (gutted and ungutted capelin).

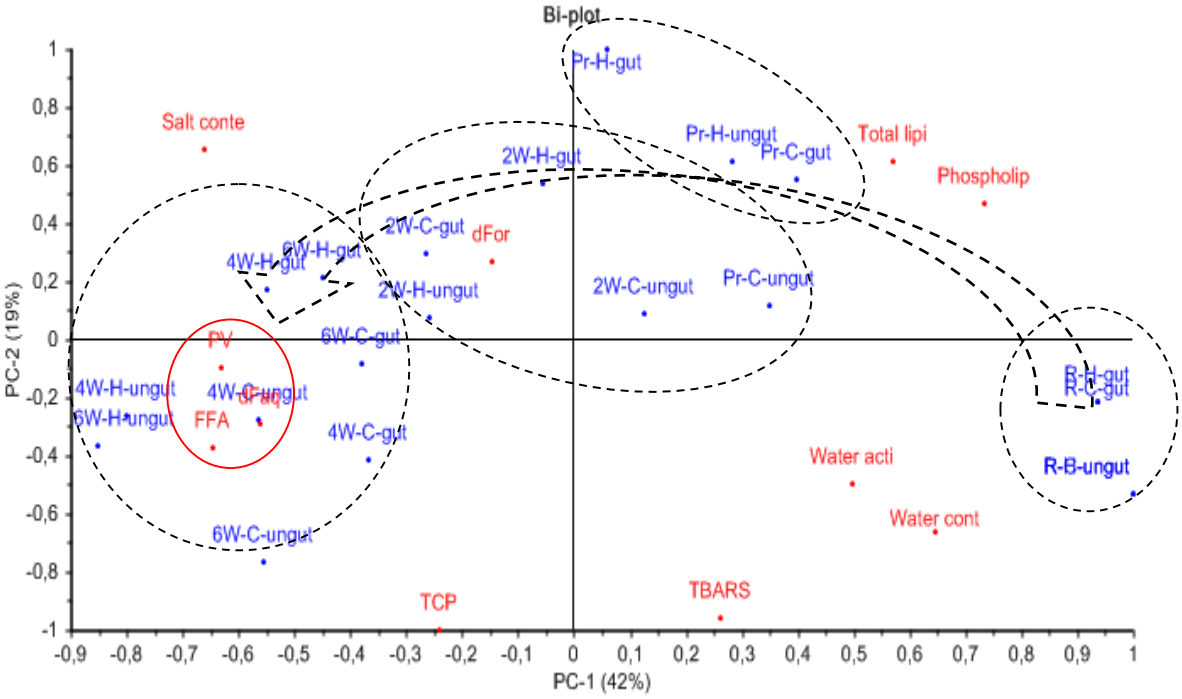


Figure 14*:* Principal component analysis (PCA) bi-plot of scores and loadings for different parameters measured during storage of capelin. Abbreviations: R, raw material; C, cold smoked; H, hot smoked; Pr, after processing; 2W, 4W and 6W, storage after processing for 2 weeks, 4 weeks and 6 weeks; gut, gutted capelin samples; ungut, ungutted capelin samples

