Dissertation for the Degree of Doctor of Philosophy

Drying and smoking of capelin *(Mallotus villosus)* and sardine *(Sardinella gibbosa)* – the influence on physicochemical properties and consumers acceptance

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Abstract

Drying and smoking are affordable fish preservation methods that are commonly used in most developing countries where poorly developed logistics limit marketing of fresh fish. In Eastern Africa dried and smoked fish are important sources of low cost stable dietary protein. Small fish, mainly sardine, is commonly blanched in brine prior to drying on the ground. The dried fish is often of low quality, restricting the sales of dried fish to low income groups shopping in open-air markets. At the same time there is an increasing demand among middle class consumers for dried and smoked small fish of high quality sold in supermarkets. Increased demand for dried and smoked small fish could be met by imports or improved processing methods. The aim of this study was to improve the quality and safety of dried and smoked small fish, and study acceptability of new products such as dried capelin caught in Icelandic waters in markets accustomed to dried small fish. The effects of blanching, drying and smoking methods on fish quality were evaluated. The influence of lipid content and packaging methods on lipid degradation, sensory properties and microbial quality during storage of dried and smoked fish was assessed, as well as the marketing potential of sardine dried under more hygienic conditions and imported dried capelin.

Blanching prior to drying of sardine and capelin resulted in low quality and sensory properties, and protein denaturation/aggregation. Fat content of capelin depends on the time of year and when capelin of 9-10% lipid content rather than 7-7.5% was used, drying rate was reduced and moisture content in the end product increased, while the fat protected proteins during blanching, drying and smoking. Drying under controlled conditions improved quality demonstrating the need for developing a commercial drier for processing of small fish. Industrially dried and smoked capelin and sardine were found to be rich in essential polyunsaturated fatty acids. Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) constituted approximately 13% and 20% of the total fatty acids of these fish respectively. Lipid content and microbial stability as well dehydration were higher in hot smoked than cold smoked capelin and sardine but hot smoking reduced the yield. Lipid content influenced hydrolysis and oxidation as well as sensory properties during storage of smoked and dried capelin. Lipid hydrolysis was higher in lower lipid capelin while lipid oxidation was higher in fatty capelin. When atmospheric oxygen was removed by vacuum packaging, dried and smoked fatty capelin became more stable during storage with less lipid degradation, less rancid odour and lower counts of microbes. Vacuum packaging had no influence on lipid hydrolysis.

Vacuum packaged hot smoked fish was stable during four weeks of chill storage. Dried capelin had moisture content <25% and water activity <0.70 and was stable and safe, during five months storage at room temperature. Improved dried sardine and capelin received high acceptability ratings, indicating consumers of traditional dried small fish might accept new dried fish products. The results from this study show that well processed and packaged dried and smoked small fish can be highly nutritious and could contribute to the reduction of malnutrition prevailing in many developing countries.

Keywords: Capelin, sardine, lipid content, lipid degradation, fish proteins, drying conditions, smoking methods, blanching, brining, microbial changes, consumers' acceptance.

Ágrip

Þurrkun og reyking eru hagkvæmar varðveisluaðferðir sem almennt eru notaðar í þróunarlöndum, þar sem vanþróaðir flutningaferlar takmarka markaðssetningu á ferskum fiski. Í A-Afríku er þurrkaður og reyktur fiskur mikilvæg uppspretta próteina í mataræði íbúa. Smáfiskur, aðallega sardínur, er venjulega settur í saltpækil og forsoðinn til að stöðva ensímvirkni og örveruvöxt áður en hann er þurrkaður utandyra. Þurrkaði fiskurinn er oft lélegur að gæðum og takmarkast sala hans við tekjulægri hópa er versla á útimörkuðum. Á sama tíma er aukin eftirspurn meðal neytenda millistéttar eftir þurrkuðum og reyktum smáfiski í stórmörkuðum sem uppfyllir gæðakröfur þeirra. Þessari eftirspurn mætti mæta með innflutningi eða bættum vinnsluaðferðum. Markmið þessarar rannsóknar var að bæta gæði og öryggi í vinnslu smáfisks og kanna viðbrögð neytenda við nýrri afurð eins og þurrkaðri loðnu veiddri við Ísland sem er ekki þekkt á mörkuðum í A-Afríku. Áhrif forsuðu, þurrkunar og reykingar á gæði afurða voru metin, ásamt áhrifum pökkunaraðferða á niðurbrot fitu. Einnig voru kannaðir skynmatseiginleikar og magn örvera í þurrkuðum og reyktum afurðum. Að síðustu var hugað að markaðssetningu á hollari þurrkaðri sardínu og innfluttri þurrkaðri loðnu.

Forsuða fyrir hefðbundna þurrkun á sardínum og loðnu leiddi til minni afurðagæða, lakara skynmats og minni próteingæða. Magn fitu í loðnu er árstíðabundið og þegar loðna með fituinnihald 9-10% í stað 7-7,5% var þurrkuð, tók þurrkunin lengri tíma og rakainnihald í lokaafurð jókst. Jafnframt dró fitan úr afmyndun próteina í vinnsluferlinu. Við stýrðar þurrkaðstæður jukust gæði afurða, en það bendir til að nauðsynlegt sé að þróa þurrkara fyrir vinnslu á smáfiski. Í þurrkaðri og reyktri loðnu og sardínum greindist hátt hlutfall lífsnauðsynlegra fjölómettaðra fitusýra eins og eicosapentaenoic sýru (EPA) og docosahexaenoic sýru (DHA), nákvæmlega 13% í loðnu og 20% í sardínum. Í heitreyktri loðnu og sardínum, var meiri fita, minni raki og aukinn stöðugleiki gegn örverum, miðað við kaldreykta afurð, en heitreyking minnkaði nýtingu. Fituinnihald hafði áhrif á vatnsrof próteina, oxun fitu og bætti skynmatseiginleika við geymslu á reyktri og þurrkaðri loðnu. Niðurbrot fitu var mest í loðnu með lágu fituinnihaldi á meðan þránun var mest í loðnu með háu fituinnihaldi. Pökkun á reyktri og þurrkaðri feitri loðnu í loftfirrtar umbúðir, leiddi til minni þránunar fitu, og færri örvera. Pökkun hafði ekki áhrif á niðurbrot fitu.

Heitreyktur fiskur í loftfirrtum umbúðum hélt upphaflegum eiginleikum sínum eftir fjögurra vikna geymslu í kæli. Þurrkuð loðna með rakainnihaldi undir 25% og vatnsvirkni undir 0,7 geymist óskemmd við stofuhita í 5 mánuði í loftfirrtum umbúðum. Bætt vinnsluferli við þurrkun á sardínum og loðnu skilaði góðum árangri og afurðinni var vel tekið hjá neytendum hefðbundins þurrkaðs smáfisks í Kenía. Niðurstöður þessarar rannsóknar sýna að þurrkaður og reyktur smáfiskur getur verið mjög næringarrík fæða, og ef verklag við vinnslu og pökkun er rétt, gætu þessar afurðir hjálpað til við að draga úr vannæringu sem er ríkjandi í þróunarlöndum.

Lykilorð: Loðna, sardína, fituinnihald, fituniðurbrot, fiskprótein, þurrkaðstæður, reykingaraðferðir, forsuða, pæklun, örverubreytingar, neytendakönnun.

List of original papers

The thesis is based on the following papers referred in the text by their respective Roman numerals. The papers are appended at the end of the thesis.

I. Cyprian, O.O., Nguyen, M.V., Sveinsdottir, K., Tomasson, T., Thorkelsson, G., Arason, S. (2015). Influence of blanching treatment and drying methods on the drying characteristics and quality changes in dried sardine (*Sardinella gibbosa*) during storage. *Drying Technology*, under review.

II. Cyprian, O.O., Nguyen, M.V., Sveinsdottir, K., Jonsson, A., Thorkelsson, G., Arason, S. (2015). Influence of lipid content and blanching on capelin (*Mallotus villosus*) drying rate and lipid oxidation under low temperature drying. *Journal of Food Process Engineering* (In Press, doi:10.1111/jfpe.12215).

III. Cyprian, O.O., Nguyen, M.V., Tomasson, T., Thorkelsson, G., Arason, S. (2015). Conformational changes in Capelin (*Mallotus villosus*) proteins during smoking and drying. *Food Chemistry*, under review.

IV. Cyprian, O.O., Nguyen, M.V., Sveinsdottir, K., Jonsson, A., Tomasson, T., Thorkelsson, G., Arason, S. (2015). Influence of smoking and packaging methods on lipid stability and microbial quality of Capelin (*Mallotus villosus*) and Sardine (*Sardinella gibossa*). *Food Science & Nutrition, 3*(5), 404–414.

V. Cyprian, O.O., Sveinsdottir, K., Jonsson, A., Nguyen, M.V., Tomasson, T., Thorkelsson, G., Arason, S. (2015). Influence of lipid content and packaging methods on the quality of dried capelin (*Mallotus villosus*) during storage. *Journal Food Science and Technology*, under review.

VI. Cyprian, O.O., Sveinsdottir, K., Jonsson, A., Tomasson, T., Thorkelsson, G., Arason, S. (2015). Marketing potential of improved dried sardine (*Sardinella gibossa*) and capelin (*Mallotus villosus*) in markets accustomed to traditional dried fish. *Journal of Sensory Studies*, under review.

The following papers were published during the study period but are not regarded as part of the thesis:

Cyprian, O.O., Sveinsdóttir, K., Magnússon, H., Arason, S., Jóhannsson, R., & Martinsdóttir, E. (2014). Development of Quality Index Method (QIM) Scheme for Farmed Tilapia Fillets and Its Application in Shelf Life Study. *Journal of Aquatic Food Product Technology*, *23*(3), 278–290.

Cyprian, O.O., Lauzon, H. L., Jóhannsson, R., Sveinsdóttir, K., Arason, S., & Martinsdóttir, E. (2013). Shelf life of air and modified atmosphere-packaged fresh tilapia (Oreochromis niloticus) fillets stored under chilled and superchilled conditions. *Food Science & Nutrition*, *1*(2), 130–140.

Cyprian, O.O., Oduor-Odote, P., Lauzon, H., Martinsdottir, E., Sigurjon, Arason. (2013). Microbiological quality and shelflife of fresh packaged Tilapia fillets stored under different chill temperatures. *Journal of Microbiology, Biotechnology and Food Sciences*, *2*(4), 2431–2455.

The author's contributions to the papers

The author designed and implemented all the experiments in collaboration with the PhD study committee (co-authors). The author carried out all the analysis and wrote the articles in collaboration with co-authors.

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Abbreviations

a*	Redness
a_w	Water activity
ANOVA	Analysis of variance
b*	Yellowness
BR	Brined
BL	Blanched in brine
CFU	Colony forming units
DHA	Docosahexaenoic acid
EPA	Eicosapentaenoic acid
FFA	Free fatty acids
GDC	Generic descriptive analysis
L*	Lightness
M_c	Moisture content
MUFA	Monounsaturated fatty acids
OFR	Fluorescence interaction compounds
O_2	Oxygen
PCA	Principal Component Analysis
PL	Phospholipid
PUFA	Polyunsaturated fatty acids
PV	Peroxide value (hydroperoxides)
R	Drying rate
r	Correlation coefficient
SFA	Saturated fatty acids
SH	Sulfhydryl
S-S	Disulphide bond
TBARS	Thiobarbituric acid reactive substances
TL	Total lipid
TPC	Total plate count
W	Weight

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1 Introduction

Fish and other marine organisms give rise to products of great economic importance all over the world. Fresh fish is highly perishable and various preservation techniques such as chilling, freezing, drying, salting and smoking are used to extend its shelf life. Drying and smoking are affordable fish preservation methods that are commonly used in many developing countries (Darvishi et al., 2013; Akintola et al., 2013; Bellagha et al., 2007). Dried and smoked fish, in particular small pelagic fish, is an important source of low cost stable dietary protein. In Eastern Africa, particularly Kenya, Tanzania and Uganda, the consumption of dried small fish is common and widespread (IOC, 2012; Oduor-Odote et al., 2010). Dried and smoked fish has a long shelf life and is sold in small portions to meet the needs of low-income consumers.

Small pelagic fish, specifically dagaa (*Rastrineobola argentea*) in Lake Victoria and sardine (*Sardinella gibbosa*) along the coast of the Indian Ocean are caught in Kenya using light attraction and small meshed seine nets (Government of Kenya, 2011). Highest catches occur during dark moonless nights when light attraction is most effective. Fishing usually takes place for 18 to 24 days in a month with a break around full moon (Government of Kenya, 2011). Superimposed on the lunar cycles of the catches, are larger seasonal and inter-annual fluctuations which are typical of short lived fish species (Thiaw et al., 2011). Fresh fish is preferred by consumers, but fluctuating catches, high perishability and poorly developed logistics restrict marketing of fresh fish to areas near landing sites and retail stores that have refrigeration and freezing facilities (Oduor-Odote et al., 2010). Dagaa and sardine are caught by artisanal fishermen. The fish is not iced on board, exposing it to ambient temperatures during fishing and transportation to landing sites (Odoli et al., 2013). This practice leads to landing of fish with reduced shelf life further restricting marketing and consumption of fresh fish to local villages. Drying and smoking are necessary if the fish is destined for distant markets.

Sardinella gibbosa is commonly blanched in brine to inhibit microbial growth and enzymatic activities prior to open air drying (Omodara & Olaniyan, 2012). The processing often results in products contaminated with soil, droppings from birds and rodents, and infested with flies. Quality uncertainty is the main barrier in accessing national retail stores restricting sales of dried fish to low price open-air markets, and people with low income. The growing middle income

groups in East Africa (African Development Bank, 2011) and reduced availability of high value fish species in local markets (IOC, 2012) has resulted in increased demand for dried small fish. Middle income consumers demand fish of high sensory quality and accessible in retail stores particularly supermarkets. East Africa member countries are also changing policies regarding cross-border trade of fish and fishery products (IOC, 2012), necessitating changes along the value chain to ensure product quality and safety.

Drying racks that are open air ventilated platforms are presently used to meet the quality standards for dried fish intended for national retail stores (Oduor-Odote et al., 2010). Since rack drying depends on weather conditions, the quality of the fish is variable. Despite this uncertainty in quality, small quantities of rack dried fresh water dagaa (*R. argentea*) have in recent years been sold in national retail stores.

Fish can be smoke-dried to extend shelf life and improve flavor. Smoking of small pelagic species has been reported in East Africa (Mhongole & Mhina, 2012). Smoking is traditionally done in simple kilns that are not energy efficient and at times result in quantitative (physical) spoilage (Oduor-Odote & Obiero, 2009).

In Africa there is a growing awareness of the nutritional and health benefits associated with fish consumption (Akintola et al., 2013). Properly processed and packaged dried and smoked fish can be an important source of stable dietary protein and also of omega-3 polyunsaturated fatty acids (Bilgin et al., 2008; Akintola et al., 2013). In 2010- 2012 a third or more of the population in East Africa suffered from malnutrition, with an estimated prevalence of 30% in Kenya, 35% in Uganda and 39% in Tanzania (FAO, 2014). Cereals and roots are reportedly supplying more than a half of the dietary energy, with meat contributing less than 3% in 2012 (FAO, 2014). Nutritional status of the population and food security can be improved if supply of correctly dried and smoked packaged fish can be enhanced in East Africa and other developing countries.

Fish and fish products produced in Kenya are marketed domestically or exported. In the year 2012, total fish production in Kenya from inland, aquaculture and marine artisanal fisheries was 154,000 MT as summarized in figure 1.1 (State department of Fisheries Kenya 2012). Fish landed from Lake Victoria was 119,000 MT, from which dagaa accounted for about 90,000 MT in 2012 (Lake Victoria Basin Commission, 2015). Dagaa landing on the Kenyan side cannot

meet the demand for small pelagic fish in Kenya (IOC, 2012). Landings of small pelagic fish mainly sardine approximated at 900 MT from the coastal fisheries cannot fill the gap. Besides, aquaculture considered to be an alternative source of fish production in Kenya has been tried with only moderate success, producing 21,500 MT in 2012 representing 14% of the national fish production Figure 1.1.

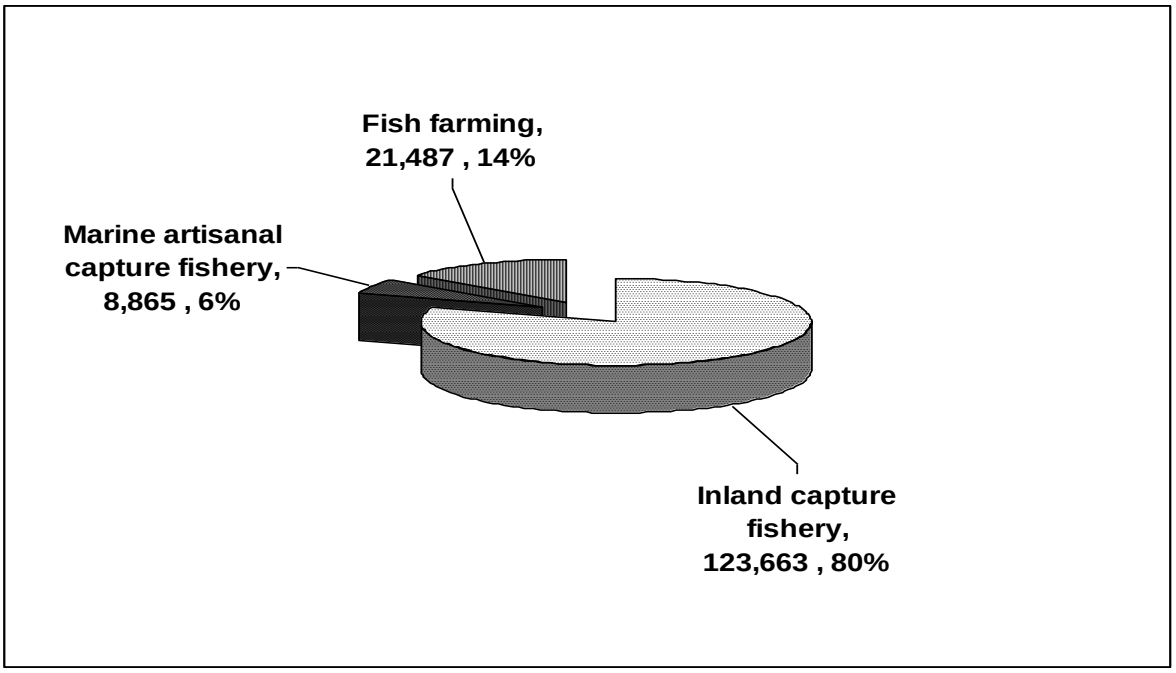

Figure 1.1 Total fish production in Kenya in 2012, quantity in MT and ratio in % (with permission from State department of Fisheries Kenya 2012).

It is a common practice in most developing countries that low value fish, mainly small pelagic, is imported while more valuable species landed from their waters is exported. In 2008 75% of fishery exports (in value terms) from developing countries were exported to developed countries, while 40% of fish they imported (in value terms) for local consumption (mainly low-priced small pelagic and also high-value species for their processing industries) originated from the developed countries (FAO, 2010). During 2012 Kenya exported a total of 10,165 metric tons of fish and fish products valued at KES 4,000 million (approximately 40 million USD) (State department of Fisheries Kenya 2012). The exported fish was mainly Nile perch, octopus, sword fish and crabs with Nile perch accounting for 87% of the total quantity and 85% of the total earnings. The fish was mainly exported to Israel (40%), Netherlands (15%), Germany (8%) and Portugal (7%) among others. Over the same period 2,622 metric tons of fish excluding dagaa imported from the

region were imported largely from Asian countries, notably India, Pakistan, Japan and Korea mainly as frozen mackerel (62.3%) and sardine (13.7%). The demand for dried small fish in Kenya can be met by reducing post-harvest losses in the small pelagic species fishery through improved processing methods and imports or producing dried fish from imported raw material like capelin (*Mallotus villosus*) caught in the North Atlantic Ocean.

Dried and smoked fish can easily be contaminated with microbes if sold unpackaged in open-air markets. Packaging is important in delivering safe and attractive dried and smoked products. Sardine and capelin that are hygienically produced under controlled drying and smoking conditions would be new products to the market. Before a decision is made to launch them, they need to be tested for consumers acceptability and pricing on the intended markets.

2 State of the art

Herein the scientific background relevant to this Ph.D. study is put into perspective giving an overview of the existing status on the drying, smoking and utilization of small pelagic fish species in East Africa. Packaging methods, lipid degradation, protein and microbial changes associated with drying and smoking of fish, distribution and marketing were studied to understand the mechanisms resulting in degradation in search of ways to enhance quality and stability. Marketing and utilization of dried and smoked small pelagic fish species are discussed with emphasis on the East African countries.

2.1 Species of interest

Two fish species were used in the study, sardine (*Sardinella gibbosa*) and capelin (*Mallotus villosus*).

2.1.1 Sardine

Sardine (*S. gibbosa*) is a species of the family Clupeidae which supports important coastal fisheries in parts of tropical waters of the western Indo-Pacific, western Indian ocean, Indonesia and off the coast of Taiwan, Korea and Australia (Okera, 1969; Froese & Pauly, 2006; Wakwabi, 1981). It occurs in shallow waters associated with coral reefs and lives at depths of up to 70 m. *S. gibbosa* grows up to 17 cm in length and dominates the sardine catches landed along the East

African coast throughout the year (Wakwabi, 1981; Okera, 1969). Almost 900 tons of sardine were reportedly landed in Kenya in 2012 being 10% of 8,947 tons of artisanal marine fish landed in Kenya (State Department of Fisheries Kenya 2012). However, personal observation at landing sites indicates that a much larger quantity than reported is landed, mostly in the southern part of the Kenyan coast. Data collection and reporting systems on marine fisheries in Kenya are often poor and landings of low valued species are often ignored or under-reported (Malleret-King et al., 2009; Gitonga, 2005), resulting in gross underestimation of production statistics. The species is utilized for human consumption mainly in dried form (Froese & Pauly, 2006; Oduor-Odote et al., 2010) by low income consumers. It is processed by washing, boiling in brine (blanching) and sun drying before bulk packaging in woven baskets and sacks.

2.1.2 Capelin

Capelin (*M. villosus*) is a small pelagic schooling fish that is economically and ecologically important in the northern hemisphere (ICES, 2009). Large populations occur in the Atlantic and Pacific Oceans, and important commercial fisheries have developed in the Atlantic (Carscadden et al., 2013). In Iceland it is caught during spawning migration in winter, from January to March. Its lipid content depends on maturity and season, and varies from about 4% to 20% body weight (Bragadóttir et al., 2002) with the highest lipid content in late fall (November-December) and lowest during the spawning season in March-April (Vilhjálmsson, 2002). Capelin catch in Iceland has exceeded half a million tons in recent years (Statistics Iceland, 2015). A small portion of it is used for human food, with about 80% reduced to fishmeal and oil (Statistics Iceland, 2015). Some female capelin is exported because of the roe while male capelin is less valued and is usually reduced to fish meal and oil. In 2012, about 570,000 tons of capelin was landed in Iceland. Fifty five thousand two hundred tons of female capelin was exported to Japan and Europe as whole fresh/frozen product (valued at ISK 87,000 per ton) and 11,500 tons exported as roe (valued at ISK 414,000 per ton) altogether valued at ISK 10 billion (approx. USD 77 million) (Statistics Iceland, 2015). The remaining 500,000 tons mainly males and females without roe was reduced to fish meal and oil valued together at ISK 40,000 per ton of raw material. There is a potential to use male capelin that is reduced to fish meal as raw material for dried and smoked small fish.

2.2 Fish drying and smoking

Sun drying and smoking are ancient and still popular methods of fish preparation for food and preserving for later consumption. Preservation is especially important both when selling to distant markets and also when supply fluctuates as is the case for most pelagic fish.

2.2.1 Fish drying

Drying is an efficient and widespread food preservation method (Dewi et al., 2011). It reduces food degradation by decreasing water activity. Drying is a process of simultaneous heat and mass transfer. The heat causes evaporation of water from the surface and mass transfer of water from the interior to the surface of the fish (Jain, 2006). Water evaporation takes place due to vapor pressure difference between the fish and the surrounding medium (Jain & Pathare, 2007; Jain, 2006). Drying is generally characterized in terms of constant and falling rate periods that are controlled by air velocity, temperature and the relative humidity (Oduor-Odote et al., 2010; Bellagha et al. 2002). In the constant rate period, drying continues at a constant rate equal to the rate of water evaporation from the surface as it is governed by evaporation from the surface or near surface areas (Reza et al., 2009). In the falling rate period of drying, water is transferred mainly by molecular diffusion. Even though water diffusivity is primarily influenced by water content and temperature (Jain & Pathare, 2007), chemical composition, particularly lipid content has also been reported to influence the diffusion of water (Cardinal et al., 2001).