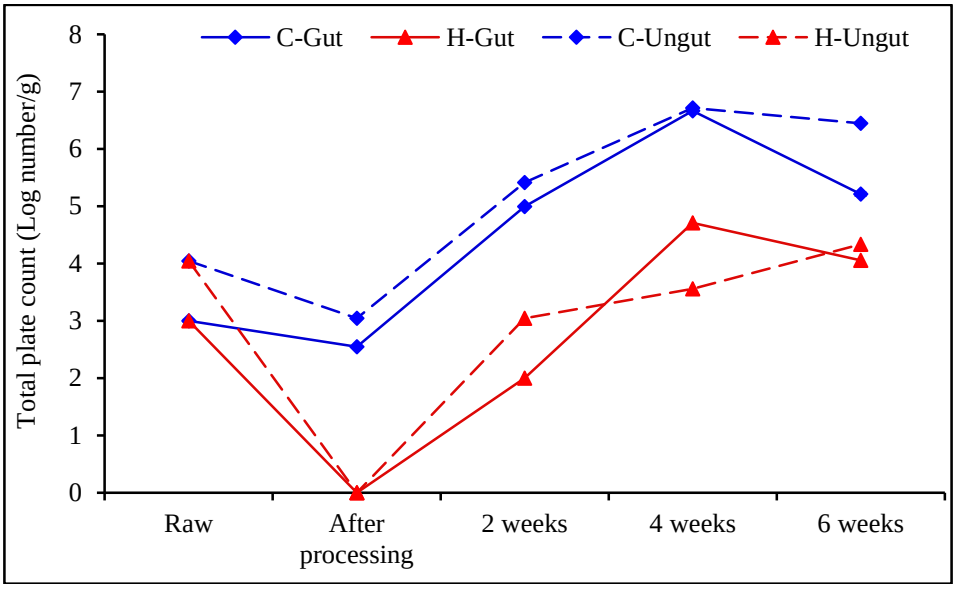
4.11 Microbial Result

4.11.1 Total plate count

The change in Total Plate Count (TPC) of capelin samples with the processing method and upon storage is shown in Figure 15a. In general, the TPC of all samples decreased remarkably ($p<0.05$) after smoking and increased significantly ($p<0.05$) again during the first 4 weeks of storage. During the last 2 weeks of storage period, the TPC tended to decrease (Figure 15a). Furthermore, the TPC of cold smoked samples (gutted and ungutted) was significantly ($p<0.05$) higher than that compared to hot smoked capelin samples throughout the processing and storage time. The TPC of ungutted samples was higher than that of gutted samples in both cold and hot smoking methods.

The TPC for all sardine samples was high initially and increased after storage for 2 weeks. However, a decrease in TPC of all samples was observed during the last 4 weeks of storage (Figure 15b). In addition, the TPC of smoked sardine showed a significantly ($p<0.05$) lower value compared to that of all dried sardine samples.

(a)



(b)

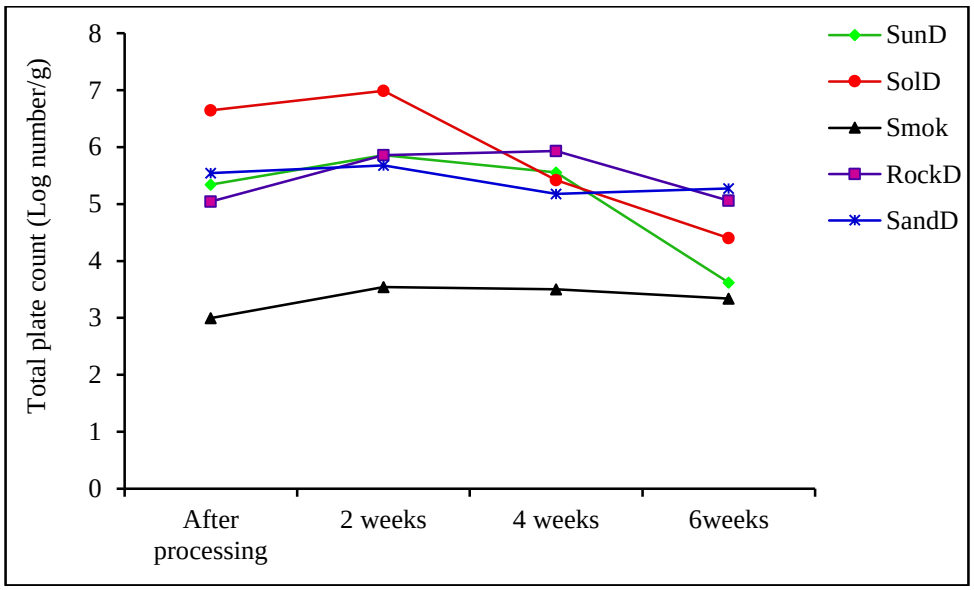


Figure 15*:* The change in bacteria count of capelin (a) and sardine (b) as influenced with the processing method and storage.

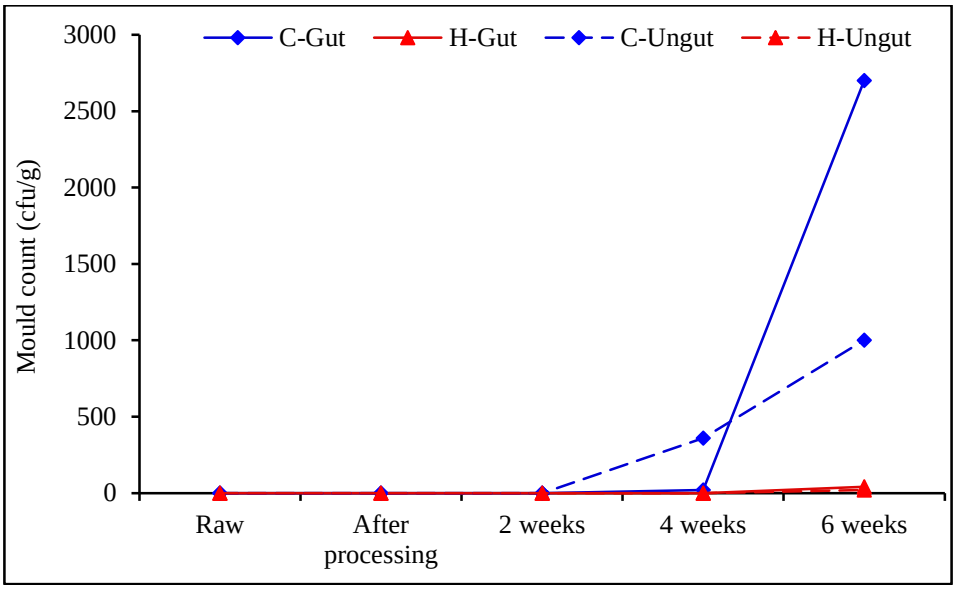
4.11.2 Mould count

In accordance with changes in TPC during processing and storage, the results of mould count for capelin showed that absence of growth was found in hot smoked capelin (gutted and ungutted) samples throughout the processing and storage period (Figure 16a). However, a growth of mould was observed after 2 weeks of storage for cold smoked ungutted capelin samples and after 4 weeks of storage for cold smoked gutted capelin.

For sardine sample, the mould count of all samples tended to decrease during storage for 6 weeks, except the rock dried samples where it increased after storage for 2 weeks and decreased again during the last 4 weeks of storage (Figure 16b). Moreover, the smoked treatment contained the lowest mould count. On the other hand, the sardine locally processed on rocks

had the highest count followed by that dried on sand. The count for solar dried and sun dried was relatively equal and higher than the count for smoked sardine (Figure16b).

(a)



(b)

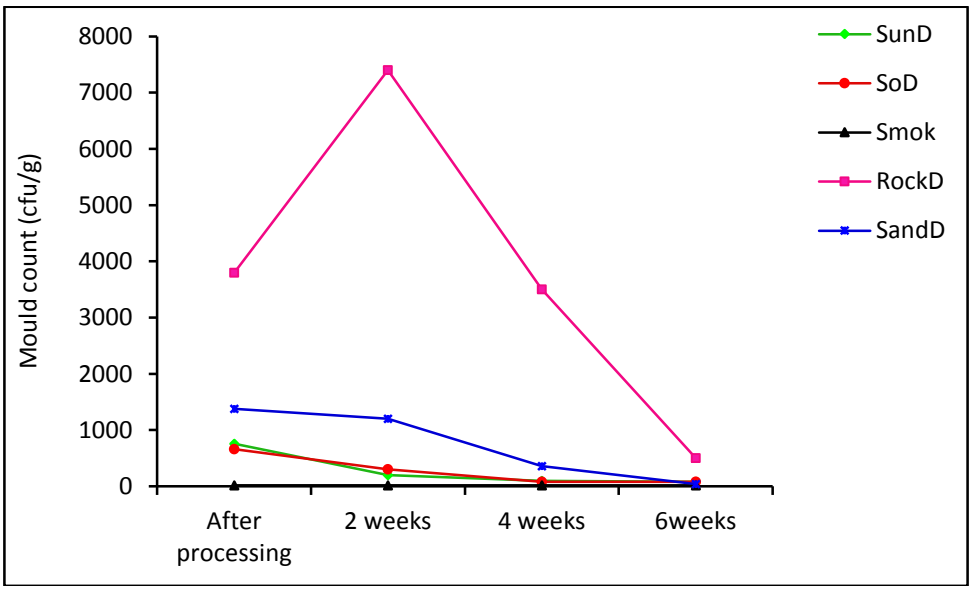
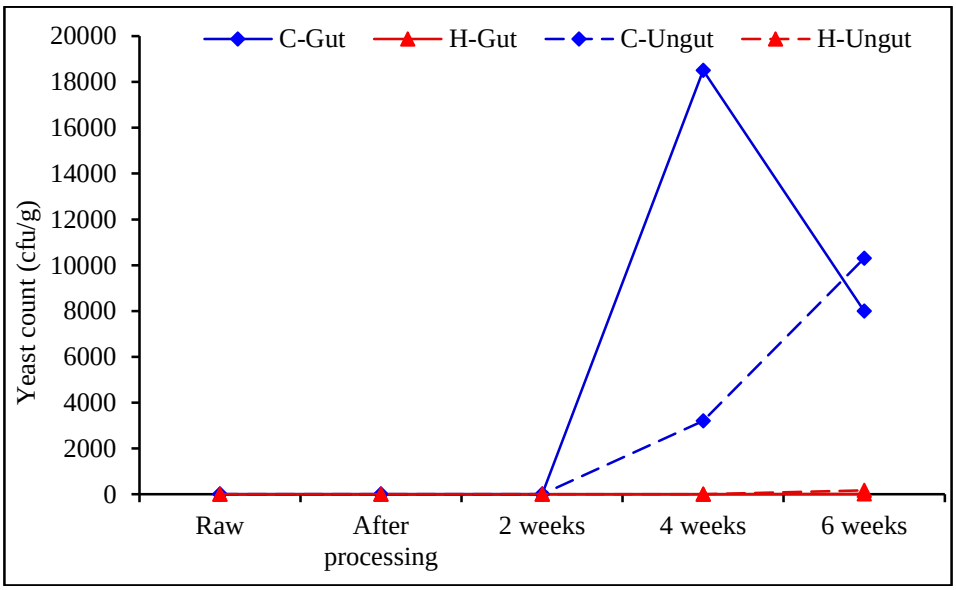