Fish drying methods vary among and within countries depending on the species used and the type of product desired. Fish may be dried to various degrees with water content in the final product ranging from about 10% to 60%. In East Africa, small pelagic species are traditionally dried in the open by spreading them directly on the ground or on mats for about 2-5 days depending on weather conditions (Ofulla et al., 2011; FAO, 1992).

The challenges faced in open sun drying prompted research into the use of solar dryers where drying takes place in an enclosed chamber (Bala & Mondol, 2001; Reza et al., 2009) and integrated solar dryers incorporating desiccants, blowers and thermal systems to continue drying during darkness (Shanmugam & Natarajan 2006). In Iceland the use of geothermal energy allows affordable year round indoor drying of fish while maintaining consistence in product quality

(Arason, 2003). The initial investments in controlled drying systems are high, but dried fish is stable during long periods of storage and safe for consumption (Reza et al., 2009).

Drying can be done at high temperatures for a short time or at low temperatures for a longer time (Arason, 2003; Lewicki, 2006). Low drying temperatures have been shown to minimize lipid oxidation and reduce protein denaturation, resulting in lower nutrient degradation (Bellagha et al., 2002; Lewicki, 2006). Shelf life of dried fish varies considerably depending on species (lean or fatty), amounts of salt, degree of drying, packaging and storage temperature.

2.2.2 Fish smoking

Smoking is one of the oldest food preservation methods. Depending on the species and type of product desired smoking is conducted under certain temperature and humidity, and smoke is usually sourced from plant material (Ahmed et al., 2013). Smoking not only increases the shelf life but also changes the appearance, taste and odor of the foods (Akintola et al., 2013; Goulas & Kontominas, 2005; Beltrin et al., 1989). Various pre-smoking treatments such as salting and drying and/or post-smoking treatments mainly cooking and marinating are applied in the smoking industry (Ahmed et al., 2013).

Smoking is not an effective preservation method and the use of salt is essential to complement the bacterial inhibitory effect of smoke by reducing water activity. The effects of salt to inhibit microbial growth increases with increasing salt concentrations, but for health and acceptability reasons, the practice is to make products with low smoke and salt content (Beltrin et al., 1989). Smoke is produced by the process of incomplete combustion of wood. It consists of numerous compounds such as aldehydes, ketones, alcohols, acids, hydrocarbons, esters, phenols, ethers, etc. (Guillen & Errecalde, 2002). These compounds are transferred to the fish during smoking by deposition on the surface and subsequent penetration into the flesh (Goulas & Kontominas, 2005). Smoking increases the shelf-life of fish as a result of the combined effects of antimicrobial and antioxidant properties of the smoke.

Traditional fish smoking methods are still commonly practiced in most developing countries. Simple kilns which use a lot of firewood are used, and the smoking conditions (temperature, humidity and smoke) cannot be controlled (Oduor-Odote & Obiero, 2009). Processors are

exposed to smoke and generally poor quality products are produced (Oduor-Odote et al., 2010). In developed countries, smoking is generally carried out under controlled conditions and can be in two forms, hot and cold smoking. Hot smoking involves cooking and can be considered mild (30-50°C) or high temperature (50-80°C) (Marc et al., 1997), but it is commonly carried out at temperatures of 70-80°C (Erkan et al., 2012). In contrast, cold smoking is achieved without cooking usually at temperatures ≤30°C resulting in lower nutrient degradation (Goulas & Kontominas, 2005).

Although small pelagic species are mainly consumed dried in developing countries (Oduor-Odote et al., 2010; Darvishi et al., 2013), they are also often smoked (Akande & Asuquo-King, 2001; Mhongole & Mhina, 2012). Improved smoking of small pelagic fish could be a promising method for species such as capelin whose existing fresh markets cannot absorb the large catches for human consumption.

2.3 Packaging of dried and smoked fish

Shelf life of dried and smoked fish is determined by the degree and method of processing as well as the storage conditions. Appropriate storage conditions should be provided to slow down deteriorative changes occurring through oxidation and microbial growth (Erkan, 2012). Dried and smoke-dried products are easily contaminated by microbes, mainly air borne molds, as they are most often processed and sold unpackaged in developing countries (Park et al., 2014; Oyero et al., 2012). When fish is processed, particularly when using traditional methods and inappropriate packaging, physical loss may occur from insect infestation and fragmentation (Abolagba & Nuntah, 2011).

Packaging is an important part of the food industry. In addition to extending shelf life, good packaging ensures the delivery of safe, whole and attractive foods to the market (Kilcast & Subramaniam, 2000). Food packaging methods vary depending on the regulations imposed at national level and market demands. Unlike other food processing industries, packaging of dried and smoke-dried fish has not been developed in many developing countries (Abolagba & Nuntah, 2011; FAO, 2003). In Africa, dried and smoked fish is mainly packaged in sacks, paper cartons, wooden and bamboo baskets (Abolagba & Nuntah, 2011; FAO, 2003). Their distribution from the processing villages to wholesale open markets and onward to the retail outlets is largely

by road transportation. Means of transportation includes bicycles, motorcycles, pick-up vehicles, buses and trucks.

In Kenya, only fish marketed in national retail stores (supermarkets) is packaged, which is a small portion of the total dried and smoke-dried fish in the country. In the traditional open-air markets where bulk of dried and smoke-dried fish is marketed, fish is sold in open bins of varying volumes and wrapped afterwards in polyethylene bags or old newspapers. This kind of packaging can expose polyunsaturated oil in dried and smoked fish to oxygen and microbial contamination.

Modified atmosphere (MAP) and vacuum packaging are commonly used in the food industry to extend shelf life and meet consumer demands for better quality products (Sivertsvik et al., 2002; Etemadian et al., 2012). MAP extends food shelf life by retarding microbial growth especially when carbon dioxide level is increased. Although most MAP might be too costly for application in artisanal fisheries, vacuum packaging could be affordable. Vacuum packaging eliminates the air and is known to improve the shelf life and overall quality of muscle foods (Etemadian et al., 2012). It inhibits oxidation and aerobic microbial growth.

Due to health reasons, consumers demand smoked fish that contain low quantities of smoke and salt (Beltrin et al., 1989). Therefore, packaging technologists should be aware of a major concern limiting the development of vacuum packaging for smoked fish, namely *Clostridium botulinum*. *C. botulinum* has often been reported in seafood products that have been packaged under regimes designed to limit the availability of oxygen (Aberoumand 2010; Sivertsvik et al. 2002). Specifically *C. botulinum* type E is of most concern in this type of packaging as it is a naturally occurring aquatic organism. Efforts should be considered to assure safety and success of vacuum packaging of smoked fish by balancing salt content and refrigeration temperature.

2.4 Degradation of dried and smoked fish

During storage of dried and smoked fish, quality deterioration such as microbial growth and lipid hydrolysis that influence the formation of oxidation products occurs (Doe, 2002).

2.4.1 Microbial spoilage

The undesirable effects of microbial activities in food are summarized in Figure 2.1. Microbial activity in food depends on its composition (intrinsic factors) such as water content and nutrients, and the physical parameters such as temperature and the surrounding atmosphere (Gram et al., 2002). During drying and smoking, the removal of water prevents the growth of microorganisms causing deterioration and minimizes many of the water mediated deteriorative reactions (Kilic, 2009). The effectiveness in preventing microbial growth in dried and smoked fish depends on water activity which is directly related to the water content. Smoked fish is more prone to microbial degradation than dried fish as smoked fish contains more water except for smoke-dried products. Smoking as a preservation method has evolved over time. As commercially practiced smoked (hot and cold) fish does not contain enough smoke and salt for effective preservation (Joffraud et al., 2006), products must be cooled immediately after smoking and stored at or below 3° C (Bilgin et al., 2008). Depending on packaging, a mixed microbial flora can develop in smoked fish during storage (Joffraud et al., 2006).

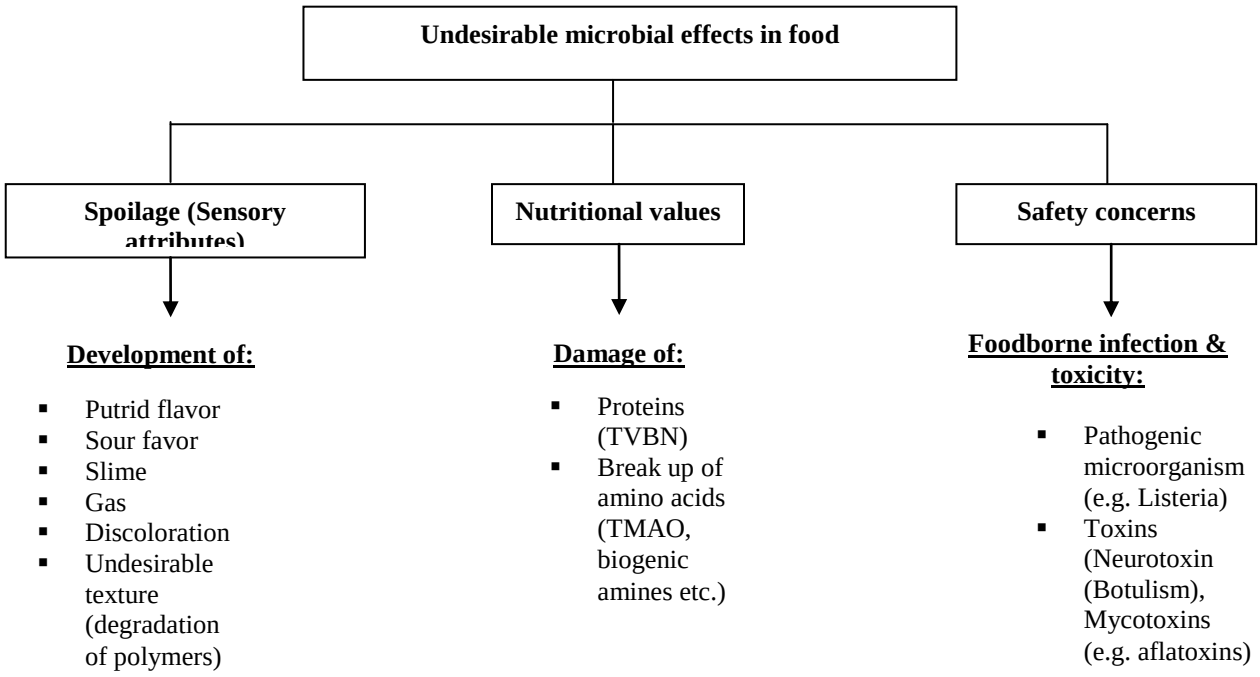

Figure 2.1 Overview of the undesirable microbial effects in food

In most developing countries, dried and smoked fish are often processed and sold in open-air markets where they are displayed uncovered on tables. Unsanitary handling by both processors and sellers, dust and insects, expose the fish to contamination (Ikutegbe & Sikoki, 2014). In dried fish products, yeasts and molds are the most important quality parameters (Kilic, 2009). Indoor drying and suitable packaging can minimize contaminations by microbes mainly air borne yeasts and molds (Park et al., 2014). Retailers in the Kenyan markets occasionally re-dry and smoke unsold products in order to extend shelf-life even further as this is observed to reduce the incidence of spoilage microorganisms.

2.4.2 Lipid degradation (oxidation and hydrolysis)

Lipids are among the most important constituents of fish muscle and may be divided into two major components: phospholipids and triglycerides. The phospholipids make up the integral structure of the cell membranes and are often referred to as structural lipids. Triglycerides are lipids used for energy storage usually within special fat cells and are often referred to as depot fat. Fish lipids differ from mammalian lipids in that they contain a higher percent of long-chain fatty acids that are highly unsaturated, explaining why they are more vulnerable to degradation mainly through oxidation and hydrolysis (Bragadóttir et al., 2002). The long chain polyunsaturated fatty acids (PUFA) especially the omega-3 have an excellent nutritional value (Stołyhwo et al., 2006).

2.4.2.1 Lipid oxidation

Due to relatively high amounts of PUFA in fish (Stołyhwo et al., 2006; Bragadóttir et al., 2002), lipid oxidation is the most important deteriorative reaction that occurs during fish drying or smoking, and distribution and marketing (Doe, 2002; Oduor-Odote & Obiero, 2009). It adversely affects nutritional quality (Underland et al., 1999), wholesomeness and sensory value of food (Ozen et al., 2011; Stapelfeldt et al., 1997; Driscoll & Madamba, 1994). The undesirable effects of lipid oxidation on food quality are summarized in Figure 2.2. Lipid oxidation depends on several factors such as the amount of lipids present, the degree of unsaturated fatty acids and their location in fish. The primary components for lipid oxidation are the PUFA, oxygen, proteins and catalysts including metals, enzymes and iron contained in proteins.

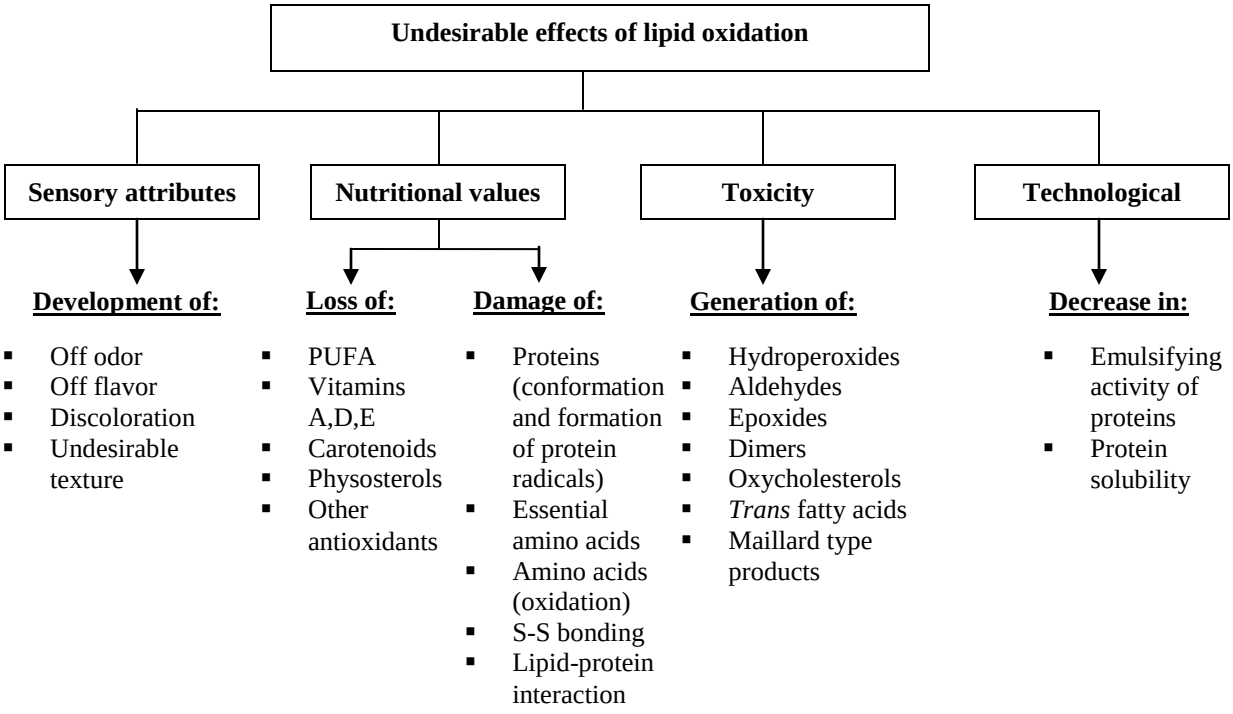

Figure 2.2 Overview of undesirable effects of lipid oxidation on food quality (modified from Kolakowska, 2003)

Lipid oxidation can be initiated by non-enzymatic (autoxidation), photogenic (photooxidation) and/or enzymatic (lipoxygenase) reactions. Photooxidation is caused by UV light, electric shining light or irradiation, whereas enzymatic initiated lipid oxidation processes are catalyzed by enzymatic activities and are influenced by temperature, pH, nature of the substrate and presence/absence of co-factors among others. On the other hand, autoxidation is the primary cause of lipid oxidation in post mortem fish and it involves a free radical chain mechanism proceeding via initiation, propagation and termination (Frankel 2005; Erickson 2002). Factors such as light, temperature, pH and water activity influence the initiation and rate of lipid oxidation. The autoxidation process of unsaturated lipid is as depicted in Figure 2.3.

Initially a labile hydrogen atom is abstracted from a methylene group adjacent to double bonds in a lipid molecule, a process triggered by an initiator (Shahidi et al., 1995). The free radical (L·) reacts with molecular oxygen to form peroxyl radical (LOO·). The peroxyl radical abstracts hydrogen from adjacent lipid molecules to form lipid hydroperoxide (LOOH) a process referred to as propagation. Lipid hydroperoxides undergoes further reactions due to metal catalysts to

form secondary intermediates of shorter chain length. The secondary autoxidation products (aldehydes, ketones, alcohols, small carboxylic acids and alkanes) are the cause of off-odor and off-flavor development during lipid oxidation (Underland et al., 1999; Huss, 1995). The free radicals of lipid oxidation terminate when their reaction are reduced by a reaction with other radicals or antioxidant.

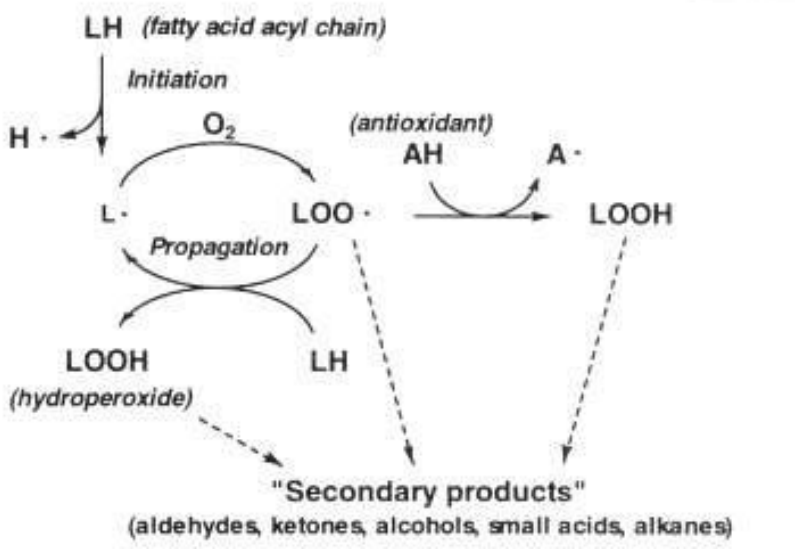

Figure 2.3 Autoxidation of polyunsaturated lipid (Adopted from Huss 1995)

2.4.2.2 Lipid hydrolysis

Lipid degradation results in the production of a range of substances that cause rancidity in food characterized by unpleasant flavor and odor thereby making it undesirable for consumers. The reactions are either non-enzymatic or catalyzed by microbial enzymes or by intracellular and/or digestive enzymes from the fish themselves (Huss, 1995). In fish lipid, hydrolysis is largely due to enzymatic activities mainly of lipase and phospholipases (Chaijan et al., 2006; Shah et al., 2009).

Hydrolysis of glycerol-fatty acid esters occurs in fish lipid with the liberation of free fatty acids (Figure 2.4) whose accumulation has been associated with unpleasant sensory properties (Chaijan et al., 2006; Lopez-Amaya & Marangoni, 2000). The main enzymes involved in fish lipid hydrolysis are triacyl lipase, phospholipase A2 and phospholipase B (Shah et al., 2009).

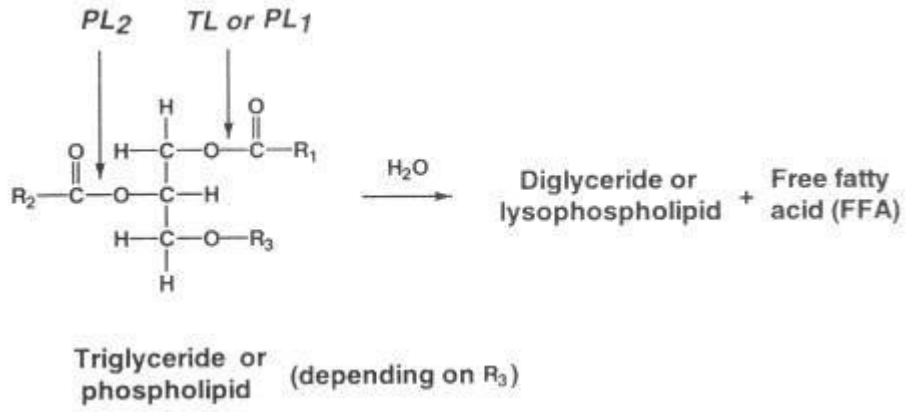

Figure 2.4 Hydrolytic reactions of triglycerides and phospholipids (Adopted from Huss 1995)

The occurrence of hydrolysis depends on fish species, temperature and also processing method. Hydrolysis is more likely to happen in un-gutted than gutted fish perhaps due to the involvement of digestive enzymes (Huss, 1995). Omodara and Olaniyan (2012) found that partial boiling (blanching) of fish before drying reduces lipid hydrolysis during drying by inhibiting microbial and enzymatic activities.

2.4.3 Interactions between proteins and lipid oxidation

Interactions between proteins and lipids have a significant effect on the progress of oxidative reactions in foods. The oxidation reactions affect the quality of food, but they also have an effect on the charge and conformation of the protein structure, leading to the loss of enzyme activity and changes in the nutritive value such as loss of essential amino acids (Finot, 1997; Underland et al., 1999; Howell et al., 2001). The three-dimensional structures of protein affect how they can interact with lipids. For example casein, due to its disordered random-coil and flexible structure, tends to cross-link in the presence of oxidized lipids more readily than the globular and more compact whey proteins (Sharma et al., 2002).

Primary and secondary lipid oxidation products react with proteins and vitamins, damaging valuable nutritionals. Reactions between the by-products of lipid oxidation cause protein oxidation leading to undesirable changes in food properties such as protein denaturation, alteration of texture and functional properties of protein (Howell et al., 2001). Protein oxidation occurs via free-radical reactions (Viljanen, 2005) in which peroxyl radicals (ROO·) formed during lipid oxidation can abstract hydrogen atoms from protein molecules (PH) (Reaction 1).

Consequently, protein radicals are formed (P·), and they can in turn create a protein net (P-P) due to the cross-linking (Reaction 2).

$ROO\cdot + PH \rightarrow P\cdot + ROOH$ Reaction 1

$P\cdot + P\cdot \rightarrow P\text{-}P$ (Protein net) Reaction 2

Lipid oxidation by-products can also physically complex with protein through hydrophobic association and hydrogen bonds to form various types of covalent bonds (Viljanen, 2005). In the covalent interaction one or more secondary lipid oxidation product can react with protein molecules present at the interface of the oil droplets in the oil-in-water emulsion. Amino acids have also been reported to react with secondary lipid oxidation products forming carbonyl groups in the proteins thereby generating fluorescent compounds and promoting polymerization (Zamora et al., 1997). In addition, the hydrophobic environment of proteins is reduced due to protein lipid interaction leading to the modification of the environment of aromatic amino acids (Viljanen, 2005).

2.5 Protein denaturation and aggregation

Processing affects the denaturation and aggregation of proteins, particularly if the processing method involves heating (Ghelichpour & Shabanpour, 2011). Fish protein denaturing temperature is mainly affected by fish species and water content. Proteins become more thermally stable with reduced water content (Rustad & Nesse, 1983). Tropical fish species contain proteins that are more thermally stable than proteins of temperate species (Poulter et al., 1985). Proteins become more thermally stable with higher habitat temperature (Meneshi et al., 1976).

Protein denaturation and aggregation is associated with the formation of disulfide bonds and conformational changes. This includes changes in the reactive groups, mainly loss of hydrophilic surface, exposure of hydrophobic areas and sulfhydryl groups that are buried or blocked in native proteins (Nguyen et al., 2011; Baylan et al., 2015; Raman & Mathew, 2014). Secondary and tertiary structures of proteins can be lost during heating due to a split in hydrogen bonds, resulting in unfolding of native conformation (Baylan et al., 2015; Raman & Mathew, 2014). The conformation of proteins plays an important role in defining their functional properties

(Ghelichpour & Shabanpour, 2011). Therefore, changes in protein conformation can lead to the loss of some of their physical and chemical properties (Skipnes et al., 2008; Rustad & Nesse, 1983) affecting the nutritional and textural characteristics of products (Raman & Mathew, 2014).