Figure 16*:* The change in mould count in capelin (a) and in sardine (b) as influenced by processing method and storage condition.

4.11.3 Yeast count

As the same pattern of changes in mould count for capelin, there was no growth of yeast in hot smoked capelin (gutted and ungutted) samples during processing and storage. However, the growth was significant in cold smoked capelin after 2 weeks for both gutted and ungutted samples (Figure 17a). For sardine sample, there was an increase in yeast count for all treatments during the first two weeks of storage (Figure 17b). Further increase was noted during week 4 in the sand dried and rock dried sardine. At the same interval of time, the yeast counts decrease in samples treated by smoking, solar drying and sun drying. At the end of the storage, yeast count decreased for the sand dried and rock dried and remained constant in smoked, solar and sun dried (Figure 17b).

(a)



(b)

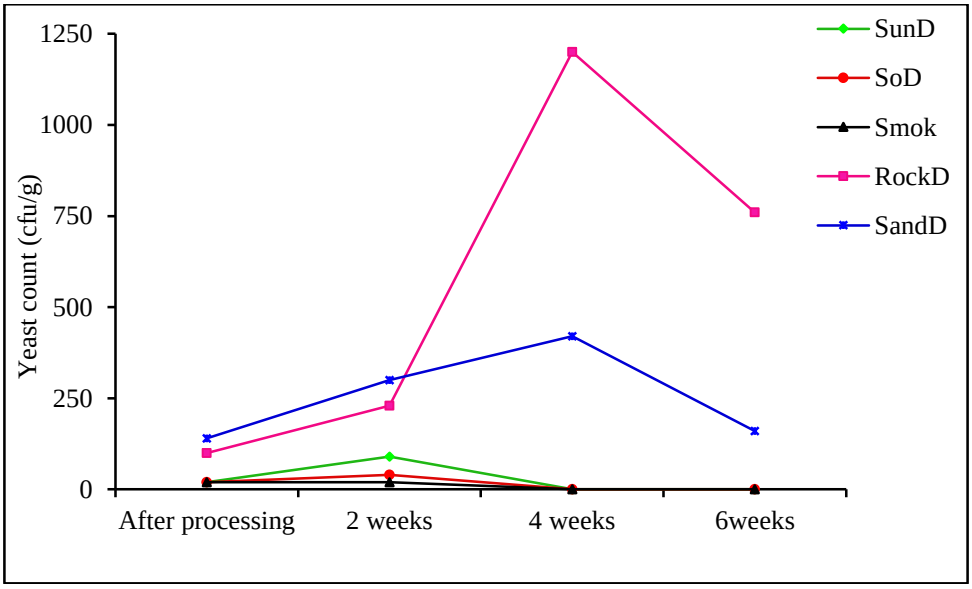


Figure 17: The growth of yeast in capelin (a) and sardine (b) as influenced by processing method and storage condition.

4.11.4 Total and faecal coliform count

Generally, all capelin treatments produced no significant ($p<0.05$) growth of total and faecal coliform after processing and during storage. Inversely, all sardine treatments showed both total and faecal coliform growth. Higher total and faecal coliform was found in the sample processed by sand drying, rock drying and solar drying. The smoked sardine had the lowest count than all other treatments. Furthermore, it was observed that mould count was high in the beginning of storage time and decreased towards the second week of storage. The count was generally constant at the end storage time (Figure 18).

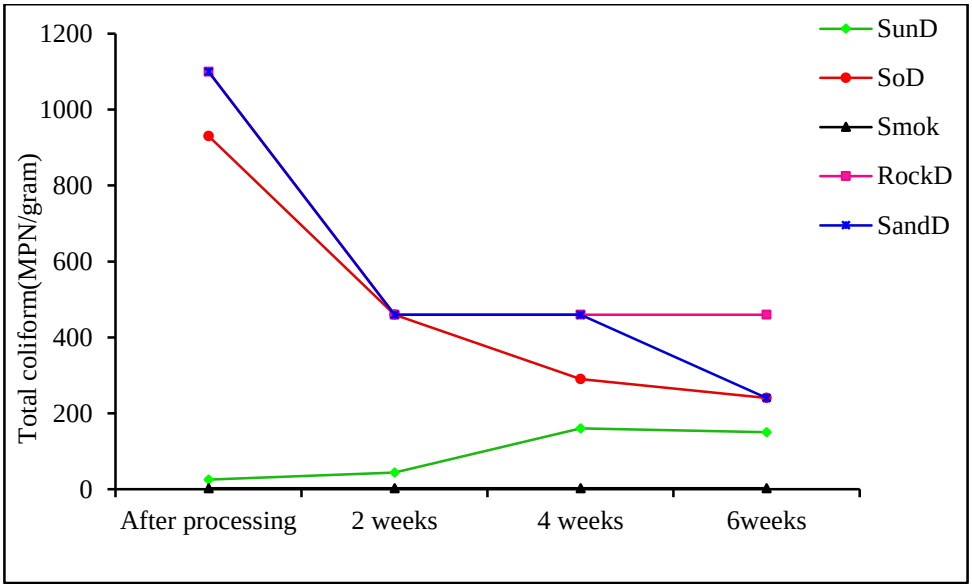


Figure 18: The change in total coliform of sardine as influenced by processing methods and storage time.

4.11.5 Salmonella

Salmonella analysis result of all samples for capelin and sardine with different treatment shows negative to salmonella with exception to one sample that showed a positive test to salmonella. The sardine locally dried on beach sand had positive salmonella test after drying. On the other hand, there was no sample that showed a positive test in all sampling points during storage.

5 DISCUSSION

5.1 Changes in water content and water activity

The water content and water activity of hot smoked capelin was lower than that of cold smoked. According to Arason (2003), heating helps to extract water form a substance, therefore the lower water content in hot smoked capelin was due to higher smoking temperature removing most of the water in fish. Lower water activity of below 0.91 limits growth of most bacteria and mould cease to grow at water activities below 0.80 (Mathlouthi 2001). This lead to the lower bacterial, mould and yeast count in hot smoked capelin compared to cold smoked (Figure 15a, 16a and 17a, respectively). Also Goulas and Kontominas (2005) reported that decrease in water activity is very important in inhibiting the growth of microorganisms in fish, and if salt

and smoke are added to the product, then the inhibition growth of microorganisms is increased. Therefore, the low microbial count in hot smoked capelin was due to the effect of low water activity, salt content and the preservative effect of smoke. Similar relationship was shown by processed sardine in which the sardine dried on sand and on rock contained higher water content and water activity, resulting in a higher bacteria microbial count compared to other dried and smoked samples. Furthermore, it was observed that the sun dried and solar dried sardine contained higher microbial counts than smoked sardine regardless of the lower water content in these samples. This may be due to preservative effect of smoke in limiting bacterial growth as it was reported by Clucas and Ward (1996). Generally, primarily controlling water activity and water content preserves both smoking and drying of fish. This is because the growth and multiplication of spoiling microorganism is dependent in the available water activity. Also the oxidation of lipid in smoked and dried fish relates to water activity of the product. Therefore, measurement of water content and water activity is typical way of determining the quality and storage time of the processed product. The results of water content and water activity in this study were in agreement with previous studies (Goulas and Kontominas 2005; Hilderbrand 2001),