2.6 Consumption of dried and smoked fish

Depending on the consumer preference, there are several forms in which fish can be consumed; fresh, dried, fermented, brined etc. The choice and acceptability of a food product are mainly based on their sensory properties. If a product has low sensory acceptability, no brand or nutritional and/or health benefit promise will manage to get it accepted by consumers (Sosa et al., 2008; Hough et al., 2006). But if a product has high sensory acceptability, there are additional issues that have to be resolved to ensure overall acceptability for instance, packaging, price, convenience and cultural habits.

Dried and smoked fish are a traditional part of the diet of a large section of the world's population (Ahmed et al., 2013; Huda et al., 2010; Reza et al., 2009; Chukwu & Shaba, 2009). Dried and smoked fish are nutritious food containing highly unsaturated fatty acids, fat soluble vitamins, essential minerals as well as proteins containing essential amino acids (Bilgin et al. 2008; Ahmed et al. 2011). Fresh fish is preferred by most consumers (Ikutegbe & Sikoki, 2014). There are however limitations such as high perishability of fish after harvest (Cyprian et al., 2008; Cyprian et al., 2013) and the distances between fishing grounds and marketing outlets that make marketing of fresh fish difficult, especially in developing nations. The challenges of extending fresh fish shelf life in many developing countries, results in a higher consumption of dried and smoke-dried fish (Oduor-Odote et al., 2010; IOC, 2012). In addition to a longer shelf-life, smoked and dried fish have a desirable taste and remain whole when cooked, making it more appealing to consumers in some market segments than fresh fish which disintegrates when cooked (Ikutegbe & Sikoki, 2014).

A considerable proportion of smoked fish is consumed. In Europe, cold and hot smoked fish products constitute about 15% of the total fish consumption (Huda et al., 2010; Stołyhwo & Sikorski, 2005). The consumer preference for these products is not only for their traditionally desirable flavor but also the preservation of nutritional quality such as the highly polyunsaturated fatty acids (PUFAs) and essential amino acids (Stołyhwo et al., 2006; Bilgin et al., 2008).

In Eastern Africa, particularly Kenya, Uganda and Tanzania dried small pelagic species are the most widely consumed fish product (IOC, 2012). Originally the main market for small pelagic fish was the animal feeds industry. However, due to an increase in human population, decline in valued species in local markets and improved drying methods, about 80% of small pelagic fish in East Africa goes for human consumption (IOC, 2012). The consumer preference for these products is not only because of the flavor, but also the reasonable price (Oduor-Odote et al., 2010).

3 Objectives of the study

The study focuses on improving the quality and safety of small fish, and determines acceptability of new products such as dried capelin in markets accustomed to dried small fish. An overview of the study is illustrated in Figure 3.1. More specifically, the aim was to:

- Investigate how drying systems (indoor and open-sun) and pre-drying treatment (brining and blanching in brine) influence drying characteristics and quality of sardine and capelin during drying (**Papers I and II**).
- Investigate the effects of lipid content and pre-drying/smoking treatment (brining and blanching in brine) on protein structure during drying and smoking (**Paper III**).
- Investigate the influence of lipid content and packaging methods on lipid degradation and microbial quality of dried and smoked fish during storage (**Papers IV and V**).
- Determine the market potential of dried capelin and improved dried sardine in Kenya (**Paper VI**).

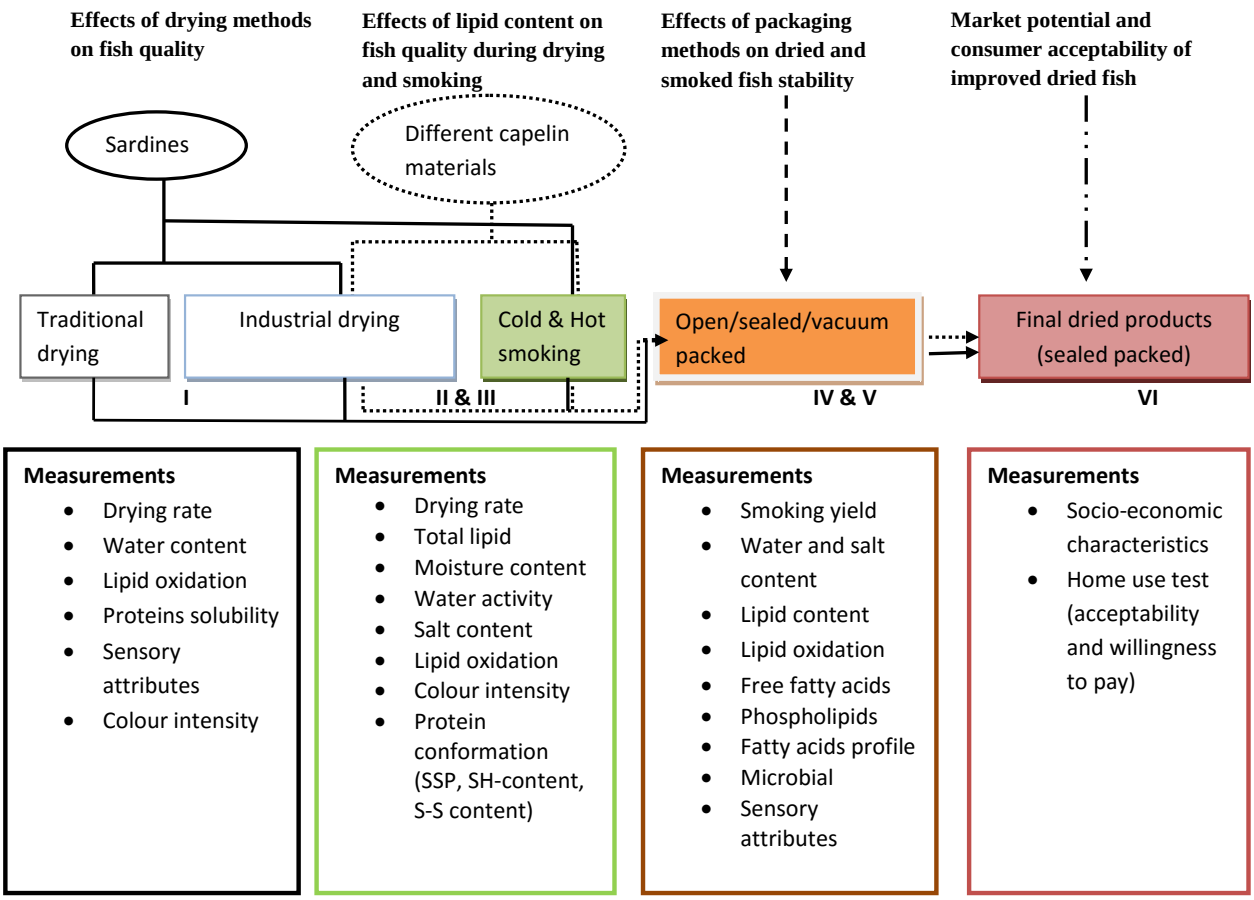

Figure 3.1 An overview of the studies contained in this thesis (Roman numerical in bold refers to original papers from the studies).

4 Materials and methods

General descriptions of the materials and methods used are presented here. More detailed descriptions of the materials and methods can be found in the original **papers I-VI** in the appendix.

4.1 Experimental design

The experimental designs of the studies contained in this thesis are described under this chapter as well as in **papers I to VI**.

4.1.1 Influence of blanching treatment and drying methods on the drying characteristics and quality changes in sardine (*Sardinella gibbosa*) during storage (Paper I)

Frozen sardine caught on 21st March, 2013 was used in the study (Figure 4.1). Sardine was thawed overnight in open air and upon thawing divided into two equal portions. One portion was blanched in brine for three minutes and the other portion brined for two hours and used as control.

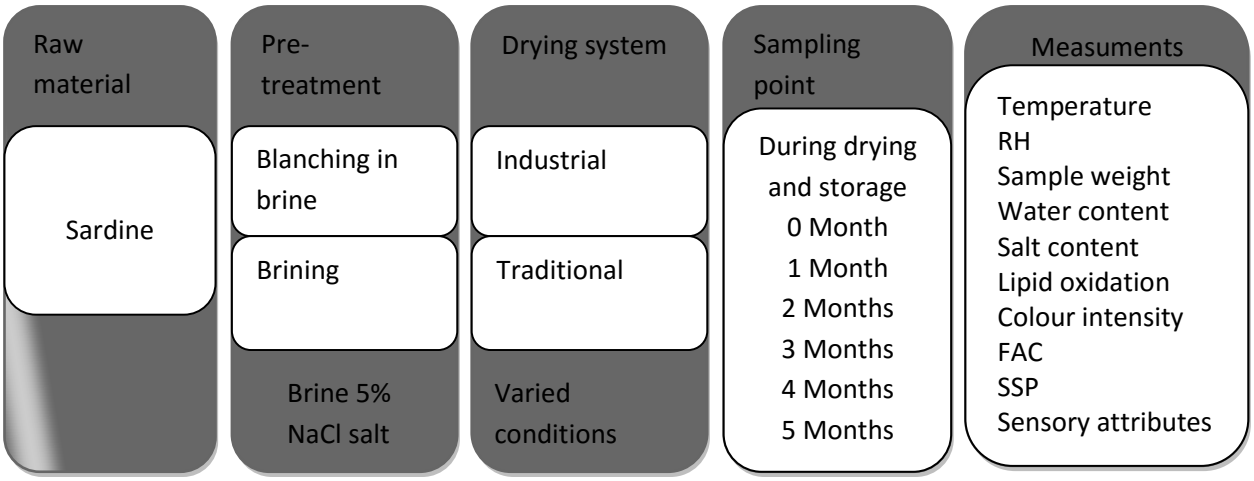

Figure 4.1 Experimental design for study on the influence of blanching and drying methods on the drying rate, quality and lipid stability of sardine (paper I).

4.1.2 Influence of lipid content and blanching on capelin (*Mallotus villosus*) drying rate and lipid oxidation under low temperature drying (Paper II)

Two batches of capelin frozen two days post catch on 13th February and 7th March, 2013, differing in lipid content were used to establish the effects of lipid content and blanching on capelin drying characteristics and oxidation (Figure 4.2). Fish was thawed overnight in open air, after which each group was divided into two equal portions for blanching in brine and brining prior to indoor drying.

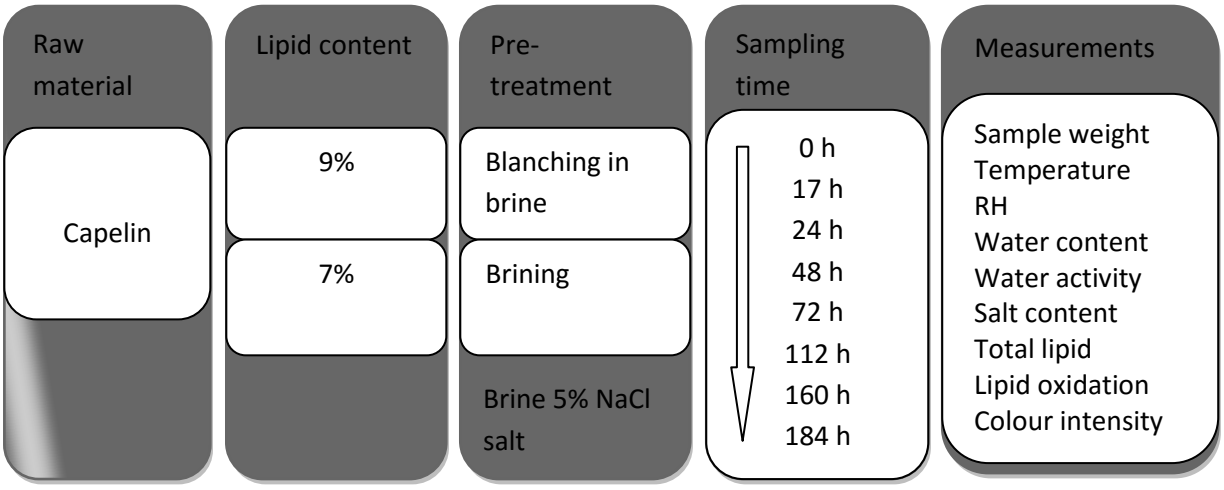

Figure 4.2 Experimental design for studying the influence of lipid content and blanching on capelin drying rate and lipid oxidation (paper II).

4.1.3 Conformational changes in capelin (*Mallotus villosus*) proteins during smoking and drying (paper III)

Two batches of frozen capelin caught on 13th February and 7th March, 2013 were used in the study to determine the effects of raw material treatments (blanching in brine and brining) and lipid content on conformational changes in proteins during fish drying and smoking (Figure 4.3).

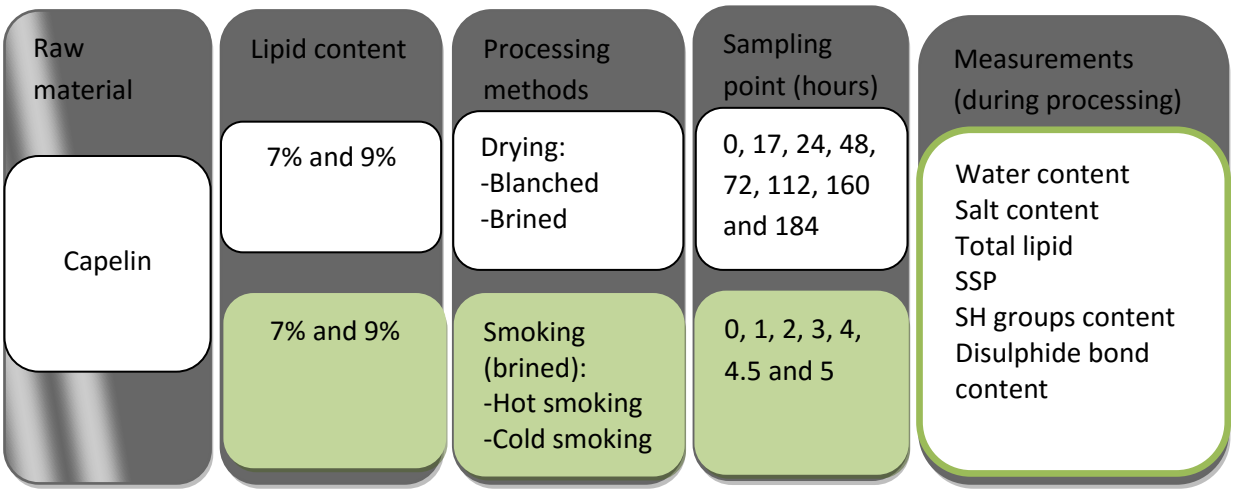

Figure 4.3 Experimental design for studying conformational changes in capelin proteins during smoking and drying (paper III).

4.1.4 Influence of smoking and packaging methods on lipid stability and microbial quality of capelin (*Mallotus villosus*) and sardine (*Sardinella gibossa*) (Paper IV)

Two batches of frozen capelin caught on 1st and 28th February, 2013 and one batch of sardine caught 21st March, 2013 were used in the study to understand the influence of smoking methods, packaging and lipid content on lipid stability and microbial quality (Figure 4.4).

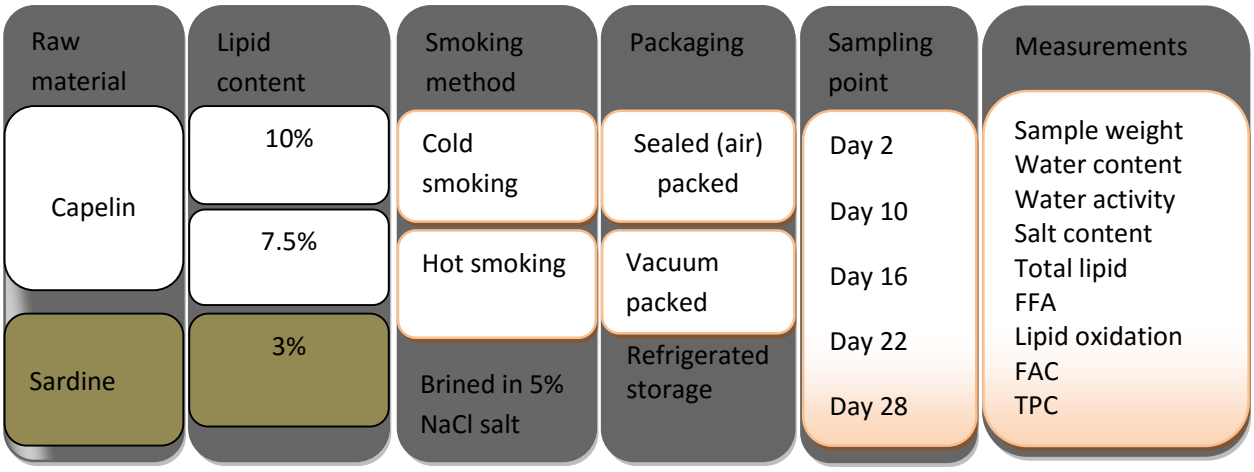

Figure 4.4 Experimental design for study on the influence of smoking and packaging methods on lipid stability and microbial quality (paper IV).

4.1.5 Influence of lipid content and packaging methods on dried capelin (*Mallotus villosus*) stability (Paper V)

Three batches of frozen capelin caught on 1st, 13th and 28th February, 2013 were used in the study to determine the influence of lipid content and packaging methods on lipid stability during storage of dried fish (Figure 4.5).

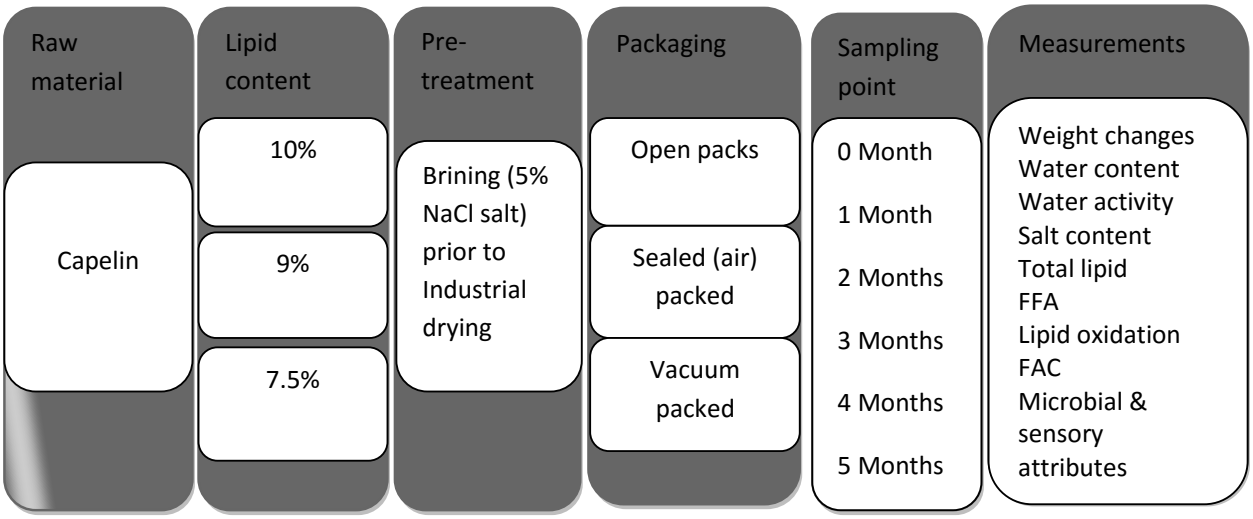

Figure 4.5 Experimental design for study on the influence of lipid content and packaging methods on dried capelin stability (paper V).

4.1.6 Marketing potential of improved dried sardine (*Sardinella gibossa*) and capelin (*Mallotus villosus*) in markets accustomed to traditional dried fish (Paper VI)

One batch of capelin caught on 15th February, 2014 and one batch of sardine caught on 20th March, 2014 were dried and packaged in sealed polyethylene bags weighing 500 g altogether and given to participants recruited among shoppers at supermarkets and open-air markets in Kenya (Figure 4.6).

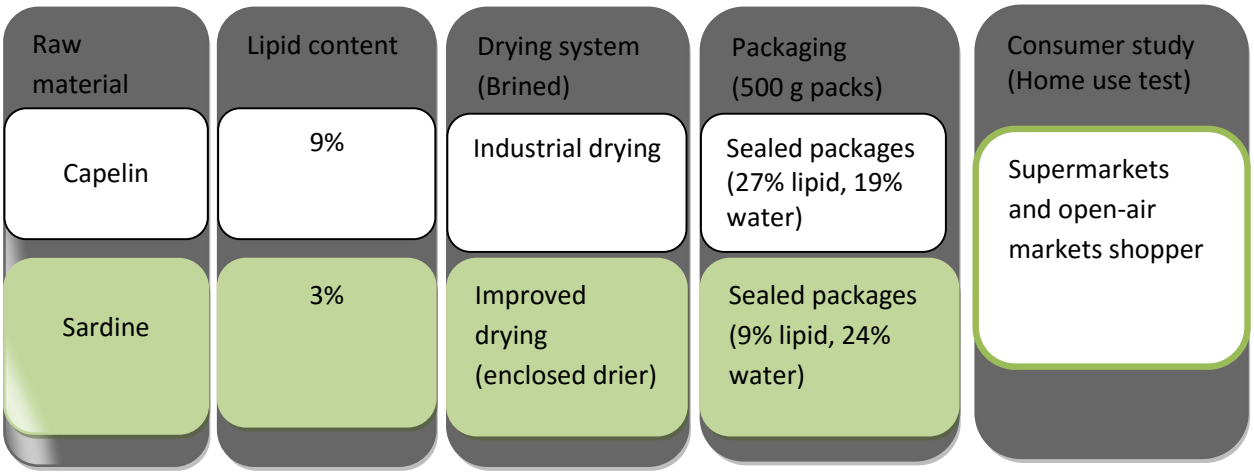

Figure 4.5 Experimental design for studying marketing potential for improved dried sardine and dried capelin in Kenya (paper VI).

4.2 Raw material and processing

Sardine caught in the Western Indian Ocean by artisanal fisherman in Mombasa Kenya were off-loaded approximately 8 hours post-catch, graded by size and placed in flake ice, and frozen within three hours in 25 kg blocks. Two blocks were transported by air freight to Iceland and the other two kept in Kenya for later studies. Frozen blocks of male capelin differing in lipid content caught in Icelandic waters were obtained from HB Grandi fishing company, Reykjavik, Iceland.

Fish drying was done under indoor (controlled) and open-sun (uncontrolled) conditions. Indoor drying was conducted in Vestfirska Hardfisksalan fish drying company in Reykjavik, Iceland. The dryer consisted of a heating chamber heated with geothermal hot water, connected directly to a centrifugal fan and drying chamber with controlled temperature, relative humidity and air velocity. Open-sun drying was conducted on open air drying racks in Mombasa, Kenya. Smoking trials were done in Matis Hornarfordur using a conventional smoking kiln equipped with an automatic control for temperature, humidity and density of wood smoke.

4.3 Evaluation of stability and quality

The drying process was evaluated by measuring temperature and relative humidity, drying rate and water content over time. Quality of products after drying and during storage was studied by

analyzing lipid oxidation (PV and TBARS, fluorescence), lipid hydrolysis (FFA, phospholipids), fatty acid composition, color (CIELAB, Lab), sensory attributes (Generic Descriptive Analysis) and protein solubility. Microbial development (total plate counts, yeasts and molds) were also measured.

The effects of treatments (blanching in brine and brining), lipid content, smoking and drying methods on the conformational changes in capelin proteins were studied by analyzing water content, salt content, salt soluble proteins, disulfide bonds content and sulfhydryl groups (total S-H and available S-H) content.

The effects of smoking, packaging and refrigerated storage on lipid changes, stability and quality of smoked sardine and capelin were studied by analyzing water, salt, water activity and lipid content. Lipid oxidation and hydrolysis as well as changes in fatty acid composition and microbial development (TPC) were also evaluated.

4.4 Marketing potential

Acceptability and willingness to buy dried capelin and improved dried sardine were investigated in a consumer study carried out in Mombasa and Kwale counties located in the southern part of the Kenyan coast. Mombasa is cosmopolitan in nature and hosts the main supermarkets along the Kenyan coast, with the majority of its population belonging to the middle class (Ipsos-Synovate 2013; Kenya National Bureau of Statistics 2015). The main sardine landing beaches along the Kenyan coast are in Kwale county where consumption of dried sardine is common. Majority of the population in Kwale county belong to low income class (Ipsos-Synovate 2013; Kenya National Bureau of Statistics 2015).

Home use test was used in the present study. Participants were recruited from shoppers in three supermarkets in Mombasa County and three open-air markets in Kwale County over a four week period. Participants were adults willing to take part in the study. Participants' details, for instance phone number and place of residence (when appropriate) were obtained. In the open-air markets a local person from each area was hired during the period of study to visit particpants in their homes to ensure complete questionnaires were returned. Participants in supermarkets who returned completed questionnaires could win a prize which was announced on the radio. A total

of 120 consumers participated; 60 supermarket shoppers and 60 open-air market shoppers. Description of evaluation protocol is as provide in **Paper VI**.