5.2 Change in salt content

Generally the salt content in all treatment of capelin showed an increase after processing (Figure 7a). The increase in salt content in all capelin samples resulted from the salt diffusion into the fish muscle during brining and the removal of water (drying) during the smoking process. It was further found that the salt content remained constant throughout the 6 weeks of storage. This was in accordance with the results of water content in which insignificant decrease was observed during storage time. Also when comparing the effect of gutting on salt content, the result revealed that the gutted raw materials had low salt than ungutted capelin, mainly due to higher water content in the viscera. After processing and during storage gutted capelin had higher value of salt content compared to ungutted capelin (Figure 7a). The higher salt content resulted into increased lipid peroxidation in gutted capelin (Figure 11a). El-Bassiounyiel and Bekheta (2005) similarly reported that lipid peroxidation increased with salt level in wheat cultivars.

On the other hand, there was no change in salt content of all sardine samples throughout the experimental period (Figure 7b). The constant salt content could be due to the equilibrium reached for water content in sardine sample. Considering individual treatment, it was observed that sun dried, solar dried and smoked sardine contained higher salt content while the rock dried and sand dried sample had the lower salt content (Figure 7b). This difference in salt content was due to addition of salt into the fish muscle during brining before sun drying, solar drying and smoking treatments. It was different from the sardine locally dried on rocks and sand in which no salt is usually added before drying. The low salt content in rock and sand dries sardine is believed the cause of high microbial count to these treatments (Figure 15b, 16b, 17b and 18b). Mensah *et al.* (2006) in the study of the effect of salt concentration in growth of *Rhizobium sp*. reported the growth of rhizobium was higher with low salt concentrations, which related to microbial growth in sardine.

5.3 Lipid oxidation.

The difference in lipid content among the different capelin samples (Figure 8a) could be due to the influence of the processing method on lipid oxidation to its components after processing and during storage. Azhar and Nasa (2006) reported that fish oils are rich in highly unsaturated fatty acids, which are susceptible to oxidation resulting into formation of free radicals and hydroperoxides. The gutted capelin contained lower percentage of total lipid than ungutted capelin. This is believed to be due to the effects of lipid present in the intestine and other internal organs of capelin. The result of total lipid in sardine (Figure 8b) shows a significant difference among the treatment at different sampling time. Total lipid in rock and sand dried sardine was the lowest. This could be due to more lipids oxidized into primary and secondary products as reflected by higher FFA (Figure 9b), lower phospholipid content (Figure 10b).

A significant ($p<0.05$) decrease in phospholipid content of all capelin samples (Figure 10a) during the storage period is mainly due to the oxidation of polyunsaturated fatty acids (PUFAs) that are the main composition of phospholipids in the fish muscle. PUFAs, particularly DHA and EPA, are highly susceptible to oxidation. A lower phospholipid content was observed in ungutted smoked samples (cold and hot smoked) may be due to the contribution of oxidative effects from the viscera. The decrease in phospholipid content was in correlation with increase in PV content as depicted in Figure 11a. As the same with results of capelin, phospholipid content of sardine samples decreased (Figure 10b). Lower phospholipid content was obtained in rock and sand dried samples, mainly due to uncontrolled drying process (i.e. higher drying temperature and longer drying time). Moreover, smoked sardine had the highest phospholipid content. This is thought to be due to the anti-oxidative effects of smoke.

Free fatty acid content at different sampling time shows that, there was extensive breakdown of lipid during the processing and storage. Lipid hydroperoxides (PV) have been identified as primary products of lipid oxidation and decomposition of hydroperoxides yields a number of secondary products such as organic acids, aldehydes, ketones, alcohols, hydrocarbons and other compounds. Generally the products of hydroperoxide decomposition are known as secondary oxidation products (Shahidi and Zhong 2005). The increase in free fatty acid in this experiment could be due to the breakdown lipid in the presence of enzymes (i.e. lipase and phospholipase). Figure 8a and 8b shows that the total lipid was decreasing with the storage time on the other hand Figure 9a and 9b shows an increase in the FFA with time for both capelin and sardine. This relationship of total lipid to free fatty acid is in agreement with previous study for sardine during frozen storage (Aubourg *et al.* 1997).