4.5 Statistical analysis

Data was analyzed using Microsoft Excel 2010 (Microsoft Inc. Redmond, Wash., U.S.A.). One way analysis of variance (ANOVA), Duncan's Multiple-Comparison Test (Post-hoc) and Pearson's correlation analysis were performed on means of the variable values using the NCSS 2000 statistical program (NCSS, Utah, USA) (**Papers I to V**). P values of < 0.05 were considered significant.

Multivariate comparison of different variables and samples was performed using Principal Component Analysis (PCA) on mean level corrected values using full cross-validation in the Unscrambler ® statistical program (Version 8.0 CAMO, Trondheim, Norway) to identify similarities and differences between samples (**Paper V**). Panelcheck V 1.4.0 (Nofima, Tromsø, Norway) was additionally used in multivariate comparison of different sensory attributes with Principal Component Analysis (PCA) (**Paper I**). Descriptive analyses were done by use of means, standard error, percentages and frequency distribution of consumers responses' using the Statistical Package for the Social Sciences (IBM - SPSS Inc. version 20.0) (**Paper VI**).

5 Main results and discussion

The main results of the study are presented in the following subchapters. More detailed results can be found in **papers I to VI**.

5.1 Factors that influence fish drying characteristics and quality

5.1.1 Drying system

Effects on drying characteristics

Average air temperature and relative humidity varied during drying in both systems (open-sun and indoor), with higher temperature in the open-sun drying (Figure 5.1 A and B). Temperature varied based on weather conditions and time of day during open-sun drying, while it was increased gradually as drying progressed during indoor drying. Relative humidity was inversely related to temperature in both drying experiments as can be expected (Oduor-Odote et al., 2010) and was highest at the start of drying in the indoor system.

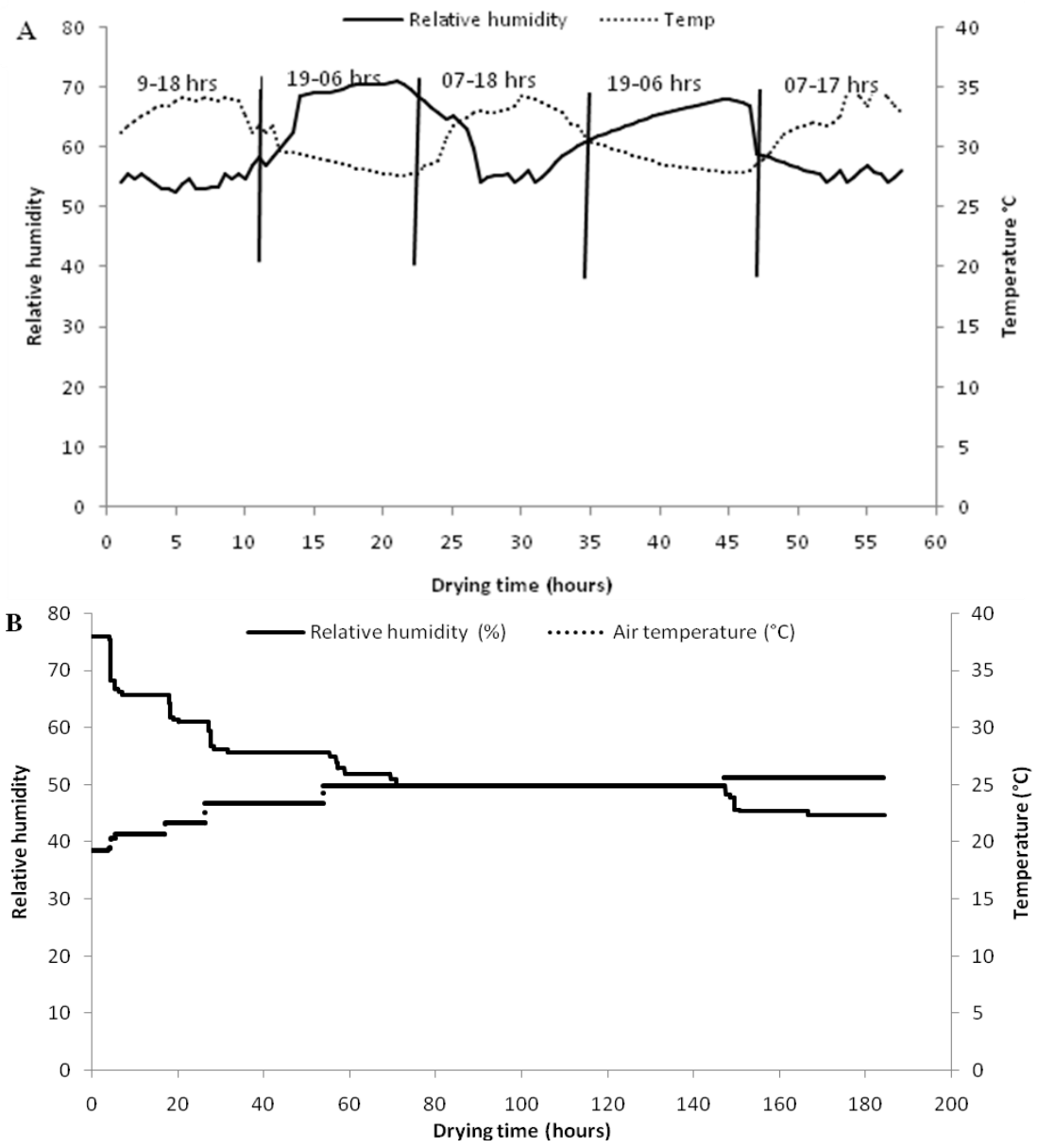

Figure 5.1 Variation of air temperature and relative humidity during open-sun (A) and indoor (B) sardine drying (n=3).

Changes in the air temperature and relative humidity affected the drying rate (Table 5.1) and consequently water content at any given time (Figure 3, **Paper I**). A higher drying rate was obtained at the start of drying in the open-sun, attributed to high moisture evaporation and diffusivity owing to low humidity levels and high temperatures. Drying is controlled by temperature, level of relative humidity and air velocity (Bellagha et al., 2007). As expected the drying rate decreased gradually with time but differently depending on the drying system (Table

5.1). Water binding in the products increases with drying and in the later drying stages water diffuses from the interior to reach the surface (Duan et al., 2005; Bellagha et al., 2002). An increase in drying rate was observed at the beginning of each day after keeping samples overnight (darkness hours) during traditional drying (after 12 hours interval in Table 5.1). This was probably due to internal diffusion in the night that allowed water to exit to the surface (with no external diffusion), resulting to an increasing rate in the drying process at the beginning of the following day. In general, the drying rate curves were similar with no constant rate period and had only a falling rate period except at the beginning of each day after keeping samples overnight under traditional drying conditions (Figure 2, **Paper I**). It took about 60 and 52 hours to dry fish to steady moisture content under indoor and open-sun drying conditions respectively. But indoor dried fish had lower moisture ratio at the end of drying than open-sun dried fish (Figure 3, **Paper I**).

Table 5.1 Variation in drying rate g water/hour for 100 g of brined and blanched sardine during open-sun (T) and indoor (I) drying. (n=10; Mean±SD)

Group	Sampling time interval (Δt) in hours during drying									
	6	12	6	6	12	6	3	3	5	14
†Bl-T	5.5±0.3^{a*}	0.0±0.0^{a}	1.4±0.1^{a}	1.1±0.1^{a}	0.1±0.0^{a}	0.5±0.0^{a}	0.0±0.0^{a}	0.0^{x}±0.0^{a}	N/A	N/A
Br-T	4.5±0.1^{b}	0.0±0.0^{a}	1.3±0.1^{a}	0.9±0.15^{b}	0.1±0.0^{a}	0.8±0.0^{b}	0.6±0.1^{b}	0.0±0.0^{a}	N/A	N/A
Bl-I	3.6±0.2^{c}	1.6±0.7^{b}	0.5±0.0^{b}	0.5±0.0^{c}	0.4±0.0^{b}	0.4±0.0^{c}	0.2±0.0^{c}	0.2±0.0^{b}	0.1±0.0	0.0±0.0
Br-I	4.6±0.2^{b}	1.4±0.5^{b}	0.4±0.0^{b}	0.3±0.0^{d}	0.2±0.0^{c}	0.2±0.0^{d}	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0	0.0±0.0
P-value	**0.028**	**0.000**	**0.005**	**0.002**	**0.015**	**0.024**	**0.018**	**0.046**	0.455	0.635

† Abbreviations: Bl = blanched; Br = brined; T = open-sun drying; I = indoor drying

* Different letters (superscript) within a column indicate significantly different values between samples ($p < 0.05$)

x 0.0 denotes values less than 0.05

Effects on fish quality

Drying system had some effect on lipid oxidation with significantly higher PV and TBARS values in open-sun dried than in indoor dried sardine (Figure 5 A and B, **Paper I**). The rate of lipid oxidation was higher during open-sun than indoor drying due to higher temperature and exposure to sunlight during open air drying. Wu & Mao (2008) reported high temperature to have accelerated lipid oxidation during drying of grass carp fillets. Upholding of the moisture ratio overnight during darkness (Figure 3, **Paper I**) might have additionally contributed to increased PV and TBARS development in fish during open-sun drying. Similarly during storage,

open-sun dried fish had a higher rate of hydroperoxide (PV) and TBARS accumulation and decomposition (Figure 5 A and B, **Paper I**) implying open-sun dried sardine was more vulnerable to lipid oxidation processes (Oduor-Odote & Obiero 2009) than when dried indoor.

Drying conditions also influenced fatty acids (FA) composition with a faster decline in unsaturated FA during open-sun drying and storage of dried sardine (Table 3, **Paper I**). The decline in unsaturated FA was most rapid in fatty acids with double bonds suggesting the impact of oxidation was higher in PUFA than in other fatty acids. Raw sardine had 26.85 g PUFA/100 g lipid. PUFA declined in value by 19% and 31% in indoor dried brined and blanched in brine sardine respectively at the end of 5 months storage. While it declined by 37% and 44% in their open-sun dried counterparts. DHA that constituted a high proportion of PUFA exhibited the biggest decline, meaning that it oxidized more readily than other PUFA. This is of concern given that PUFA, especially EPA and DHA are beneficial to the health of consumers (Bilgin et al., 2008; Stołyhwo et al., 2006).

The sensory attributes (flavor, odor and texture) of dried fish were not much different between systems (Figure 7, **Paper I**). Even though it took longer for the fish to attain stable moisture during indoor drying, the final products were of better quality and more stable during storage.

5.1.2 Raw material treatments (brining and blanching in brine)

Effects on drying characteristics

Drying rate and water content changes were highest at the start of drying in capelin blanched in brine (Tables 5.2 and Figure 5.2). Higher drying rate and water loss in blanched capelin was attributed to denaturation and hydrolysis of myofibrillar proteins that might have occurred during blanching, affecting muscle water holding capacity (Rustad & Nesse, 1983). More so, high temperature encountered during blanching may have increased the activity of the water molecules accelerating their diffusivity. This contradicts our other results obtained during sardine drying, where no significant difference in drying rate and moisture ratio was obtained between blanched in brine and brined groups (Table 5.1; Figure 2, **Paper I**). This could probably be because the blanching time of 2- 3 minutes used was not long enough to cause greater denaturation and hydrolysis in sardine proteins since capelin is a temperate species unlike

sardine caught in tropical waters. Tropical fish species contain thermal stable proteins than temperate species (Poulter et al., 1985) because the tissue proteins increase in stability with increase in their habitat temperature (Meneshi et al., 1976).

Although the final water content was not significantly different based on the treatments at the end of drying, blanched in brine fish were drier than brined only (Figure 5.2**;** Figure 3, **Paper I**). The difference in moisture content between the groups at the end of drying can be explained by the fact that equilibrium vapor pressure on the surface of a food is determined not only by the temperature and the water content in food but also by the interactions of water molecules with solutes as blanched fish had a stronger salt concentration (Figure 1, **Paper II**). It should be noted that blanching in brine lasted for 2-3 minutes but resulted in salt uptake that was higher than or equal to when fish was brined for 2 hours. This means blanching enhanced the salting-in effect.

Table 5.2 Changes in drying rate (g water/hour for 100 g) in brined and blanched capelin during indoor drying (n=10; Mean±SD).

	sampling time interval (Δt) in hours during drying						
Group	**17**	**7**	**24**	**24**	**40**	**48**	**24**
†C1-BR	1.6±0.1^{a*}	0.8±0.1	0.4±0.0	0.2±0.02^{a}	0.1±0.0^{ab}	0.1±0.0	0.0±0.0
C1-BL	1.7±0.1^{ab}	0.7±0.0	0.3±0.1	0.2±0.0^{a}	0.1±0.0^{a}	0.1±0.1	0.0±0.0
C2-BR	1.7±0.1^{ab}	0.7±0.1	0.4±0.0	0.2±0.0^{ab}	0.2±0.0^{b}	0.1±0.0	0.0±0.0
C2-BL	1.9±0.1^{b}	0.7±0.0	0.3±0.0	0.2±0.0^{bc}	0.1±0.0^{ab}	0.0±0.0	0.0±0.0
p-value	**0.046**	0.862	0.079	**0.045**	**0.015**	0.095	0.575

† Abbreviations: C1 = high lipid capelin; and C2 = low lipid capelin; Bl = blanched; Br = brined; T = open-sun drying; I = indoor drying

* Different letters (superscript) within a column indicate significantly different values between samples ($p < 0.05$)

x 0.0 denotes values less than 0.05

Water activity (a_w) was similar between the groups at the start of drying (Figure 5.2). However, blanched fish with the highest water reduction and stronger salt concentration, obtained low a_w. Furthermore, it was conspicuous towards the end of drying that groups had different a_w, even those under similar treatment. Here, it can particularly be said that, salt whose uptake by fish depended mainly on lipid content (Figure 2, **Paper II**) contributed to the differences within the same treatment, because salt as a solute is known to decrease the foods a_w due to its binding effects on free water (Grummer & Schoenfuss, 2011). Dried fish had the required water activity

as intermediate water foods should have 15–50% water content and a_w of 0.60–0.85 (Kilic, 2009).

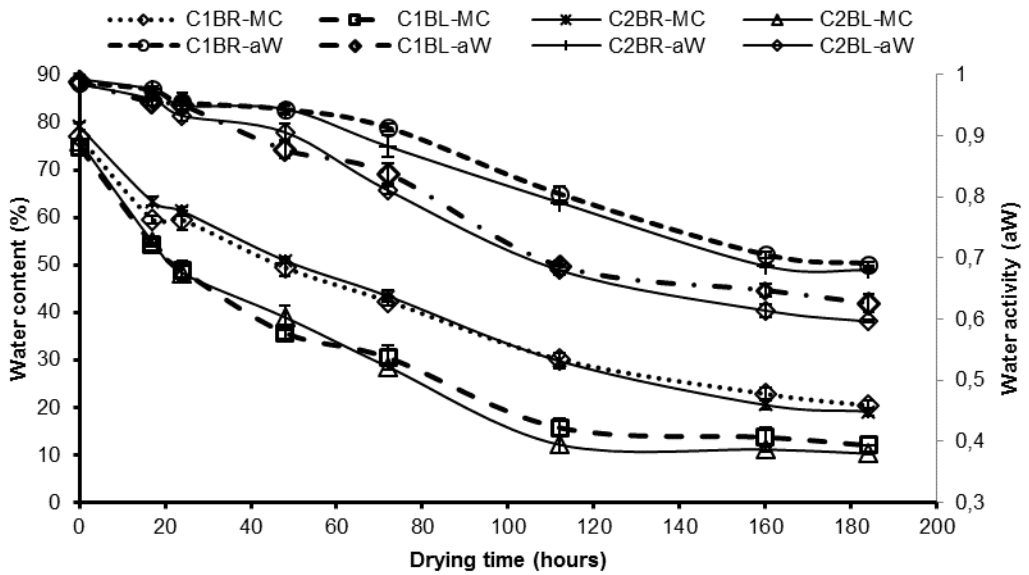

Figure 5.2 Variation in water content (MC, n=3)) and water activity ($a_{w,}$ n=3) in brined (BR) and blanched (BL) capelin as a function of drying time. C1 = high lipid capelin; and C2 = low lipid capelin.

Effects on fish quality

Oxidative deterioration during drying of capelin occurred at different times depending on blanching and lipid content (Figure 5.3 A). Early in drying (17 hours), when water content and drying rates were high, PV decreased in all groups. However after 24 hours of drying when water content was about 48% and a_w 0.95 (reduced drying rate) in blanched fish, the PV increased rapidly to maximum values of 370 and 281µmol/kg (fatty and less fatty capelin). In brined fish maximum values were reached (325 and 285µmol/kg) when water content and a_w were 50-45% and 0.944-0.92 respectively. These results suggest that there could be major changes in the capelin muscle structure at 45-50% water content, leaving lipids more accessible to oxygen and catalysts than at either higher or lower water content promoting formation of hydroperoxides. PV increased and subsequently decomposed fastest in blanched fish. Hydroperoxides are unstable and readily decompose into secondary products of oxidation that contribute to off flavor of foods (Underland et al., 1999; Stapelfeldt et al., 1997).

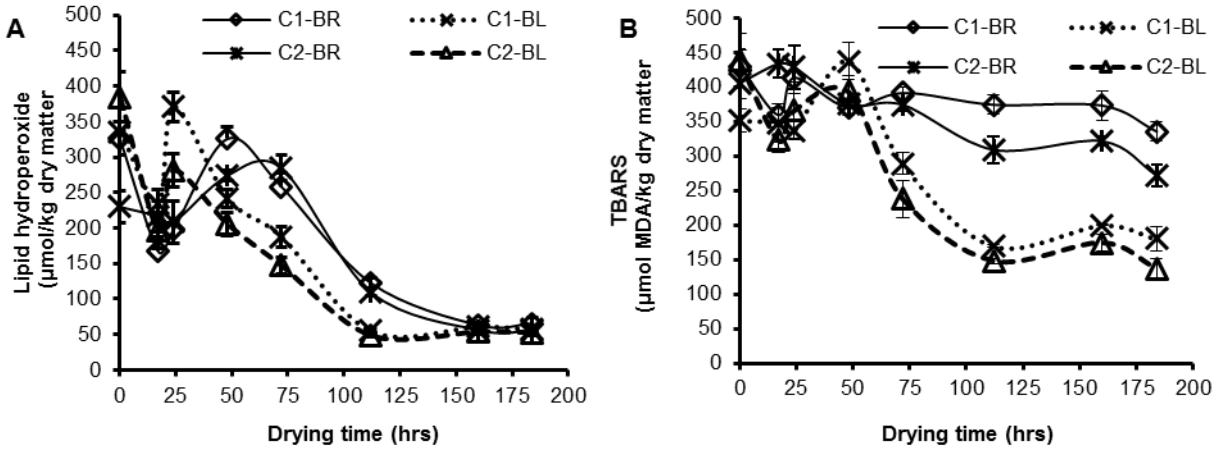

Figure 5.3 Level of lipid hydroperoxide (A) and thiobarbituric acid reactive substances (B) in brined (BR) and blanched in brine (BL) capelin fish as a function of drying time (n=3). C1 = high lipid capelin; and C2 = low lipid capelin.

TBARS, a secondary product of lipid oxidation generally decreased as drying progressed except in blanched groups where it increased briefly after 24 hours drying (Figure 5.3 B). TBARS then decreased more rapidly in blanched than brined fish possibly because of cross-linking of malomaldehyde with amino acids to form amidine linkages (Karlsdottir et al., 2014; Nguyen et al., 2012). Similar results were obtained during a storage study of dried sardine, where a slower increase in PV and TBARS was observed in brined than blanched fish (Figure 5, **Paper I**). Secondary lipid oxidation products have been reported to have a profound impact on both sensory and functional properties of foods (Stapelfeldt et al., 1997). This indicates that brined fish groups that showed less change in lipid degradation during drying (Figure 5.3) and storage (Figure 5 & Table 3, **Paper I**) could possess the desired sensory properties of dried fish rather than when blanched. Blanching accelerated lipid degradation during drying and storage.

Changes in color during fish drying and storage were negatively influenced by blanching (Figure 5.4; Figure 4, **Paper I**). After treatments, blanched groups (fatty and less fatty capelin) obtained stronger yellowness than brined groups, and vice versa in lightness. The stronger yellowness in blanched fish groups was in harmony with high lipid oxidation (Figure 5.3; Figure 5 A and B, **Paper I**). In dried fish, yellowness is associated with lipid oxidation, enzymatic browning and long storage (Nguyen et al., 2011). Color is an important quality attribute influencing consumer choices (Murat & Onur, 2000; Driscoll & Madamba, 1994), but blanching as practiced by small

scale dried fish processors results in undesirable color. Quality and shelf life of blanched fish is therefore limited.

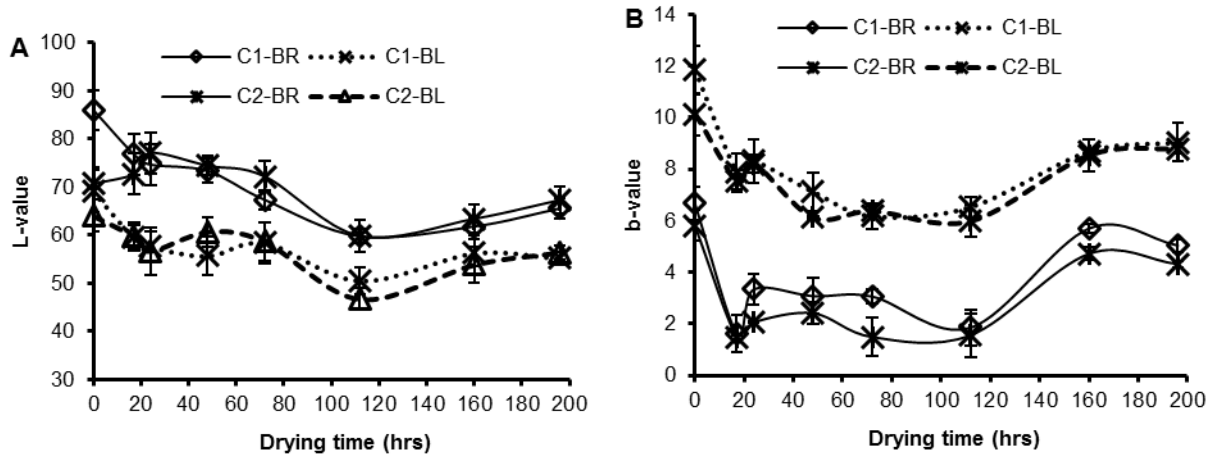

Figure 5.4 Changes in the color parameters; lightness (A) and yellowness (B) in brined (BR) and blanched in brine (BL) capelin fish as a function of drying time (n=15). C1 = high lipid capelin; and C2 = low lipid capelin.

5.1.3 Lipid content

Effects on drying characteristics

Lipid content affected capelin drying, with a slower water loss in fatty groups (9%) (brined and blanched in brine) than in less fatty (7%) (Figure 5.2). Less fatty fish lost water faster during drying even when the water content was evidently less than that of fatty fish later on during drying. It can be understood that lipids played an important role as a limiting factor in the drying steps either by replacing the aqueous phase reducing water transfers or acting as a physical barrier to heat transfer that causes evaporation and diffusivity of water.

Lipid content had an effect on the salt uptake during brining and blanching in brine, with a stronger salt concentration in less fatty fish than fatty fish (Figure 2B, **Paper II**). The salt concentration in raw fish was rather similar with values of 0.68% and 0.72% in the capelin groups. Higher salt uptake in less fatty capelin can be explained by water content and lipid relationship in fish. Water and lipid contents are inversely related in fish (Henderson et al., 1984), suggesting the muscle fluid in less fatty (C2) fish had less solutes concentration than fatty

(C1) fish resulting in more salt ions diffusion into C2 than C1. Salt concentration influenced water activity (a_w) in fish which was reduced with increasing salt concentration (Figure 5.2). Salt as a solute is known to decrease the a_w due to its binding effects on free water (Grummer & Schoenfuss, 2011).

Effects on fish quality

Lipid content had no influence on lipid oxidation during capelin drying (Figure 5.3). Fatty and less fatty capelin generally had similar trends of hydroperoxide (PV) and TBARS buildup and breakdown during drying, with relatively high TBARS levels in fatty capelin groups towards the end of drying. Although fatty fish are particularly susceptible to lipid degradation, capelin batches used in the study were of medium lipid content (7- 9%) possibly explaining why we did not obtain differences between the groups. A decrease in hydroperoxides as a result of decomposition was noted, but could not have influenced fish quality since hydroperoxides are known to have no impact on sensory properties of food (Stapelfeldt et al., 1997). TBARS known to contribute to off flavor in foods (Underland et al., 1999) remained moderately stable during drying.

Changes in color were not significantly different between fatty and less fatty fish, despite a strong yellowness in fatty groups (Figure 5.4). This suggests that changes in yellowness during drying might have been caused mainly by enzymatic browning since a weak correlation between yellowness and PV ($r \leq 0.02$), yellowness and TBARS ($r \leq 0.05$) was obtained in all groups. Lightness decreased in all groups during drying.

5.1.4 Summary

- Blanching enhanced fish salt uptake and water loss during drying thereby resulting in low water content and water activity desired for product stability.
- Blanching and open-sun drying had a negative influence of fish quality as demonstrated in development of undesirable yellowness, faster rate of lipid oxidation and decline in PUFA.
- Indoor drying conditions and fish batches studied produced dried products with required moisture content and water activity for stability during storage.
- A stable dried product of superior quality was produced from un-blanched fish dried under controlled conditions (indoor system), demonstrating the need for developing of controlled drying systems for commercial small fish drying.
- Lipid content affected the drying rate and products moisture content, with less fatty fish exhibiting faster drying, stability in quality during drying and lower moisture content.

5.2 Conformation changes in capelin proteins during drying and smoking

Capelin harvested at two different times was used to evaluate the effects of brining and blanching in brine on conformational changes in the proteins during drying and smoking and how the changes were affected by variation in lipid content. The lipid content in fatty capelin was 9%, whereas less fatty capelin had 7%.

5.2.1 Changes in lipid, water and salt content

Changes in lipid, water and salt content were measured as they are believed to influence protein conformation. Blanching reduced lipid content of capelin more than brining (Table 5.3). This might be due to exudation of lipid during blanching and drip drying. Similarly, water content was reduced after the treatments, and more after blanching, a phenomenon attributed to the reduced water holding capacity due to protein denaturation (Rustad & Nesse, 1983).

Contrary to lipid and water contents, salt concentration increased after fish treatments, increasing most in less fatty and blanched brined capelin (Table 5.3). This could be due to the differences in raw material water and salt contents. Moreover, higher salt concentration in less fatty capelin after brining and blanching in brine imply that lipid act as a barrier to salt uptake since the uptake was slower in fatty fish. Lipid and salt concentration increased after drying and smoking, with significant differences between the groups reported only in salt concentration. This could be explained by the dehydration level and loss of lipid during blanching. The drier the fish became the higher the salt content.

Table 5.3 Lipid, salt and content of raw and processed (dried and smoked) capelin differing in lipid content (n=4; Mean±SD)

Capelin batch	Variable	Raw material	Treatment		Dried products		Smoked products	
			Brined	Blanched-Brined *	Brined	Blanched-Brined	Brined-Cold	Brined-Hot
High lipid (C1)	Lipid[a]	9.1±0.2[a]	9.±0.4[a]	7.4±0.0[b]	28.1±0.5[c]	27.1±0.7[c]	-	-
	Salt	0.7±0.0[Δa]	2.0±0.2[b]	2.2±0.0[b]	6.2±0.2[c]	7.6±0.1[d]	-	-
	Water	76.8±0.1[a]	76.9±1.1[a]	74.9±0.9[b]	20.4±1.0[c]	12.0±0.5[d]	-	-
Low lipid (C2)	Lipid	7.0±0.3[a]	6.8±0.1[a]	6.3±0.2[b]	27.2±0.1[c]	27.4±0.8[c]	-	-
	Salt	0.7±0.0[a]	2.4±0.0[b]	2.4±0.1[b]	8.8±0.1[c]	9.6±0.3[d]	-	-
	Water	78.2±1.1[ab]	79.1±1.6[a]	76.1±1.0[b]	19.1±0.6[c]	10.3±0.7[d]	-	-
High lipid (C1)	Lipid	9.1±0.2[a]	9.00±0.4[a]	-	-	-	16.0±0.5[b]	17.8±0.6[c]
	Salt	0.7±0.0[a]	2.0±0.2[b]	-	-	-	3.5±0.4[c]	3.8±0.4[c]
	Water	76.8±0.1[a]	76.9±1.1[a]	-	-	-	59.1±1.5[b]	54.5±0.8[c]
Low lipid (C2)	Lipid	7.0±0.3[a]	6.75±0.1[a]	-	-	-	13.2±0.7[b]	15.2±1.0[c]
	Salt	0.7±0.0[a]	2.4±0.0[b]	-	-	-	4.3±0.3[c]	4.7±0.2[c]
	Water	78.2±1.1[a]	79.1±1.6[a]	-	-	-	57.8±1.2[b]	53.7±0.8[c]

[a]Abbreviations: Lipid (% lipid content), Salt (% lipid content), (% content).

*Treatment done only for drying trials.

-Reported earlier/later.

[a-d]Different letters within a row indicate significantly different values between samples ($p < 0.05$).

[Δ]Values equal to 0.0 are values less than 0.05

5.2.2 Changes in protein solubility (salt soluble proteins)

Protein solubility (SSP) was reduced in all groups as drying progressed and was lowest in blanched brined less fatty capelin (Figure 5.5). The significant decrease in SSP after blanching in brine and during drying (Figure 5.5 A) is thought to be due to conformational changes in the proteins. Denaturation and hydrolysis of myofibrillar proteins result in increased hydrophobicity. This might have occurred during blanching thereby reducing the content of thermolabile compounds such as amino acids (Ghelichpour & Shabanpour, 2011; Finot, 1997). Although blanched brined capelin contained more salt than brined capelin after the treatments (Table 5.3), it was not significantly different. Therefore influence of salt on protein solubility was not detected mainly due to low salt concentrations after treatments (< 2.5%). Similar results were obtained before smoking in which case only brined capelin groups were used (Figure 5.5 B).

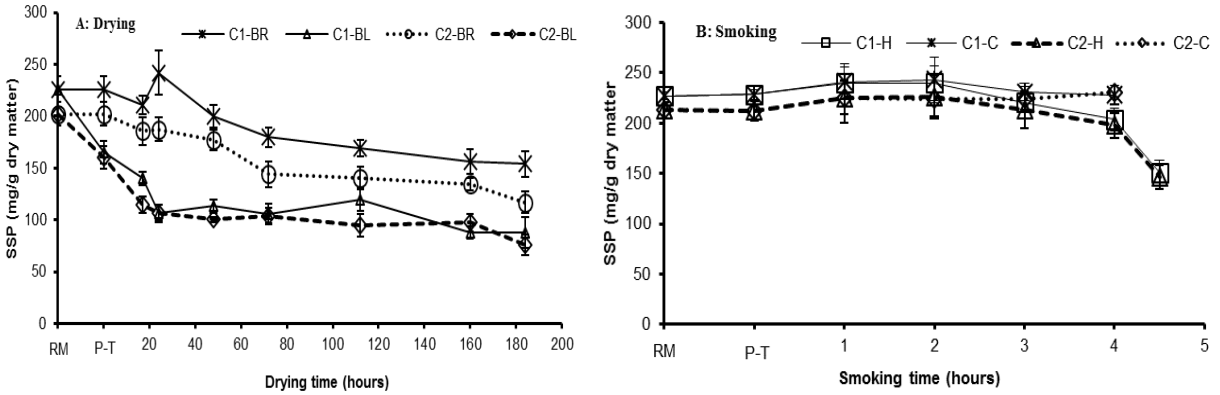

Figure 5.5 Changes in salt soluble proteins (SSP) in high lipid (C1) and low lipid (C2) capelin during drying and smoking (n=3; Mean±SD). RM = raw material; P-T = pre-treated; –BR = brined; -BL = blanched; -H = hot smoking; -C = cold smoking. Extraction of the protein fraction was performed in 1M salt solutions.

As water content is reduced during drying (Table 5.3), the distance between protein chains diminishes, altering protein-water interactions and exposing protein molecules to an organic environment that is less polar than water (Rustad & Nesse, 1983). This can result in reduced proteins solubility as a result of a tighter network (cross-linkages) formed, perturbing protein structure and integrity with the formation of protein aggregates (Wang et al., 2010; Fennema, 1977). Reduced protein solubility may also be linked to lipid oxidative products that form complex interactions through cross-linking with protein components (Underland et al., 1999). Secondary lipid oxidation products were higher in the start of drying, decreasing as drying progressed especially in blanched fish (Figure 5.3B).

Protein solubility was less influenced by brining and was apparently stable during smoking in both groups except for a slight increase during the first two hours of smoking. Significantly lower solubility was obtained in the batches towards the end of hot smoking (Figure 5.5 B). The slight increase in solubility during the first two hours of smoking might be due to protein hydrolysis (caused by enzymatic activity) into components of less molecular weight (Stoknes et al., 2005).

In general, these results indicate that during early smoking when the temperature in both cold and hot smoking was around 30°C, slight protein denaturation occurred and the difference in protein solubility between the batches could be accounted for by differences in lipid content.

Lipid seems to have a protective effect on protein as protein solubility was highest in fatty capelin. This may be due to lipid's low heat transfer coefficient, leading to protection of the proteins from heat during blanching, drying and smoking. Dyer & Dingle (1961) reported a rapid decrease in protein extractability in smoked lean fish (< 1% fat) than fatty fish (3-10% fat). Lipid acted as a barrier to salt uptake in the current study with fatty fish absorbing less salt than less fatty fish (Table 5.3). Salt is known to have a major effect on fish protein solubility and water holding capacity (Nguyen, Thorarinsdottir, et al., 2011) and could have contributed to low protein solubility especially as drying progressed in blanched brined capelin.

Decrease in protein solubility (SSP) during hot smoking was confirmed in both batches after 2 hours (Figure 1 B), when the temperature was ≥40°C and significantly during the last stage of hot smoking that lasted 30 minutes at 75°C. Since protein solubility was stable during cold smoking, the results imply changes in solubility observed during hot smoking were influenced by temperature rather than salt that was less than 5% in the groups. A significant positive correlation was obtained between SSP and water content (Table 3, **Paper III**) during drying and smoking confirming that protein solubility decreased with decreasing water content.

5.2.3 Changes in sulfhydryl (SH) groups and disulfide bond content

Total sulfhydryl content

Total sulfhydryl (SH) content of the soluble proteins decreased during drying and smoking, except during cold smoking where it remained relatively stable (Figure 5.6). The decrease in total SH content is thought to be due to the exposure of sulfhydryl groups buried in native proteins making them vulnerable to oxidation (Nguyen et al., 2011; Rawdkuen et al., 2010; Zuazaga et al., 1984). Masking of sulfhydryl groups by protein aggregates formed due to dehydration could have also contributed.

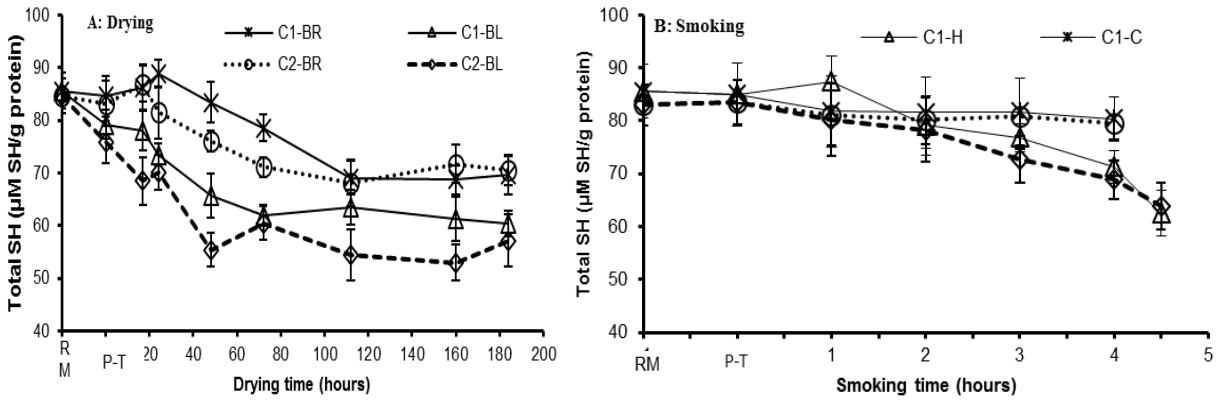

Figure 5.6 Changes in total sulfhydryl content in high lipid (C1) and low lipid (C2) capelin during drying and smoking (n=3; Mean±SD). RM = raw material; P-T = pre-treated; –BR = brined; -BL = blanched; -H = hot smoking; -C = cold smoking. Extraction of the protein fraction was performed in 1M salt solutions.

Brined fatty capelin obtained higher and blanched less fatty lower total SH content than other treatments. The brined fatty capelin with the higher SH content had consequently lower disulfide bond content than blanched less fatty fish (Figure 4 A, **Paper III**). Blanching exposes sulfhydryl groups buried in native proteins to reactive oxygen or secondary oxidative by-products, whereas lipid offers protection against denaturation that causes the exposure of sulfhydryl groups to reactive oxygen or by-products of lipid oxidation. Blanching then triggers oxidation of sulfhydryl groups with the formation of hydrogen and hydrophobic bonds (disulfide interchanges) (Baylan et al., 2015; Raman & Mathew, 2014; Hsu et al., 2007).

Smoke contains phenolic compounds that have antioxidant properties (Guillen & Errecalde, 2002) implying that changes in sulfhydryl content obtained during hot smoking (Figure 5.6) may have been caused by aggregation of proteins rather than oxidation factors. Hot and cold smoking of capelin resulted in reduced lipid oxidation. (**Paper IV**). It can therefore be postulated that masking of sulfhydryl groups by protein aggregates could have decreased the total sulfhydryl content during hot smoking, since the content was relatively stable in indentured capelin proteins during cold smoking. Significant correlations were obtained between SH and water content during drying and smoking, and also between SH and temperature during smoking (Table 3, **Paper III**), supporting the explanation that sulfhydryl groups might have been masked by

protein aggregates. The influence of lipid content was not pronounced during smoking since groups within the same smoking method were not significantly different.

Available sulfhydryl content

Available SH content increased after blanching, during early drying of brined capelin and during smoking, but raised most after blanching and towards the end of hot smoking (Figure 3, **Paper III**). Proteins were denatured during blanching exposing the sulfhydryl groups that are unavailable (buried in native proteins) thereby increasing the proportion of available SH in the total SH content. The increase in available SH content in brined capelin during early drying may be related to protein hydrolysis caused by enzymatic activity or the groups increased salt content in the fish muscle resulting in protein swelling (salting-in effect), exposing SH groups (Nguyen et al., 2011; Baylan et al., 2015). It may also be due to changes in the reactive groups, mainly loss of hydrophilic surface and exposure of hydrophobic areas (Raman & Mathew, 2014). Available SH content in blanched brined capelin decreased during early drying, stabilizing towards end of drying in all groups. The decrease in available SH was related to the masking of sulfhydryl groups by protein aggregates.

During smoking, available SH increased but significantly during hot smoking when temperature was higher than 40°C (Figure 3, **Paper III**). Although high temperature during hot smoking exposed SH groups buried in native proteins, the reactive SH groups were less oxidized and underwent minimal disulfide interchanges as revealed in disulfide content changes that were not significantly different during smoking (Figure 4 B & Table 3, **Paper III**). This can be explained by the antioxidant properties of the smoke (Guillen & Errecalde, 2002). Generally available SH content correlated strongly with disulfide content.

Disulphide bond content

The disulfide bond content was mainly influenced by raw material treatments, with blanched brined capelin obtaining relatively higher content (Figure 4 A, **Paper III**). Disulphide content increased after blanching, whereas brining had no effect. Only a slight increase in disulphide content was observed during early drying. The disulphide content remained apparently stable during later stages of drying. The results are in agreement with total SH content in that higher

disulphide content was obtained in blanched brined capelin, that had lower total SH content and vice versa in brined capelin. This is because sulfhydryl groups get oxidized when they are exposed and subsequently undergo disulfide interchanges (Baylan et al., 2015; Raman & Mathew, 2014; Hsu et al., 2007), resulting in reduced total SH content and increased disulphide content.

The results suggest that as proteins aggregate after blanching, disulfide bonds increase and the total SH content is reduced. Disulfide bond content became stable in the later stages of drying, corresponding with the stability in SH groups. Although lipids had some effect on the available SH and disulfide content during drying and smoking of capelin, the effect was not significant considering changes in disulphide bond content.

5.2.4 Summary

- SSP and total SH content were reduced while available SH and disulfide content increased during drying and hot smoking.
- Blanching reduced protein solubility and SH content due to high protein aggregation during drying that might have reduced the eating quality of dried fish mainly due to change in texture.
- Conformational changes occurred in early drying when water content and dehydration rate were relatively high.
- Protein conformation in particular during hot smoking was attributed to protein denaturation that resulted in reduced yield and possibly loss in nutritional value.
- Lipids have a protective effect on capelin proteins as less conformational changes were observed in fatty fish after blanching and during drying and smoking.
- Blanching and drying had greater influence on protein conformational changes than brining and smoking.

5.3 Stability of dried and smoked capelin and sardine

Capelin harvested at three different times and sardine was dried and smoked, and used to evaluate the effects of lipid content and packaging methods on stability of dried and smoked fish. Quality changes in hot and cold smoked fish were evaluated during refrigerated storage of fatty and less fatty capelin batches and sardine, packaged in sealed (air) and vacuum packages (**Paper IV**). Changes the three batches of dried capelin (fatty, moderate and less fatty) stored in open, sealed and vacuum packages at room temperature were evaluated (**Paper V**).

5.3.1 Influence of lipid content and smoking methods on fish stability

Smoked fish

Lipid content and smoking method had no influence on salt concentration (3.18- 3.87%) (Table 1 A, **Paper IV**). Salt is important as in addition to the preservative effects, it inhibits growth of food poisoning organisms, particularly *C. botulinum,* that is of particular concern in vacuum packaged fish. Lund & Peck (1991) reported a concentration of 3% to be the minimum level needed to inhibit the growth of *C. botulinum* type E. Smoked fish had water activity values of 0.944-0.953 (Table 5.4 A), that are below the critical level of 0.97 for the formation of botulinum toxin (Lund & Peck 1991). It can also be mentioned that for health reasons salt concentration should be kept low in food and salt level of 4% was found to be acceptable (Cardinal et al., 2001). Capelin and sardine in this experiment had salt content of 3.18- 3.87% and met that criterion.

Lipid content increased in all treatments after smoking compared to raw materials, with highest increase in hot smoked fish (Figure 1, **Paper IV**). The increase in lipid was also influenced by the initial lipid level as the greatest increase was in fatty capelin. No changes in lipid content occurred during storage. Similar results with nominal variations in total lipid were reported during storage of dried fish (migaki-nishin) (Shah et al., 2009) and sardine stored in ice (Chaijan, 2009).

Fresh capelin batches differed in raw phospholipids (PL) content. Both differed from sardine PL, with sardine and less fatty capelin having relatively high PL proportion (Table 5.4). The main lipid components in fish are triacylglycerols and PL. Diet of the fish affects the proportion of PL by either increasing or decreasing triacylglycerols (Standal et al., 2010; Burri et al., 2012), thereby increasing or decreasing total lipids (Mørkøre et al., 2007) and explaining why PL content is relatively stable. All groups had low proportion of PL indicating that majority of the lipid in capelin and sardine is in the form of triglycerides. PL hydrolysis occurred during smoking, with accelerated hydrolysis in hot smoked fish. This might be due to the greater activities of phospholipases at high temperatures (Chaijan et al., 2006; Shah et al., 2009). PL generally declined during storage. Hydrolytic activity was highest in less fatty capelin and sardine that had relatively high proportion of PL (Table 5.4). However, during storage, hot smoked fish had a reduced lipolytic enzymatic activity.

Table 5.4 Phospholipids changes in smoked fish as a function of storage time based on Duncan multiple range tests. ± = standard deviations (n=3; Mean±SD).

Storage time (days)		**2**	**10**		**16**		**22**		**28**	
Group	**RM**	**AŦ**	**Air**	**Vac**	**Air**	**Vac**	**Air**	**Vac**	**Air**	**Vac**
C1H	5.8±0.5	4.9±0.5	4.7±0.4	5.0±0.1	4.3±0.8	4.4±0.5	4.0±0.9	4.5±0.0$^{\Delta}$	4.4±0.3	3.7±0.2
C1C* **	5.8±0.1^{a}	5.2±0.6^{ab}	4.1±0.6^{b}	4.3±0.7^{b}	3.6±0.0^{c}	4.2±0.6^{bc}	2.3±0.5^{d}	2.7±0.7^{d}	1.7±0.2^{d}	1.9±0.5^{d}
C2H*	10.2±0.2^{a}	7.4±0.4^{b}	5.7±0.3^{c}	7.2±0.2^{b}	5.2±1.1^{c}	5.7±0.3^{c}	5.1±0.1^{c}	5.6±0.2^{c}	5.5±0.3^{c}	5.8±0.7^{c}
C2C* **	10.2±0.2^{a}	8.2±0.5^{b}	6.3±0.4^{c}	6.5±0.6^{c}	6.1±0.2^{c}	6.0±0.8^{c}	2.0±0.6^{d}	2.1±0.4^{d}	2.0 ±0.4^{d}	2.1±0.2^{d}
SH**	6.7±0.0^{a}	4.8±0.1^{b}	3.8±0.0^{c}	3.0±0.1^{c}	3.3±0.7^{c}	3.3±0.6^{c}	2.3±0.4^{cd}	3±0.0^{c}	2 ±0.1^{d}	2.6±0.3^{d}
SC**	6.7±0.0^{a}	5.6±0.2^{b}	2.9±0.3^{c}	3.0±0.1^{c}	2.4±0.4^{ab}	2.3±0.0^{ab}	1.1±0.3^{c}	1.6±0.1^{ac}	1.2±0.2^{c}	1.7±0.5^{ac}

RM = Raw material; A^{T} = Analyses before packaging; Air = Air packaged; Vac = Vacuum packaged; C1 = high lipid capelin; C2 = low lipid capelin; S = sardine; H = hot smoked; C = cold smoked; $^{\Delta}$ Values equal to 0.0 are values less than 0.05; ***** significant difference at a level $p < 0.05$; ******* significant difference at a level $p < 0.001$. Different letters (superscript) indicate significantly different values between samples within a row.

Fresh capelin had lower proportion of free fatty acids (FFA) than sardine (Figure 2, **Paper IV**), indicating that hydrolysis of glycerol-fatty acid esters occurred to some extent during post-catch handling of sardine. The sardine was obtained from artisanal fishermen that did not chill the fish. Increase in FFA during storage was highest in cold smoked fish and in less fatty capelin and sardine that had high PL hydrolytic activity. This suggests that temperature of 75°C for 30 minutes during hot smoking may have denatured most lipolytic enzymes that are responsible for lipid hydrolysis with the liberation of FFA. Generally, FFA evolution was in agreement with the PL hydrolytic trends. Whilst, FFA increased progressively during storage, PL decreased. The

results demonstrate rapid development of FFA during chilled storage of cold smoked fish as well as fish with high PL ratio.

Content of lipid oxidation indicators (PV and TBARS) was different between groups of fresh fish. Fatty capelin had higher values than less fatty capelin (Figure 5.7; Figure 3 D-F, **Paper IV**). Sardine had unexpectedly the highest PV value (313µmol/kg) despite low lipid content, but did not differ from the capelin groups in TBARS (Figure 3 D-F, **Paper IV)**. It is likely that faster lipid oxidation occurred during post-catch handling of sardine as earlier discussed with PL and FFA content.