There was a significant increase in PV observed in all sample treatments. The increase in PV was due to the generation of primary products caused by the presence of oxidation initiators such as enzymes, iron from the blood and oxygen and hence the processing methods such as drying drive oxidation of phospholipids during early stages of oxidation (Hernández-Herrero *et al*. 1999; Rosenthal and Kanner 1992). For example, cold smoking could activate enzymatic oxidation since it cannot inactivate the enzymes. A significance ($p<0.05$) difference in PV was observed between the cold smoked capelin and hot smoked where cold smoked contained higher PV than hot smoked whether gutted or ungutted (Figure 11a). This may be due to the internal organ of fish such as liver, intestine and kidney can influence the PV of fish (Sheridan 1988). The increase in PV was in correlation with the increase in FFA and fluorescence shift both in organic and aqueous phase. These results are in agreement with that reported by Nguyen *et al*. (2012) in brining and dry salting of cod. The peroxide value for sardine indicates a difference among the five processing methods. The sun dried found to contain larger values,

followed by the solar dried and smoked while the sand dried and rock dried contained less value (Figure 11b). The lower PV content in sand and rock dried sardine could be due to high temperature of the rock and sand as well as uncontrolled drying time leading to further oxidation of hydroperoxides to form secondary and tertiary lipid oxidation products. The rapid change may be caused by the higher microbial level in these samples leading to enzymatic oxidation of lipid.

It is interesting to note that in contrast to other parameters examined (FFA, PV, δFaq, etc.) Formation of thiobarbituric acid reactive substances did not show a continuous tendency during storage for any of the capelin treatments studied for the first two weeks (Figure 12a), so that very irregular pattern distributions were obtained and a clear effect of the processing and storage treatment could not be clearly inferred. At week 4 of storage an increased pattern was shown to all treatment. This could be due to unstable hydroperoxide to decompose into alkoxy radicals that can undergo carbon-carbon cleavage to form breakdown products including aldehydes, ketones, alcohols and esters (Rosenthal and Kanner 1992). Similar result was obtained by Goulas and Kontominas (2005) in the study of the keeping quality of chub mackerel but disagree with the result reported by Nguyen *et al.* (2012) in salted cod where there was an increase in TBARS throughout storage. The differences in TBARS result could be due to the difference in the fish species and processing method undertaken in the experiment.

Theoretically the formation of fluorescent compounds is resulted from interactions between carbonyl compounds (electrophilic molecules), formed during lipid oxidation, and amine compounds (nucleophilic molecules) that are present in fish muscle (Kikugawa and Beppu 1987). The results in this study observed an increase in proportion of the fluorescence shift of both the organic phase and aqueous phase after two weeks of capelin storage; then a decrease was found the end of storage. The trend shown by δFaq had positive correlation with that of FFA and PV during the 4^{th} and 6^{th} weeks of storage as clearly shown by principle component analysis (Figure 14). The difference in δF , during time of storage, is due to the fact that at first the fluorescent compounds responsible for the δF value were mostly lipid soluble; however, as lipid damage increased, these kinds of compounds became progressively more soluble in the aqueous phase (Shahidi and Zhong 2005). The results were in agreement with those reported by Aubourg *et al*. (1998) for frozen sardine which are related to capelin. However, the results obtained in this study were in disagreement with those published by Nguyen *et al.* (2012, 2013) for salted cod during processing and storage. They demonstrated the increase in fluorescence intensity of organic phase is in significant correlation with the increase in lipid oxidation products and storage time. This might be due to the different fish species used (i.e. fatty and lean fish) and different processing conditions.