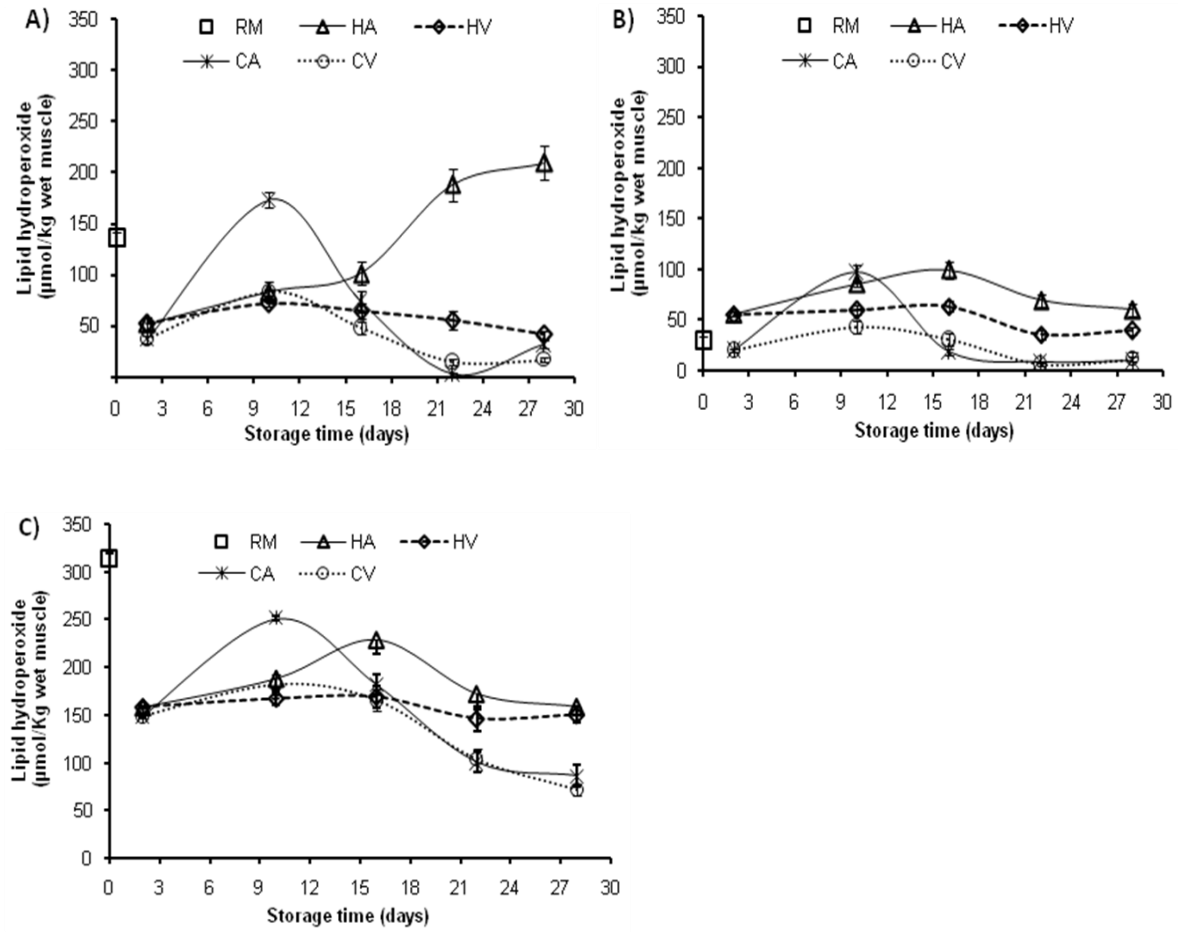

Figure 5.7 Lipid hydroperoxides development (A, B & C) in capelin (high lipid (C1) =A; low lipid (C2) = B) and sardine (S = C) muscle lipid (RM = raw material) upon smoking and chilled storage of hot smoked (Air = HA & vacuum = HV) and cold smoked (Air = CA & vacuum = CV) packaged (n=3).

PV and TBARS decreased in all groups unlike PL and FFA except for PV in less fatty capelin (C2) that had the least PV in raw fish. Although smoking entails high temperature generally known to accelerate lipid oxidation (Marc et al., 1997), results show the influence of temperature on lipid oxidation during smoking may have been surpassed by the effects of smoke antioxidant properties (Guillen & Errecalde, 2002). But temperature differences and water content during hot and cold smoking, contributed to the differences in PV and TBARS obtained in the groups. Hot smoked fish was more dehydrated and thus saturated with lipid than cold smoked fish.

Cold smoked sardine and fatty fish were more vulnerable to lipid oxidation during storage shown by the fast accumulation and decomposition of hydroperoxides and TBARS. The vulnerability to lipid oxidation during chilled storage of cold smoked fish was in harmony with the changes in phospholipids and FFA. There was an elevation of PV and TBARS during early storage of sardine, probably due to the high content in fresh fish (Chaijan et al., 2006). Generally less fatty capelin that had high phospholipid content accumulated less PV and TBARS throughout the storage time.

The aerobic plate count in raw fish was log 2 - 2.7 CFU/g and 4 CFU/g for capelin groups and sardine respectively (Figure 5.8). Decline in total plate counts was observed after smoking in all groups and under limit of detection in hot smoked fish (log 1 CFU/g). This occurrence could be attributed to the effects of dehydration and antimicrobial properties of the smoke constituents (Rorvik, 2000) besides the high temperature during hot smoking. Microbial counts increased in all groups during storage. Lipid content had no influence on microbial development of smoked fish.

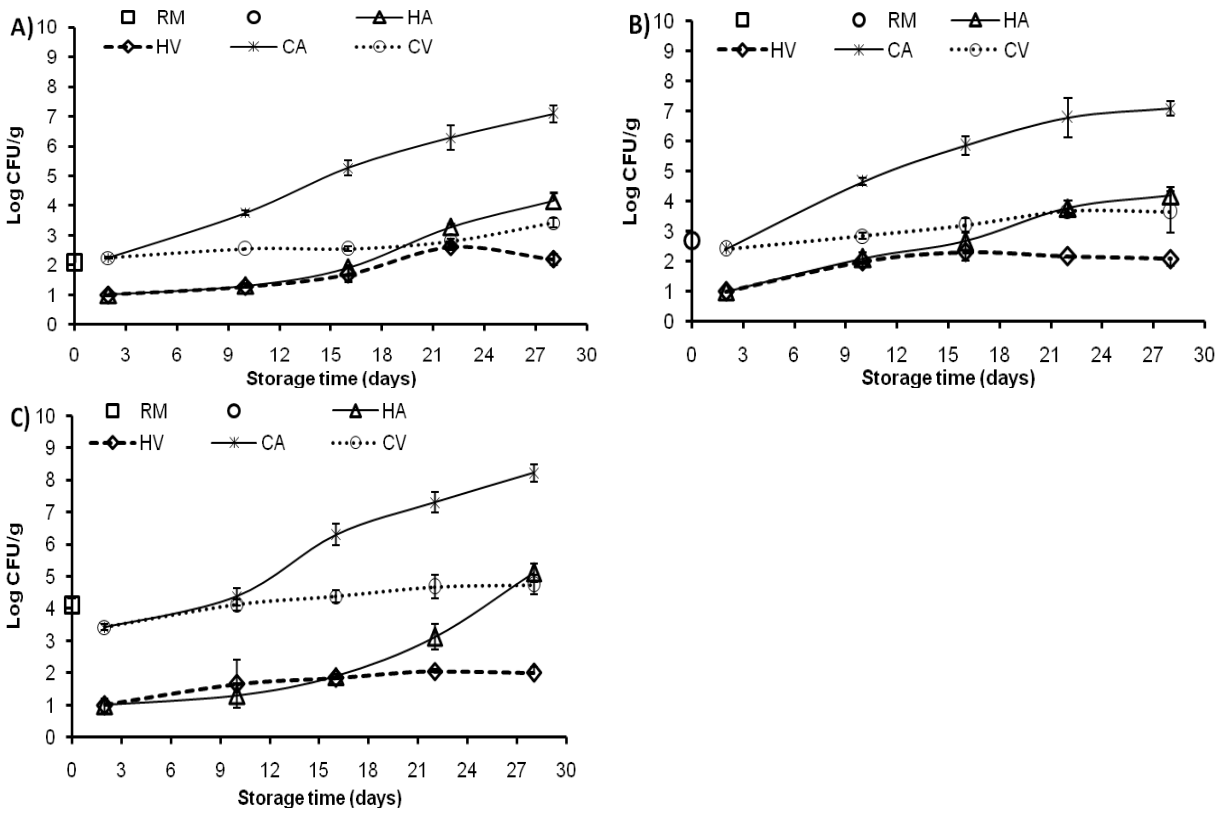

Figure 5.8 Total plate count in capelin (A =high lipid (C1) & B = low lipid (C2)) and sardine (C = S) fish (RM = raw material) upon smoking and chilled storage of hot smoked (Air = HA & vacuum = HV) and cold smoked (Air = CA & vacuum = CV) packaged (n=3).

Dried fish

Dried capelin batches differed significantly in lipid content with values of 31%, 28% and 25%, and corresponding water activity of 0.758, 0.683 and 0.676. Water activity was < 0.80 which is sufficient to inhibit growth of harmful bacteria and ensure product safety (Kilic, 2009). Lipid and water content of dried packaged capelin did not change during storage. Monoenic (MUFAs) fatty acids constituted the majority FAs of total lipid in capelin after drying followed by saturated (SFAs) and polyunsaturated fatty acids (PUFAs) (Table 1, **Paper 5**). Proportions of PUFAs were significantly higher in less fatty and moderate fatty compared to fatty capelin. This can be explained by higher proportion of PL with lower fat content.

Proportion of phospholipids (PL) was significantly lower with increased lipid content of dried capelin being 25%, 15% and 8% of total lipid in less fatty, moderate fatty and fatty capelin

respectively (Figure 5.9 A, B & C). Phospholipids are membrane lipids with relatively constant absolute content, explaining why the proportion of PL is higher in less fatty than fatty capelin. PL hydrolysis in dried capelin was rapid in all batches during early storage and most rapid in less fatty capelin with the highest proportion of PL (Figure 5.9 A, B & C). The relatively higher PL content at the beginning of storage declining as storage progressed explains the reduced rate of hydrolysis with time. Rapid PL hydrolysis in less fatty capelin may be attributed to the occurrence of substrate specific enzymes such as phospholipases that catalyze cleavage of hydrophilic phospho-diester bonds but not of lipophilic triacylglycerols. Also, a higher proportion of the highly susceptible PUFAs especially n-3 PUFAs in PL than triacylglycerols (Parmentier et al., 2007) can explain the difference in rate of hydrolysis, why rapid hydrolytic activities occurred with high PL content.

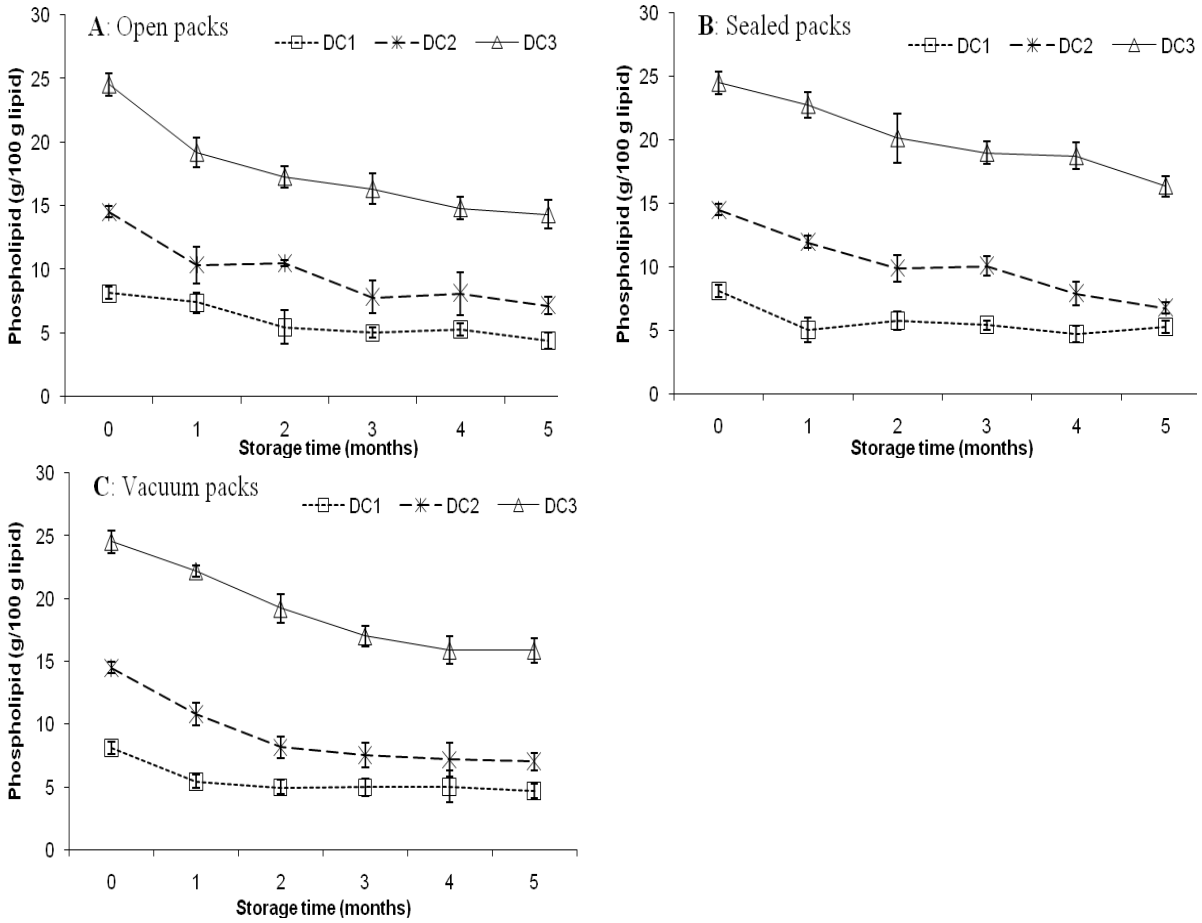

Figure 5.9 Changes in phospholipid (A, B & C) in packaged dried capelin during 5 months of storage at ambient temperature (n=4). DC1= dried capelin of high lipid; DC2 = dried capelin of moderate lipid; DC3 = dried capelin of low lipid.

Free fatty acids (FFA) content was highest in dried capelin with the lowest fat content and highest proportion of PL (Figure 2 D-F, **Paper V**). FFA evolution in dried fish during storage was in agreement with smoked fish results (Figure 2, **Paper IV**), in that FFA increased in all groups and most rapidly during early storage and in less fatty batch. Moreover, FFA content increased while PL content declined ($r = -0.54$) indicating that FFA evolution was mainly due to PL degradation. The evolution trends of FFA in different groups can be explained by the corresponding PL hydrolysis.

Similar to smoked fish, lipid content affected lipid oxidation in dried fish. PV developed fastest and TBARS were highest in fatty capelin (Figure 5.10; Figure 3 D-F, **Paper V**). Generally PV increased whilst TBARS decreased during storage. Hydroperoxides are known to accumulate during the initial oxidation process but decrease later as the rate of cleavage and reactions exceed their formation (**Paper IV**). On the other hand the decrease in TBARS during storage may be explained by the low decomposition of hydroperoxides into secondary products of lipid oxidation as PV progressively increased ($r=-0.27$). The interactions of malonaldehyde with protein components to give tertiary lipid oxidation products might have contributed to decrease in TBARS (Nguyen et al., 2012; Underland et al., 1999).

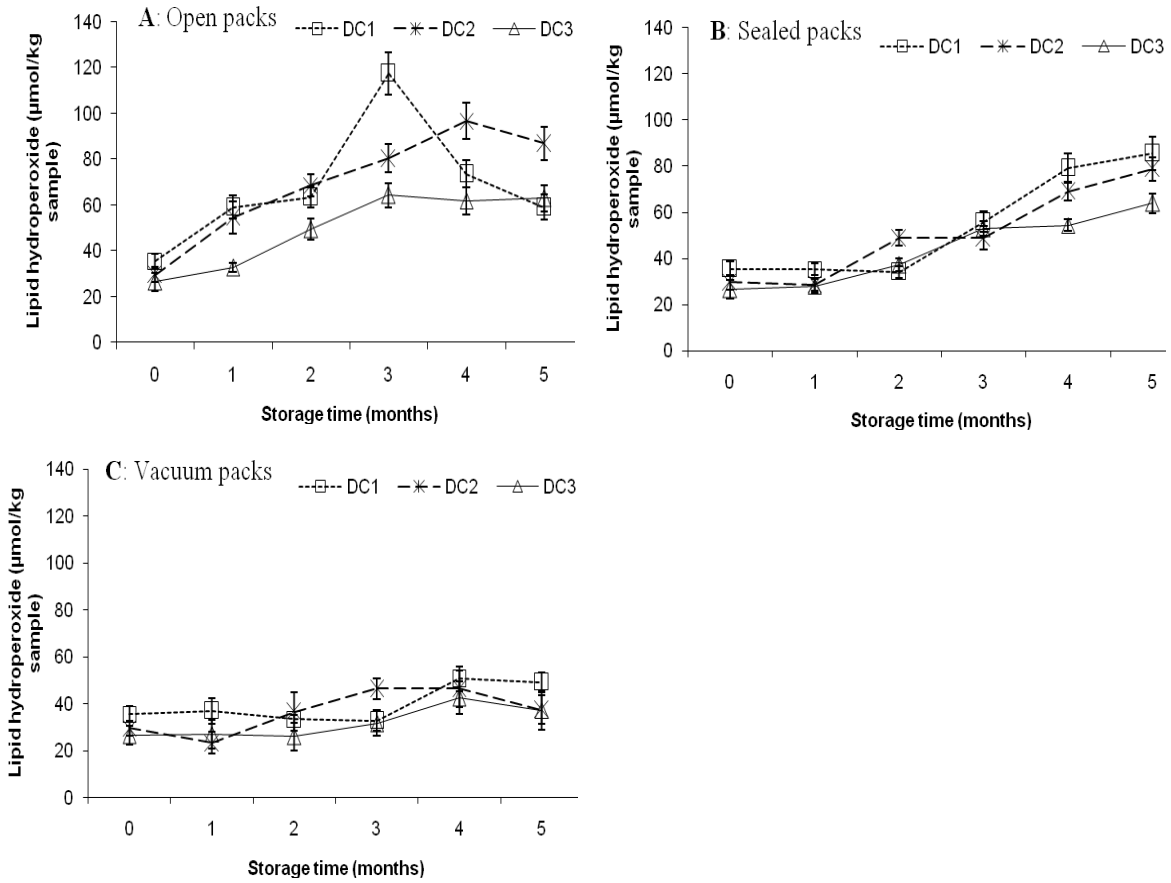

Figure 5.10 Lipid hydroperoxides development (A, B & C) in packaged dried capelin during 5 of months storage at ambient temperature (n=4). DC1= dried capelin of high lipid; DC2 = dried capelin of moderate lipid; DC3 = dried capelin of low lipid.

Slow microbial growth was observed during storage of dried capelin with TPC increments of less than log 1 colony-forming unit by the end of five months storage. At the beginning of storage, total plate count (TPC) was log 5.48, 5.24 and 5.38 CFU/g in fatty, moderate fatty and less fatty capelin respectively. Slow microbial growth during storage may be explained by the low water content (< 25 %) and water activity (< 0.80) in dried capelin (Kilic, 2009). Lipid content like in smoked fish study had no effect on TPC development. Yeast and molds were generally not detected in the dried capelin except after extended storage in open packages in fatty and moderate fatty fish.

5.3.2 Influence of packaging methods on fish stability

Smoked fish

Packaging method had as expected no influence on phospholipids (PL) and FFA during storage (Table 5.4 and Figure 2, **Paper IV**). PL hydrolysis occurs mainly due to enzymatic activities (Chaijan et al., 2006) that are not influenced by access to oxygen. The increase in FFA is mainly derived from PL hydrolysis.

Packaging methods had significant effects on lipid oxidation during storage of smoked fish (Figure 5.7; Figure 3 D-F, **Paper IV**). Lipid oxidation was more intense in open packaged than vacuum packaged capelin. Vacuum packaging limits access to molecular oxygen thereby preventing lipid oxidation. Packaging methods and lipid content influenced FA composition during storage (Table 2, **Paper IV**). Fish lipids are easily oxidized because of high proportion of PUFAs (Stołyhwo et al., 2006). PUFAs declined during storage. More rapid decline was observed in air packed than vacuum packed fish. This related well with the formation and decomposition of lipid oxidation indicators suggesting the losses might be due to oxidation. Vacuum packaging can ensure smoked products stability from lipid oxidation, but not enzymatic related lipid hydrolysis.

A lag phase in bacterial growth was observed in all packaged smoked fish groups during early storage (Figure 5.8), probably due to cold shock on the microbes and also the occurrence of antimicrobial smoke constituents. During late storage high microbial growth was evident in air packages, attributed to the diminishing intensity of antimicrobial smoke constituents, presence of oxygen and re-establishment or succession by cold loving bacteria. Total counts reached log 7 CFU/g & log 4 CFU/g in cold and hot smoked air packaged capelin groups and log 8 CFU/g & log 5 CFU/g in cold and hot smoked sardine respectively at the end of storage. Aerobic counts of log 5 CFU/g has been used in smoked fish as limit for consumption (Leroi et al., 2000; Hansen et al., 1995). This indicates air packaged cold smoked fish in all batches had surpassed the limit for consumption on day 28 during chilled storage. On the other hand aerobic counts in air packaged hot smoked capelin groups did not reach log 5 CFU/g consumption limit on day 28, while hot smoked sardine had reached the limit on day 28.

Vacuum packaged groups showed delayed growth ($p<0.05$) obtaining lower aerobic counts throughout the storage not reaching log 5 CFU/g consumption limit. This could be due to low O_2 level, retention of smoke with antimicrobial properties and to a lesser extent the low storage temperature used in the study that may have inhibited aerobic micro-flora development.

Dried fish

Phospholipid hydrolysis occurred during storage of packaged dried capelin, but was not influenced by packaging methods (open, sealed and vacuum) (Figure 5.9 A, B & C). Supporting the earlier explanation that PL hydrolytic processes were largely driven by enzymatic activities. Similarly FFA evolutions obtained in dried capelin was not influenced by packaging methods (Figure 2 D-F, **Paper V**). Generally, the results are in agreement with those of smoked fish that lipid hydrolysis was mainly influenced by PL content.

Packaging methods affected the lipid oxidation, with accelerated oxidation occurring in dried fish with access to oxygen (Figure 5.10; Figure 3 D-F, **Paper V**). Peroxide value increased during storage in open and sealed bags except towards the end of storage when it decreased in the fatty and moderate fatty capelin in open bags (5.10 A, B). Vacuum packaged dried capelin was stable during storage (Figure 5.10 C).

Degradation of TBARS in dried capelin was higher when fish is packaged in open and sealed bags than vacuum bags, but with an increase in open bags towards the end of storage (Figure 3 D-F, **Paper V**). Increase in TBARS in dried fish packaged in open bags towards the end of storage may be attributed to faster decomposition of existing hydroperoxides into TBARS since the corresponding PV were decreasing. Packaging methods had therefore some impact on TBARS decomposition with capelin being more stable when vacuum packaged than in open and sealed bags. The results agree with PV data in explaining the importance of limiting access to oxygen to minimize lipid oxidation.

Packaging method influenced microbial development in dried capelin as the lowest total plate counts (TPC) were found in vacuum packaged capelin, a fact attributed to the air elimination retarding growth of aerobic microorganisms. As earlier observed yeasts and molds were generally not detected in dried capelin, except in fatty and moderate fatty capelin after extended

storage in open bags. This may in addition to the low water content be explained by the drying conditions and packaging methods used. Indoor drying, sealing and vacuum packaging minimized contamination by microbes mainly air borne yeasts and molds (Park et al., 2014). The results indicate that vacuum packed dried capelin was microbiologically stable during storage.

Lipid content and packaging methods affected rancidity (Table 2 A & B, **Paper V**). A stronger rancid odor was detected in fatty as well as open packaged samples. These results are in agreement with primary lipid oxidation indicator showing a positive correlation ($r = 0.67$ with PV). Rancid odor was below 20 on the scale of 0-100, a limit that has been used to indicate that samples are becoming rancid (Magnússon et al., 2006). The stock-fish odor was slightly reduced as storage time progressed, with more reduction in fatty and open packaged capelin (Table 2 B, **Paper V**). The changes were however, not significantly different ($p > 0.05$) between groups (lipid levels, packaging methods) during storage. This can be explained by slow accumulation of TBARS with time but they have a profound impact on food sensory properties (Stapelfeldt et al., 1997). Stock-fish odor was inversely correlated to PV and TBARS ($r = -0.22$ and -0.21 respectively).

To gain an overview of the similarities and differences between sample groups over storage time principal component analysis (PCA) was carried out (Figure 5.11). Microbial and fatty acid composition data analyzed at the beginning, middle and the end of storage were not included in the model. The samples varied mainly in phospholipid, total lipid, water content and rancidity along the first principal component (PC-1), explaining 49% of the variations in the samples. Less fatty (DC3) samples were located to the left of the scores plot along PC-1 described by phospholipid and stock-fish odor variables whereas fatty (DC1) samples were located on the right side described mainly by the total lipid, water content and rancid odor. The variables describing less fatty were negatively correlated to those that described fatty capelin. Capelin that had moderate lipid content were centrally located implying they were marginally described by the variables that described both less fatty and fatty capelin.