5.4 Microbial growth during storage

The results for the determination of microorganism growth in fish sample processed by different method are important for determining the effectiveness of the processing method. It was observed that both capelin and sardine that was processed by hot smoking contained lowest value of TPC, yeast and mould counts. Hilderbrand (2001) reported that the lower count in smoked fish may be due to high temperature killing the food poisoning and spoilage bacteria as well as the chemical compounds contained in smoke that can inhibit the growth of bacteria. In addition of the effect of temperature and smoke, addition of salt and low water activity are important for inhibiting the growth of microbes in food as it was reported by Goulas and Kontominas (2005). The TPC in cold and hot smoked capelin was lower at the beginning of

storage and were increasing with the storage time. The increased TPC correlate with an increase in PV and FFA indicating that microorganism plays a role in the initiation of oxidation reaction due to influence in enzymes. The results of storage study for mould and yeast growth in capelin (Figure 16 and 17) show no growth for the first two weeks of storage and an increase in mould and yeast counts was observed at week 4 and week 6 of storage for cold smoked capelin. This can be concluded that both cold and hot smoked capelin stored at 2 °C become unfit for consumption after 2 weeks storage.

6 CONCLUSION AND RECOMMENDATIONS

The results from this study indicate that both processing methods and storage time significantly affect the quality of fish. The hot smoked sardine and capelin contained low microbial count throughout the storage period. The high TPC correlated with PV, FFA and δFaq. Sardines that were locally processed by drying on sand and rock contained highest microbial count, free fatty acid and fluorescence. This means the product is not good for consumption after a short storage time. The storage time has little effect on water and salt contents, since these parameters were constant throughout the storage time. Gutting of capelin influenced negatively lipid content and free fatty acid formation. Also the δFaq was highly correlated with lipid oxidation indicators (PV and FFA) during storage. This shows the importance of fluorescence measurement as a promising technique to assess the secondary and tertiary lipid oxidation products. Future research should target on specific packaging materials and antioxidants that can prevent or inhibit lipid oxidation in capelin and sardine as well as establishment of acceptable limits of oxidation in capelin and sardine based on safety value, wholesomeness and nutritional values. Also research should be done on how the fishing/catching season affect rate of lipid oxidation.

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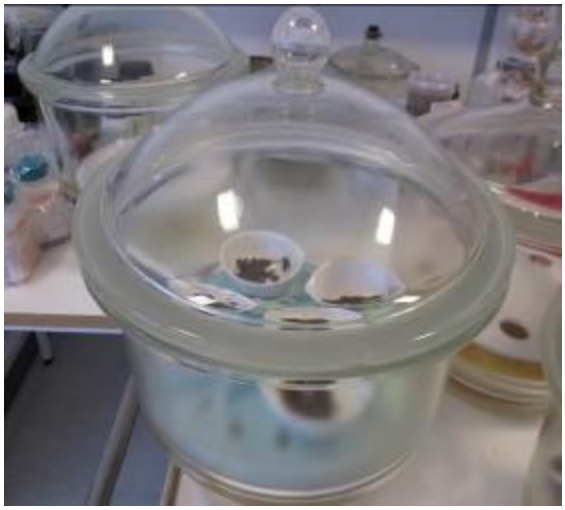
APPENDIX I:

PARTIAL INSTRUMENTS USED IN THIS PROJECT

(a): Novasina AW-Meter; (b): Desiccator with oven dried sample for water content); (c): Water bath (d):Salt determination meter, (e): Centrifuge; (F): Fluorescence meter

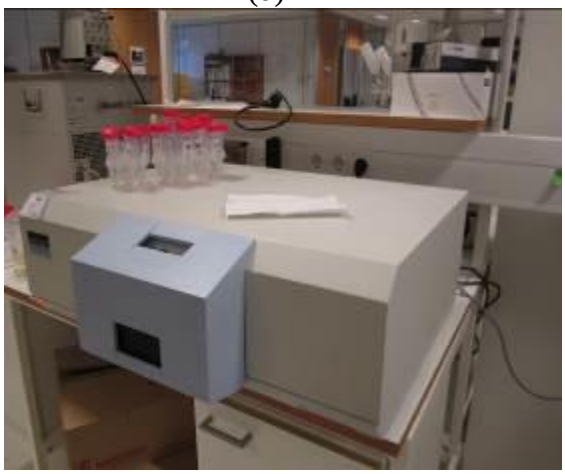
(a)



(b)

(c)

(d)



APPENDIX : II

PICTURES OF FRESH AND DRIED SAMPLES

(a): Side view of smoking house; (b): Front view of smoking house (c): Raw capelin (d):Smoked capelin, (e): Minced capelin for lipid extraction (F): Extract for lipid oxidation analysis

(a)

(b)

(c)

(d)