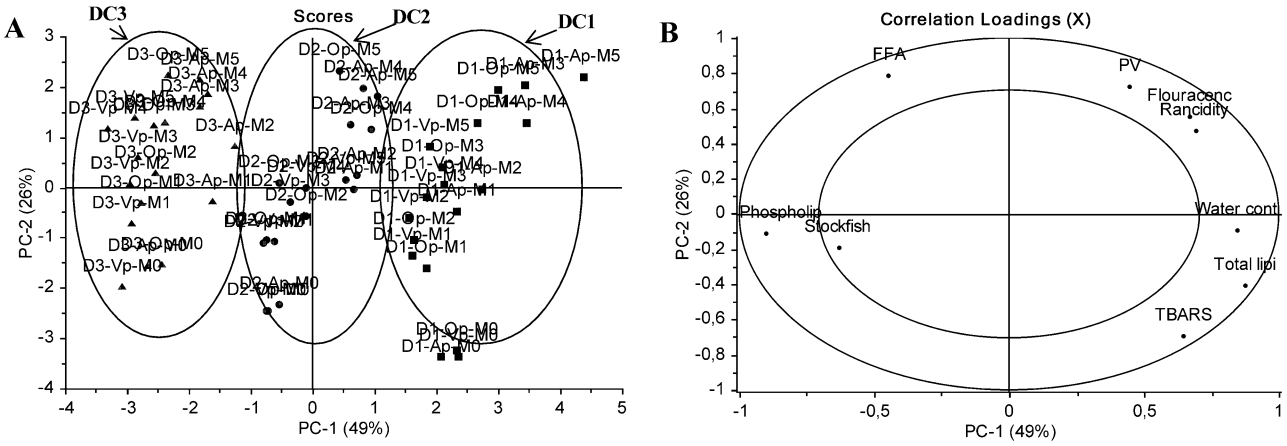

Figure 5.11 Principal component analysis (A) scores and (B) correlation loadings describing dried capelin samples based on parameters evaluated at all sampling points. PC1 49% vs. PC2 26%. DC1 = dried capelin of high lipid; DC2 = dried capelin of moderate lipid; DC3 = dried capelin of low lipid; Op = open packs; Ap = air packaged; Vp = vacuum packaged; M = month of storage.

Samples also varied in FFA, PV and TBARS along the second principal component (PC-2) accounting for 26 % of the total variation. Samples located on the lower side of PC-2 were described by early storage and TBARS but also total lipid while samples on the upper side were described by later storage and FFA, PV, and fluoresce (Figure 5.11). TBARS were negatively correlated with the variables that described samples during later storage. The influence of packaging methods was not clearly portrayed in the model.

5.3.3 Summary

- Lipid degradation was greater during hot smoking than cold smoking of fish (capelin and sardine), but hot smoked fish was more stable during chilled storage.
- Capelin and sardine are valuable sources of essential PUFAs, but lipids in smoked sardine are less stable during chilled storage than lipids in smoked capelin (all batches).
- The spoilage of lipids in dried and smoked fish was both due to oxidation (PUFAs and oxygen) and hydrolysis (enzyme activity).
- Lipid hydrolysis was fastest in the lowest lipid capelin, while lipid oxidation was relatively higher in fatty capelin.
- All capelin batches were of medium lipid content (7.5- 10 %) and a stable safe dried product at room temperature with water activity of less than 0.70 could be produced from the raw materials.
- Dried and smoked fatty capelin was more stable during storage if oxygen in the atmosphere was excluded by vacuum packaging.

5.4 Marketing potential of dried capelin and sardine

Improved dried sardine (water content 24%, fat 9%) and indoor dried capelin (water content 19.5%, fat 27%) packaged in sealed polyethylene bags weighing 500 g each were used in a marketing study carried out among shoppers in open-air markets and supermarkets in Kenya.

5.4.1 Dried fish consumption patterns

Majority of the respondents shopping in open-air markets (72%) had only elementary education, followed by high school level (28%) with none of the respondents having obtained a university degree (Table 1, **Paper VI**). Majority of respondents shopping in supermarkets had completed high school (63%), followed by those with a university degree (30%) and elementary education (7%).

Respondents shopping in open-air markets consumed dried fish more frequently (33%, more than or equal to four times a week) than those shopping in supermarkets (5%, four times a week or more often) (Table 5.5). This indicates that shoppers in open-air market consume dried fish on a regular basis. Consumption of dried fish was negatively influenced by education, with high consumption frequency among less educated consumers (Table 5.5). Educated consumers are generally more aware of the health and other benefits associated with fish consumption. In a study on preferences for fish and seafood using an 'evoked set' analysis education was found to positively influence consumers preference for fish (Kinnucan et al,. 1993). Health benefits are however questionable when it comes to products of low quality that are contaminated. Kenyan consumers consider dried small fish an inferior/low quality product sold mainly in rural areas (Peter Oduor-Odote et al., 2010), where a majority of the population are poor with limited education. A majority of respondents shopping in the supermarkets are most likely middle class as they were mainly working for private companies or government and had relatively small households (Table 1, **Paper IV**). Therefore, they are able to purchase more expensive protein sources than dried fish whose quality is uncertain and not often available in the supermarkets.

Table 5.5 Dried fish consumption pattern among respondents at the coast of Kenya divided by shopping location and education level.

Education level/shopping location	% respondents consumption frequency					
	Less than once a month	Once a month	2-3 times a month	Once a week	2-3 times a week	More often
Elementary education	4.3	6.4	14.9	10.6	29.8	34
Secondary education	22.2	5.6	38.4	11.1	11.6	11.1
University degree	25.6	20.9	18	18.2	8.2	9.1
Village markets	1.7	8.3	10	16.7	30	33.3
Supermarkets	30	8.3	33	11.7	11.7	5

5.4.2 Acceptability rating

The products were generally well received with acceptability rating scores ranging between seven and eight (Table 5.6). Although the overall acceptability values were relative for both products, respondents shopping in open-air markets rated both products higher than those shopping in supermarkets. Open-air shoppers rated the products higher as they are familiar with and regular consumers of dried fish. The result is in agreement with a study by Boutrolle et al. (2005) who reported that greater familiarization with a product resulted in higher acceptability ratings. Dried capelin had significantly ($p<0.05$) higher appearance rating irrespective of the respondents' shopping location. On the other hand, sardine obtained the highest flavor rating. Texture was not significantly different ($p>0.05$) between the two locations, with a higher rating for capelin than sardine. Capelin appears to be attractive to consumers but the flavor was moderately ranked and needs to be improved. Both dried capelin and improved processed sardine were acceptable in the Kenyan markets and could in general be accepted in East African markets accustomed to dried small fish.

Table 5.6 Product acceptability of average (Std. Error) values on 9-point hedonic scale ranging from 1 (dislike extremely) to 9 (like extremely) (n=60)

Sensory attribute	Capelin		Sardine	
	Open market	Supermarket	Open market	Supermarket
Overall acceptability*	8.0 (0.2)[a]	7.1 (0.3)[b]	7.7 (0.2)[a]	7.0 (0.2)[b]
Appearance***	8.4 (0.2)[a]	7.8 (0.2)[b]	6.8 (0.2)[c]	6.8 (0.2)[c]
Flavour***	6.7 (0.2)[a]	6.3 (0.2)[a]	7.6 (0.2)[b]	7.4 (0.20)[b]
Texture	7.2 (0.2)	7.4 (0.2)	7.0 (0.2)	7.1 (0.2)

[a] Different letters (superscript) indicate significantly different values between samples within a row. *p < 0.05, ***p < 0.001.

Most respondents shopping in the supermarkets commented that poor quality dried products (contaminated with soil) and unavailability in national retail stores limited dried fish consumption among the group. Even though the majority of respondents shopping in supermarkets were irregular dried fish consumers, they were familiar with dried small fish. This implies that they might accept new dried fish products such as capelin if quality could be improved. Gender, religion and household size had no significant influence on product acceptability.

5.4.3 Willingness to buy

Consumers were willing to buy the products irrespective of the shopping location (Table 5.7). The average values of willingness to buy capelin and sardine were relatively high and very close, but those shopping in open-air markets were more willing ($p<0.05$) to purchase the products than those shopping in supermarkets. This may be because consumers shopping in open-air markets were more familiar with dried fish.

Table 5.7 Willingness to buy (1 = "very unlikely" to 9 = "very likely") capelin and sardine at specified amount (I USD =90 KES), average values (Std. Error)

Willingness to buy	Capelin		Sardine	
	Open market	Supermarket	Open market	Supermarket
Unlikely/likely to buy***	8.3 (0.2)[a]	7.3 (0.2)[b]	8.0 (0.1)[a]	7.2 (0.2)[b]
At KSH 200***	8.6 (0.2)[a]	8.8 (0.1)[a]	7.9 (0.1)[b]	7.8 (0.2)[b]
At KSH 400***	6.7 (0.3)[a]	7.5 (0.2)[b]	6.1 (0.2)[a]	5.2 (0.3)[c]
At KSH 600***	4.0 (0.2)[a]	6.4 (0.2)[b]	3.2 (0.2)[c]	2.1 (0.2)[d]
At KSH 800***	1.4 (0.1)	3.8 (0.3)[a]	1.2 (0.1)	1.2 (0.1)

[a] Different letters (superscript) indicate significantly different values between samples within a row. ***p < 0.001.

On the specific amount of money the respondents were willing to pay for 500 g of the product, open-air markets and supermarkets shoppers had high ratings for both products at KSH 200, but significantly higher rating was obtained for capelin than sardine (Table 5.7). Consumers were not willing to pay more than the reference price of KSH 400 for improved dried sardine, but supermarket shoppers were willing to pay up to KSH 600 for dried capelin (Table 5.7). The unwillingness of consumers to pay more than the reference price for sardine could be that they were not able to see the difference of improved dried sardine from traditionally dried. Sosa and others (2008) reported food product choice and acceptability to be based on sensory properties. Sardine was dried in enclosed drier that depended on weather conditions and may have only reduced contaminations from the environment (such as soil) but not lipid oxidation that affects color development during drying resulting in unattractive products. Also the reference price used in the study was based on the supermarket price of similar product that are believed to be improved and more expensive than traditionally dried sardine sold at about KSH 300 in open-air markets.

5.4.4 Summary

- Education level influenced consumption of dried fish, with more regular consumption among low income classes who had limited education.
- Dried capelin and improved dried sardine received relatively high acceptability ratings.
- The products differed in definite attributes with capelin obtaining high ratings for appearance and texture, while sardine obtained the highest flavor ratings.
- Middle income consumers were willing to pay up to KSH 600 for 500 g of capelin, while consumers class was both not willing to pay more than KSH 400 for improved dried sardine.
- There is market potential for dried capelin and improved processed sardine in the Kenyan markets accustomed to small dried fish.
- Overall quality, flavor and appearance need to be stressed in market promotion to enhance consumption and attract new consumers especially among the middle income class.

6 Conclusions and future prospects

The overall aim of the work was to find ways to improve the quality and safety of dried sardine, and determine acceptability of new products such as dried capelin in markets accustomed to dried small fish.

Dried sardine and capelin with water activity low enough to inhibit harmful microorganisms (<0.7), assure stability during distribution and storage can be produced with blanching and indoor drying. The quality products produced under controlled indoor conditions demonstrate the need for developing practical controlled drier for commercial drying of small fish in Kenya and elsewhere. Fresh water dagaa from Lake Victoria marketed mainly in dried form is an important fishery in east African region with annual landing of more than 500,000 tons in recent years that could supply raw materials for making valuable dried fish products.

Blanching in brine as it is done in traditional drying for ensuring safety affects quality adversely mostly through lipid hydrolysis and oxidation. But it cannot be excluded from the process as the infrastructure for cold chain from fishermen to consumers is lacking with unavailability of ice and potable water. Brining fish for up to two hours at room temperature and in none-potable water to attain appropriate salt concentration is not realistic compared to blanching in brine for 2-3 minutes. Changes in processing might greatly compromise safety but more research is needed to optimize blanching.

Male capelin currently mainly reduced to fish meal and oil can if harvested when fat content is appropriate be used as a raw material for producing dried products. High fat content slows down drying and increases drying time, but at the same time it offers some protection against conformational changes in fish proteins during blanching, drying and smoking. Blanching may not be suitable for commercial capelin drying due to conformational changes and the drying should be conducted at low temperature to maintain protein quality and sensory properties. The effects on tropical fish protein might be different and further studies on the effect of blanching in brine before drying of tropical fish and product acceptance is recommended.

Hydrolysis was fastest in less fatty (7- 7.5% lipid content) capelin but oxidation was fastest in fatty (9- 10% lipid content) capelin. When oxygen in the atmosphere was excluded by vacuum

packaging dried and smoked fatty capelin became stable during storage with less lipid degradation (lipid hydrolysis and oxidation), odor and amount of microbes. A stable dried nutritious product of improved quality can be produced from unblanched fish dried under controlled conditions. Dried fish was more stable while retaining the essential PUFAs during ambient storage when vacuum packaged. All capelin batches used in the study (7- 10% lipid content) could produce a stable safe dried product if vacuum packaged.

Dried and smoked capelin and sardine are rich in essential PUFAs. Cold smoking may be superior to hot smoking when producing capelin for chilled distribution and storage. Hot smoking resulted in greater lipid degradation and dehydration during processing influencing both nutritional value and processing yield. Stability during storage was however greater in hot smoked than cold smoked fish. Lipids in smoked capelin were more stable than in smoked sardine.

The consumers seem to be willing to buy improved dried sardine and indoor dried capelin as both products were well received. Products differed in definite attributes that resulted in high acceptability rating for capelin especially among the consumers shopping in supermarkets. Middle income classes were willing to pay up to KSH 600 for 500 g of dried capelin and sardine at up to KSH 400, while low income classes who consume dried fish on regular basis were willing to buy 500 g of both products at up to KSH 400 which was used as the reference price. The unwillingness of consumers to pay more than the reference price for sardine could be that they were not able to see the difference of improved and traditionally dried sardine. Also the reference price used in the study was based on the supermarket price of similar product that are believed to be improved and more expensive than traditionally dried sardine sold at about KSH 300 in open-air markets. This indicates that consumers, especially among the middle income classes, might well accept new dried fish products if the overall quality could be guaranteed. Even though there is a market potential for dried capelin and improved processed sardine, the exact price for the products was not established. A follow-up study covering a large area is recommended to assess business feasibility prior to commercialization.

In general dried and smoked small fish was found to be nutritious and could contribute to the reduction of malnutrition prevailing in most developing countries where it’s consumed, if quality

and consumption is enhanced. The studies were carried out on capelin of medium lipid content and one sardine batch. It would be more interesting if the capelin batches studied were in the category of fatty, semi-fatty and lean as the lipid content of capelin varies from about 4% to 20% depending on the season.

7 Industrial application

Indoor drying of unblanched fish and controlled smoking, results in the production of nutritious fish products that are consistent in quality and stable during storage. Indoor drying system will eliminate the current dependency on weather conditions during drying as practiced in most developing countries, but the raw materials used must be of good quality highlighting the need to improve post-catch handling in the sardine fishery.

Together with storage temperature, vacuum packaging of dried and smoked capelin, will overcome the problem of when to catch capelin intended for human consumption since its lipid content varies over the catching period (spawning migration in winter) January to March in Iceland. Vacuum packaging assures stability during storage, even though all the batches in the study were of medium lipid content (7-10 %), implying that capelin landed over that catching period can be dried and smoked for human consumption if vacuum packaging is applied.

Although dried and smoked fish quality is related to processing method, it should also be emphasized to processors, distributors and retailers to take advantage of existing opportunity in the use of vacuum packaging technology to maintain quality and extend shelf life to overcome the degradation and marketing challenges of dried and smoked small fish in developing countries. Dried and smoked small pelagic fish are primarily sold in open-air markets and consumed by the low income classes. Vacuum packaging will improve the product presentation and accessibility to national retail stores as well as the retention of nutritional value (PUFAs), increasing consumption of dried and smoked small fish, and also attract new consumers especially among the middle income classes. Marketing of the products in national stores will also raise the value of the fishery thereby creating more employment and higher income along the value chain.

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9 Appendices

PAPER I

I

Influence of blanching treatment and drying methods on the drying characteristics and quality changes in dried sardine (*Sardinella gibbosa*) during storage

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Drying Technology

Under review

Influence of blanching treatment and drying methods on the drying characteristics and quality changes in dried sardine (*Sardinella gibbosa*) during storage

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Abstract

The aim was to examine the drying characteristics of blanched and unblanched sardine during indoor and open sun drying conditions, and the changes in quality of products during storage. High drying rates were obtained in samples at the start of drying, with the highest rate in blanched sardine during open sun drying. Blanching slowed down free fatty acids progression during products storage but it adversely affected colour, lipid composition through oxidation, and sensory properties. Although sardine dried faster under open sun than indoor conditions, it dried for a longer time under indoor drying conditions to attain a stable moisture ratio that was lower than in open sun dried samples. Indoor drying produced a quality stable product with less lipid oxidation and the desired moisture content, lipid composition and sensory properties. Therefore, a stable dried product of improved quality can be produced from unblanched sardine dried under controlled conditions.

Key words: Sardine, dried, blanched, drying rate, quality

1 Introduction

Fish and other marine species give rise to products of great economic importance all over the world. Fresh fish is highly perishable and drying, smoking and salting have traditionally been used for preservation [1]. In many developing countries, dried fish is an important source of low cost and stable dietary protein [1,2]. In East Africa, dried sardines are among the most widespread fishery products with a comparatively long shelf life, sold in small portions to meet the needs of the low income groups [3]. *Sardinella gibbosa* (locally known as 'sim sim') dominate the catches of sardine species landed along the Kenyan coastline (Wakwabi E, unpublished).

Traditionally, sardines are open sun dried on the ground for about 2-3 days depending on weather conditions. Fish dried in open sun are of variable and more often low quality with limited shelf life [1]. Contaminations from soil, insects and birds droppings during drying also pose a problem. Poor product quality is a barrier to high end markets restricting dried sardine marketing to local community areas. For this reason, drying racks which are raised ventilated platforms in open air have been introduced [3]. Nevertheless, rack drying depends on weather

conditions and therefore makes the results uncertain. Similar challenges were reported to have prompted research in the use of integrated solar dryers incorporating desiccants, blowers and thermal systems to continue drying during darkness hours [4]. Initial investments and running costs increase the price, but the product is stable during storage and safe for consumption.

Considering the rising of middle income groups in East Africa as well in many developing countries [5] where such products are consumed, premium dried fish products could be marketed in high end markets. The use of geothermal energy in Iceland has allowed affordable year round indoor fish drying while maintaining consistence in product quality [6]. Kenya is the top geothermal energy producer in Africa [7] but development in geothermal utilization is nonetheless limited. To add value and ensure food safety in the sardine fishery, the use of modern drying technologies such as systems utilizing geothermal energy are inevitable. There is a need to conduct drying trial in such system and evaluate product quality alongside open sun dried fish for advising prospective investors in the industry.

Sardine in particular *Sardinella gibbosa* is commonly partially boiled (blanched in brine) prior to drying to inhibit enzymatic and microbial activities [8]. The effect of fish blanching before drying on the drying characteristics and quality of product is poorly studied. The most important chemical reaction that occur during fish drying and storage of dried products is lipid oxidation that results in nutrient degradation and changes in sensory properties [3,9]. Marine fish lipids are rich in n-3 long chain polyunsaturated fatty acids [10] which are of excellent nutritional value. However, they are prone to oxidation that adversely affects nutritional quality, wholesomeness and sensory value. The present study was designed to investigate the effect of blanching treatment and drying methods on the drying characteristics, lipid composition and oxidation, and sensory quality of sardine during drying and storage.

2 Materials and methods

2.1 Raw material, processing and sampling

Sardine caught in the Western Indian Ocean by artisanal fishermen from Mombasa, Kenya on 21st March, 2013 was landed approximately 8 hours post-catch and graded by size

(20–25 g). The graded fish (100 kg) was placed in flake ice and transported to the Kenya Marine and Fisheries Research Institute laboratory within 3 hours. The fish was immediately frozen upon arrival in blocks weighing 25 kg and kept frozen at -25 °C. Two frozen blocks were transported by air freight to Iceland and the other two kept in Kenya for later studies.

The frozen fish was thawed overnight in open air at 20±2 °C. Upon thawing, fish was divided into two equal portions. One portion was blanched in brine (BL) and the other brined without blanching (unblanched, Co). The blanched portion was put in a perforated metallic pan and immersed in boiling brine (103±0.5 °C) with pre-determined concentration of 5% fine NaCl-salt solution for two minutes to attain about 2% salt content in the fish muscle. The blanched fish was then spread on meshed trays to cool and remove excess surface water. The unblanched portion was immersed in similar brine solution (5% fine NaCl-salt) for 2 hour at 2 °C to attain about 2% salt content in the fish muscle. The unblanched fish was then spread on meshed trays to remove excess surface water as in blanched. Ten individual fish from each portion were tagged and weighed individually. Drying trials were done under indoor (I) and open sun (T) drying conditions. The fish was arranged in a single layer on plastic meshed trays before drying.

The indoor drying was conducted in Vestfirska Hardfisksalan fish drying company in Reykjavik, Iceland. The dryer used consisted of hot water flow heat exchanger with a centrifugal fan, connected directly to the drying chamber. The heat exchanger and drying chamber were equiped with a regulator that controlled the drying temperature (19-25 °C) and relative humidity (76-49%) respectively. Air velocity in the drying chamber was kept constant at about 3.6 m/s. Open sun drying was conducted on open air drying racks in Mombasa, Kenya. During open sun drying, fish was covered with plastic sheets at night as commonly practiced for protection against rodents and rain. Once dried, fish was transported to the laboratory and each portion air packaged equally in 10 polyethylene bags and stored at room temperature (24±3 °C). Sampling was done approximately once a month for 5 months. At each sampling point, 2 bags per portion were picked randomly for analyses.

2.2 Analyses

Temperature and humidity

Temperature was measured and recorded using temperature data loggers (iButton DS1922L, CA, USA) with an accuracy of ±0.5 °C. While relative humidity values were measured and recorded using humidity data loggers (digital infrared humidity meter) with reading accuracy of ±0.01%. The loggers (n=3) were placed at different points during the experiments and the readings automatically recorded every 15 minutes.

Drying kinetics

Equilibrium moisture content was determined at two temperatures 25 °C and 33 °C, and relative humidity levels of 55% and 49%, obtained at the end of (unblanched and blanched) sardine drying under indoor and open sun conditions respectively. Vemuganti equation applied in calculating equilibrium moisture content (EMC) in agriculture products was used Eq. [1] [11]

$$\mathrm{Me} = \frac{1}{C1}\ln\left(\frac{B1}{A1}.\ln RH\right) \qquad [1]$$

Which

A1 = - (6.65596 F + 20.146 N)

B1 = 1.987 (T+2.395 P + 0.8176 N)

C1 = - (0.46997 F + 0.22625 N)

Where Me is the equilibrium moisture content; RH is the relative humidity; T is the temperature (°C); F is the fat %; N is nitrogen % and P is the protein %.

The moisture content of drying sample at any time was transformed to the moisture ratio (MR) using Eq. [2] [11, 12]

$$MR = \frac{M_t - M_e}{M_0 - M_e} \qquad [2]$$

Where M_t is the moisture content at any time of drying (kg water/kg dry basis); M_0 is the initial moisture content (kg water/kg db) and M_e is the equilibrium moisture content (kg water/kg db).

The drying performance is generally estimated using the drying rate (DR), which is proportional to the difference in moisture content between the material to be dried and the equilibrium moisture content. Mathematically, it can be expressed using Eq. [3] [13]

$$DR = \frac{dM}{dt} = \frac{M_t - M_{t+\Delta t}}{\Delta t} \qquad [3]$$

Where DR is the drying rate at any time of drying (Kg water/Kg db. hour; dM is the change in moisture content (Kg water/Kg db); dt is the change in time (Hour); $M_{t+\Delta t}$ is the moisture content at $t = t + \Delta t$, and Mt is moisture content at $t = t$.

During sample drying, diffusion is generally accepted as the main mechanism for the transportation of moisture from the internal layers to the surface layer to be evaporated. The effective moisture diffusivity can be determined using Eq. [4] [13, 14]

$$\ln(MR) = \ln\left(\frac{W - W_e}{W_0 - W_e}\right) = \ln\left(\frac{6}{\pi^2}\right) - \left(\frac{\pi^2 D_{eff}}{L^2}\, t\right) \qquad [4]$$

Where D_{eff} is the effective moisture diffusivity (m^2/s); L is the sample thickness (m); t is the drying time (s).

Colour measurement

The colour intensity of dried sardine was measured using Minolta CR-300 chromameter (Minolta Camera Co., Osaka, Japan) in Lab* system (CIE, 1976) with CIE IlluminantC. The appliance recorded the L^*-value (lightness), a^*-value (redness) and b^*-value (yellowness) on CIELAB colour scale. Three positions on fish along the lateral line area were measured. The average $L^*$and b^*-values of five fish measurements (n=15) were used.

Chemical composition

Total lipid were extracted from 25 g samples (80±1% water) with methanol/chloroform/0.88 % $KCl_{(aq)}$ (at 1/1/0.5, v/v/v) according to the [15] method. The lipid content was determined gravimetrically and the results were expressed as % wet muscle basis. Protein was determined by Kjeldahl method. The organic matter was digested by sulphuric acid in the presence of a catalyst. The reaction product was rendered alkaline, and the liberated ammonia was distilled and titrated with hydrochloric acids [16]. The salt content (NaCl) was

determined based on [17] and expressed as % muscle weight. Initial moisture content (M_0) in fish was calculated as the loss in weight during minced sample drying at 105 °C for 4 h [18]. Results were expressed as Kg water/Kg db. Weight loss measurements taken during drying were used in calculating moisture content of samples at any given time based on the initial weight and moisture content.

Lipid oxidation

Lipid oxidation was measured as lipid hydroperoxides (PV) and thiobarbituric acid-reactive substances (TBARS) on dried samples during storage. PV were determined using the ferric thiocyanate method described by [19] with modifications according to [10], except that 3±0.5 g of sample was used instead of 5 g, and after extraction and centrifuging, 200 µL of the chloroform layer was collected and mixed with 800 µL of chloroform: methanol solution. The results were expressed as µmol lipid hydroperoxides/kg muscle weight.

TBARS were measured as described by [20] with modifications. A 3±0.5 g muscle sample was homogenized with 10 ml of trichloroacetic acid (TCA) solution (7.5% TCA, 0.1% propyl gallate and 0.1% EDTA mixture) using an Ultra-Turrax homogenizer at 8000 rpm for 10 s. The homogenate was then centrifuged at 5100 rpm for 20 min at 4 ^{o}C (TJ-25 centrifuge, Beckmann Coulter, USA). A 100 µL of supernatant was collected and mixed with 900 µL of 0.02 M thiobarbituric acid solution in 1.5 mL Eppendorfs and heated in a water bath for 40 min at 95 °C. The samples were cooled down on ice after which, 200 µL were placed in duplicate into a 96-well micro plate reader for absorbance reading at 530 nm (Sunrise Micro plate Reader, Tecan GmbH, A-5082 Grödig, Austria). The results were expressed as µmol of malomaldehyde diethylacetal/kg muscle weight.

Free fatty acid and fatty acid composition

Free fatty acid (FFA) content was determined on 3 mL of the lipid extract based on a complex formation with cupric acetate-pyrimidine [21], followed by absorbance reading of the upper layer at 710 nm. The FFA concentration was calculated as µM quantities of oleic acid, based on a standard curve spanning a 2-22 µmol range. Results were expressed as g FFA/100 g lipid.

The fatty acid composition was determined following derivatisation of extracted total lipid to fatty acid methyl esters (FAME) by gas chromatography (Varian 3900 GC, Varian, Inc., Walnut Creek, CA, USA) equipped with a fused silica capillary column (HP-88, 100 m x 0.25 mm x 0.20 µm film), split injector and flame ionisation detector (FID) based on [22] method. The result of each fatty acid was expressed as g fatty acid/100 g lipid.

Sensory evaluation with Generic Descriptive Analysis

Ten sensory panel members were trained in three sessions during a preliminary study. The panel was trained to recognize sensory characteristics of the dried fish and describe the intensity of each attribute. An unstructured scale from 0 to 100% [23] was used with vocabulary which defined sensory attribute for dried sardine as described in Table 1. Sample evaluations were conducted according to international standards [24]. From each fish one piece, about 5 cm long and 3 cm wide, was prepared by removing the head and tail. The pieces were placed in plastic boxes coded randomly with three digit numbers that did not indicate treatment or storage time before being served to the panel. Each panelist evaluated duplicates of samples in a random order in two sessions. Data recording was done manually on a 10 cm line provided on a paper.

2.3 Statistical analysis

Statistical analysis was done using by Microsoft Excel 2010 and NCSS 2000 (NCSS, Kaysville, UT). Analysis of variance (ANOVA) was carried out using Duncan's multiple-comparison test for stepwise comparison at the 95% significance level. Multivariate comparison of different sensory attributes was performed with Principal Component Analysis (PCA) using Panelcheck V 1.4.0 (Nofima, Tromsø, Norway).

3 Results and discussion

3.1 Temperature and relative humidity profile

Significantly higher temperature was recorded during open sun (27-35 °C) than indoor (18-25 °C) drying (Figure 1). Whereas temperature varied based on weather conditions and time of day in open sun drying, it increased as drying progressed in indoor drying. Relative humidity

varied between 72% and 50% during open sun drying and declined from 76% to 49% during indoor drying. Relative humidity was inversely related to temperature in both drying experiments as can be expected [3]. When the warm-dry air moves over fish while drying, heat is transferred to the fish and water on the surface layer evaporates. This creates moisture gradient between the air and fish muscle as the temperature of fish increases causing moisture to diffuse from the inner layers to the surface layer. In indoor drying, low temperature and high humidity at the beginning of drying was used to prevent formation of hard surface layer (crust) due to imbalance in diffusion resulting from higher external diffusion than internal diffusion. However as drying progressed, the temperature was increased with a corresponding decrease in relative humidity to continue the drying process as the fish were becoming drier. The low temperature (18-25 °C) used during indoor drying has been shown to reduce nutrient degradation by minimizing lipid oxidation and protein denaturation [25,26]. Unlike indoor drying, ambient temperature in the open sun drying was lowest during hours of darkness (when samples were kept overnight) and highest during the day. As discussed later this might have contributed to low quality of the open sun dried products.

3.2 Drying rate and moisture ratio

Drying method and blanching, both affected drying rate and consequently the moisture content (Figure 2). Drying rate was significantly ($p < 0.05$) higher during open sun drying than indoor drying in the early stages of drying when moisture content was relatively high. This was attributed to higher moisture evaporation and diffusivity owing to low humidity levels and high temperatures exhibited at the start of drying under open sun conditions. Drying is controlled by temperature, level of relative humidity and air velocity [1]. However, under the same drying trial, blanched sardine obtained a high drying rate that was not significantly ($p>0.05$) different from unblanched counterpart at the start of dying. This could be because sardine used was small and thin, implying the distance for internal diffusion was shorter resulting in the obtained insignificant different between groups. On the other hand, higher drying rate in blanched than unblanched may be attributed to denaturation and possibly hydrolysis of myofibrillar proteins thereby affecting muscle water holding capacity. Although this finding is in agreement with an earlier study on blanched and unblanched capelin during cold air drying, the difference in drying rate between capelin groups was statistically significant [25]. Apart from size difference, the effect

of blanching on proteins between species may have differed as capelin is a temperate species unlike sardine caught from tropical waters. Tropical fish species contain thermal stable proteins than temperate species [26] because the tissue proteins increase in stability with increase in their habitat temperature [27]. Also, low moisture content in the blanched groups at the start of drying ((2.8 Kg water/Kg db versus 3.1 Kg water/Kg db) could have contributed since drying rate has been reported to depend not only on temperature, relative humidity and air speed but also the moisture content [1]. As expected the drying rate decreased gradually with reducing moisture content but at different rates depending on the drying conditions. Water binding in the products increases with drying and in the later drying stages water diffuses from the interior to reach the surface [25].

An increase in drying rate was observed during open sun drying when moisture content was between 0.8 and 0.9, and between 0.5 and 0.7 Kg water/Kg db. The occurrences were obtained at the beginning of each day (at 25 and 46 hours in Figure 1) after keeping samples overnight (darkness hours). This might be due to internal diffusion in the night that allowed water to exit to the surface (with no external diffusion), resulting to an increasing rate in the drying process at the beginning of the following day. In general, the drying rate curves were similar with no constant rate period and had only a falling rate period except at the beginning of each day after keeping samples overnight under open sun drying conditions.

It took about 60 and 52 hours to dry sardine to stable moisture content under indoor and open sun drying conditions respectively (data not shown). Generally, the moisture ratio (MR) reduced with drying time (Figure 3). MR reduced at different rates depending on the drying method. Whereas a systematic reduction in MR happened during indoor drying, it apparently remained constant when samples were kept overnight (during darkness) during open sun drying. This phenomenon as earlier explained could be related to arrest of the external diffusion during hours of darkness (overnight storage) thereby upholding the moisture ratio. This indicates open sun drying conditions could have resulted in a shorter drying time than the reported 52 hours had it not been the overnight sample storage dictated by conditions. Dried sardine had moisture ratio of about 0.1 at the end of drying under both trials, indicating the moisture loss was not significantly different ($p > 0.05$) between the groups.

3.3 Effective moisture diffusivity

The effective moisture diffusivity (D_{eff}) values were not significantly ($p > 0.05$) different and ranged between 1.1×10^{-9} and 1.5×10^{-9} m^2/s (Table 2). D_{eff} was slightly higher in samples during open sun drying (Bl-T and Co-T) than indoor drying (Bl-I and Co-I). This is in agreement with drying rates result where, higher rates occurred during open sun drying. Blanching was found to have insignificantly ($p > 0.05$) influenced the drying rate and also D_{eff} probably due to the size of sardine as the fish used was small and thin. The D_{eff} values reported in this study are within the general range for food materials 10^{-11} to 10^{-9} m^2/s [28].

3.4 Colour changes

Both blanching and drying methods had considerable impact on colour. After drying lightness was not significantly ($p>0.05$) different between the groups although blanched sardine had lower lightness (L^*-value) than that of other groups (Figure 4 A). Whereas, yellowness (b^*-value) was significantly ($p < 0.05$) higher in blanched than in unblanched groups (Figure 4 B). During storage, lightness declined whereas, yellowness increased in all groups and was statistically significant ($p < 0.05$) in blanched sardine that recorded higher increments towards the end of storage. In dehydrated foods colour is among the most important quality attribute regarding consumers purchase decision and also an effective quality indicator [25,29]. In dried fish, yellowness is undesirable as it is related to lipid oxidation and long storage [29]. Shelf life of blanched sardine is then limited.

3.5 Chemical composition

Lipid content of raw sardine was 3.0±0.08%. Generally, lipid content decreased after blanching and brining but significantly ($p < 0.05$) after blanching to 2.4±0.13%, but was 2.9±0.20% after brining. The corresponding water content was 3.08 Kg water/Kg db (75.5±0.50% wet basis (wb)) which did not differ from brined samples but reduced to 2.82 Kg water/Kg db (73.8±0.62%, wb) after blanching. The results indicate there was lipid and water loss during blanching and brining before drying onset. It can be understood that blanched sardine lost lipid through exudation during blanching and/or with the drip water while cooling to remove excess surface water. At the end of the drying process under both indoor and open sun

conditions, lipid content did not differ significantly ($p > 0.05$) between the groups, with values ranging between 0.29 and 0.39 Kg water/Kg db (9.0±0.74% to 10.3±50% wb). This may be explained by the extent of dryness in the fish, as blanched groups were drier at the end of drying as reflected in the groups' moisture ratio (Figure 3). The protein content in raw sardine was 13.52±0.94% while salt content was 0.65±0.04%. After blanching and brining in NaCl solution, blanched and unblanched samples had protein content (14.33±0.62% and 13.53±0.55%, respectively) and salt content (2.12±0.06% and 2.05±0.05%, repectively) that were not significantly ($p > 0.05$) different.

3.6 Lipid oxidation

Lipid oxidation of the dried sardine was studied by measuring primary (PV) and secondary (TBARS) oxidation products (Figure 5 A & B). Drying methods had a greater impact on the PV than blanching. Significantly ($p < 0.05$) higher PV values were recorded in open sun dried sardine compared to indoor dried sardine in both blanched and unblanched samples (170 vs. 100 µmolMDA/kg and 131 vs. 90 µmolMDA/kg, respectively). The PV values were also higher in blanched than in unblanched dried sardine, with significant ($p < 0.05$) difference in open sun dried sardine. Blanching and open sun drying increased the rate of lipid oxidation due to higher temperature during blanching and open drying. High temperature speeds up the rate of lipid oxidation. [30] Reported high temperature to have accelerated lipid oxidation during drying of grass carp fillets.

During storage, PV increased during early storage but significantly ($p < 0.05$) during the second month in open sun dried blanched (Bl-T) and third month of storage in other groups (Figure 5 A). Later towards the end of storage, the PV decreased but at different times in the groups except in indoor dried unblanched fish (Co-I) which showed increased PV values throughout the storage. Open sun dried fish had higher rate of hydroperoxide (PV) accumulation attaining peak values 292 µmolMDA/kg in blanched and 267 µmolMDA/kg in unblanched samples earlier than their counterparts, implying that open sun dried sardine is more vulnerable to lipid oxidation processes than indoor dried. Reduced PV content measured as the storage time progressed is in agreement with [3] conclusion that rapid build-up of hydroperoxides occurs during the initial oxidation process, however with extended storage time, the rate of hydroperoxides

cleavage and reactions exceed the rate of formation. This implies that at the end of the study, indoor dried unblanched fish had not reached the level where hydroperoxides cleavage and reactions exceeded formation.

After drying, TBARS were significantly ($p < 0.05$) different between the groups with higher values in open sun dried fish (Figure 5 B). During storage, slow TBARS accumulation was observed in all groups except open sun dried blanched fish (Bl-T) that showed an increase in TBARS from the beginning of storage attaining significantly ($p < 0.05$) higher values in the third and fourth month, and a decline at the end of storage period. The slow TBARS accumulation recorded in other groups implied delayed formation especially in indoor dried fish that had lower values. The faster accumulation in Bl-T and subsequent decrease at the end of storage time is an indication of rapid decomposition of hydroperoxides into secondary oxidation products and afterwards decomposition of the secondary products during the advancement of oxidation [31]. Generally open sun drying and blanching treatment significantly ($p < 0.05$) accelerated lipid oxidation during drying and storage.

3.7 Free fatty acids and fatty acid composition

After drying, the free fatty acid (FFA) content was different between the groups, but a significantly ($p < 0.05$) higher FFA content was observed in unblanched sample compared to that of blanched (Figure 6). This result indicates that higher hydrolysis of glycerol-fatty acid esters occurred during drying of unblanched fish with the liberation of free fatty acids. Hydrolysis of glycerol-fatty acid esters is known to be caused mainly by enzymatic activities [31], which might have been inhibited by blaching process [8]. During storage, the FFA content increased moderately in unblanched groups but remained apparently stable in the blanched groups. Unlike other quality indicators, FFA results demonstrate the importance of blanching treatment in terms of enzymatic activity inhibition.

The major fatty acids groups in sardine were saturated fatty acids (SFA), followed by polyunsaturated fatty acids (PUFA) and monounsaturated fatty acids (MUFA) (Table 3). Amongst the SFA, palmitic acid (C16:0) was predominant, with oleic acid (C18:1n9) and DHA dominate in MUFA and PUFA respectively. During drying and storage, unsaturated fatty acids declined, and there was a corresponding increase in SFA.

Fatty acid composition was influenced by drying methods, blanching treatment and storage time. Significant ($p < 0.05$) difference was observed between indoor and open sun dried sardine with respect to storage time. Open sun dried and blanched sardine exhibited faster decline in unsaturated FA than indoor and the unblanched dried groups. The decline in unsaturated FA upon drying and during storage was most rapid in the fatty acids with double bonds suggesting the impact of oxidation was higher in PUFA. The PUFA had declined in value by 19% and 31% in indoor dried unblanched and blanched sardine, 37% and 44% in open sun dried unblanched and blanched sardine, at the end of storage from the 26.85 g PUFA/100 g lipid in raw fish. DHA that constituted a high proportion of PUFA exhibited the biggest decline, meaning that it oxidized more readily than other PUFA. Other PUFA including EPA had similar trends but less pronounced. The higher decline in PUFA reported in blanched and open sun dried fish is of concern given that PUFA, especially EPA and DHA, are beneficial to the health of consumers [10].

3.8 Sensory Evaluation

Figure 7 shows how sample groups of dried sardine were described by the sensory attributes with storage time. Sample grouping were evident on each side of the PC1-axis, with samples located on the left side of scores plot in early storage and on right side in later storage time indicating they were easily distinguished. The sensory attributes of dried fish (flavour and odour, and texture: dry and sticky) detected at the beginning of storage on the left side of loadings plot along the 1st principal component (PC1) characterised newly dried fish. The sensory attributes colour muscle, flavour and odour rancidity were more predominant after extended storage.

Samples varied mainly with regard to differences in flavour, odour and colour attributes along the PC1 explaining 66% of the variation between them. The main difference occurred with the storage time as well as blanching treatment, as the sample groups are located to the left side at the beginning of storage dominated by unblanched groups but on the right side after extended storage dominated by blanched groups. This indicates that unblanched groups were more characterized by the desired attributes and appeared to keep the characteristics longer. Dried sardine varied also with regard to differences in texture mainly sticky attribute along the 2nd

principal component (PC2), explaining 14% of the variation between the samples. Rancid odour and flavour did not significantly contribute to the differences in the sample groups during storage. This can be explained by slow accumulation of TBARS in the study. TBARS have been reported to have a profound impact on food sensory properties [32,33].

4 Conclusions

Blanching had influence on sardine drying rate and also slowed down free fatty acids progression during drying and products storage. However, it adversely affected the products quality mainly lipid composition (oxidation), colour and sensory properties. In ideal situation, blanching of sardine before drying is done for longer time than the blanching described in this paper and more research is needed to optimize the process for quality reasons. Although a relatively higher drying rate was obtained during open sun drying, indoor drying produced a quality stable product with desired moisture content, lipid composition and sensory properties. This has reinforced the need for development of a controlled drying system that utilizes the affordable available energy sources such as solar and geothermal energy for commercial sardine drying.

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Table 1: Sensory vocabulary for dried Sardine

Sensory attribute	Scale (0-100)	Short name	Description of attribute
Odour			
Characteristic	none | strong	O-Characteristic	Characteristic odour for dried fish
Rancid	none | strong	O-Rancid	Rancidity, rancid fish oil odour
Appearance/Colour			
Muscle	none | strong	C-muscle	Flesh dark brownish
Flavour			
Characteristic	none | strong	F-Characteristic	Characteristic for dried fish (stock fish)
Rancid	none | strong	F-Rancid	Rancid fish oil flavour, cod liver oil
Texture			
Dry	none | strong	T-Dry	When chewed, pulls liquid from mouth
Sticky	none | strong	T-Sticky	Glues together teeth when biting the fish muscle

Table 2 Effective moisture diffusivity (Deff) of unblanched (Co) and blanched (Bl) sardine during industrial (I) and traditional (T) drying.

Group	$D_{eff}(m^2/s)(x10^{-9})$
Co-T[†]	1.41
Bl-T	1.45
Co-I	1.12
Bl-I	1.13

[†] Abbreviations: Co = unblanched fish; Bl = blanched fish

Table 3 The Fatty acid composition (g fatty acids/100 g lipid) present in highest concentrations in the lipid extracted from the muscle of raw and dried air packaged sardine stored at room temperature (24±3°C) for 5 months. ± = standard deviations (n=3)

			Indoor dried (I)				Open sun died (T)			
Fatty acid	**Co-RM**[†]	**Bl-RM**	**Co-M0**	**Bl-M0**	**Co-M5**	**Bl-M5**	**Co-M0**	**Bl-M0**	**Co-M5**	**Bl-M5**
C14:0	3.92±0.03^{a}	4.02±0.03^{b}	4.05±0.02^{b}	4.18±0.07^{c}	4.83±0.22^{d}	5.43±0.16^{e}	4.33±0.24^{c}	5.00±0.28^{de}	5.13±0.22^{de}	5.13±0.34^{de}
C15:0	0.88±0.01^{a}	0.98±0.11^{a}	0.89±0.01^{a}	0.96±0.02^{b}	0.98±0.00^{c}	1.11±0.03^{d}	1.05±0.07^{cd}	0.98±0.00^{c}	0.08±0.00^{e}	1.10±0.04^{d}
C16:0	25.8±0.02^{a}	26.2±0.20^{a}	27.8±0.18^{b}	28.42±0.14^{c}	29.55±0.34^{d}	32.59±0.55^{e}	28.92±0.41^{cd}	31.75±0.32^{e}	30.35±0.54^{d}	33.22±0.17^{f}
C17	1.63±0.01^{a}	1.73±0.12^{a}	1.62±0.01^{b}	1.81±0.05^{c}	0.37±0.01^{d}	1.93±0.03^{e}	1.68±0.16^{bc}	1.79±0.06^{c}	0.37±0.01^{d}	0.39±0.02^{d}
C18:0	8.59±0.1^{a}	8.79±0.23^{a}	9.62±0.11^{b}	9.68±0.38^{bc}	9.99±0.05^{c}	10.62±0.39^{d}	10.53±0.11^{d}	10.96±0.06^{e}	10.99±0.05^{e}	12.00±0.18^{f}
Saturated	**40.82±0.96^{a}**	**41.98±0.28^{a}**	**44.5±0.51^{b}**	**45.55±0.58^{bc}**	**46.62±0.62^{ce}**	**50.44±1.12^{d}**	**46.79±0.14^{e}**	**50.84±0.37^{d}**	**46.92±0.62^{e}**	**51.84±0.36^{d}**
C16:1n7	2.66±0.06^{a}	2.70±0.11^{a}	2.57±0.03^{a}	2.54±0.07^{a}	2.52±0.15^{a}	2.46±0.15^{a}	2.31±0.09^{b}	2.22±0.08^{b}	2.49±0.20^{a}	2.52±0.15^{a}
C18:1n9	6.36±0.07^{a}	6.32±0.06^{a}	5.85±0.09^{b}	5.2±0.13^{cf}	4.93±0.01^{d}	3.84±0.07^{e}	5.52±0.09^{c}	5.03±0.06^{f}	5.75±0.06^{b}	4.93±0.01^{d}
C18:1n7	2.89±0.03^{a}	2.87±0.01^{a}	2.26±0.03^{b}	2.81±0.07^{a}	2.16±0.00^{c}	2.13±0.04^{c}	2.73±0.18^{a}	2.89±0.01^{a}	2.33±0.04^{b}	2.16±0.00^{c}
C20:1n9	0.76±0.04^{a}	0.77±0.00^{a}	0.56±0.05^{b}	0.49±0.05^{b}	1.27±0.07^{c}	2.00±0.20^{d}	1.68±0.17^{e}	0.55±0.08^{b}	0.33±0.07^{f}	0.27±0.07^{f}
C22:1	4.29±0.03^{a}	4.11±0.13^{a}	3.81±0.17^{b}	3.70±0.28^{b}	3.59±0.18^{b}	4.36±0.19^{a}	3.34±0.04^{c}	3.31±0.10^{cd}	3.13±0.16^{d}	3.59±0.18^{b}
Unsaturated	**18.23±0.29^{a}**	**18.00±0.32^{a}**	**17.05±0.56^{b}**	**16.69±0.75^{bc}**	**16.12±0.27^{c}**	**15.31±0.56^{cd}**	**16.76±0.06^{b}**	**16.07±0.26^{c}**	**15.84±0.38^{cd}**	**15.12±0.27^{d}**
C18:2n6	1.37±0.00^{a}	1.29±0.00^{a}	1.24±0.02^{b}	1.02±0.00^{c}	1.22±0.01^{b}	1.19±0.04^{b}	1.32±0.01^{d}	1.26±0.21^{b}	1.35±0.04^{a}	0.83±0.01^{e}
C20:5n3 (EPA)	4.54±0.02^{a}	4.23±0.02^{a}	3.51±0.02^{b}	3.38±0.12^{b}	2.91±0.02^{c}	2.31±0.04^{d}	2.91±0.02^{c}	2.57±0.10^{e}	2.01±0.10^{f}	2.0±0.00^{f}
C22:5n3	2.95±0.01^{a}	2.72±0.01^{a}	2.03±0.01^{b}	1.88±0.04^{c}	1.53±0.03^{d}	1.30±0.07^{e}	1.53±0.03^{d}	1.51±0.01^{d}	1.32±0.02^{e}	1.41±0.10^{de}
C22:6n3 (DHA)	16.39±0.25^{a}	15.82±0.25^{a}	14.38±0.07^{b}	12.61±0.63^{c}	10.19±0.61^{d}	8.35±0.72^{e}	12.19±0.61^{c}	10.23±0.29^{d}	9.18±0.46^{de}	8.41±0.09^{e}
Polyunsaturated	**26.85±0.75^{a}**	**25.61±0.55^{a}**	**23.13±0.42^{b}**	**22.29±0.58^{be}**	**19.72±0.52^{c}**	**17.99±0.84^{df}**	**21.72±0.52^{e}**	**18.56±0.03^{d}**	**16.86±0.65^{f}**	**15.05±0.25^{g}**

[†] Abbreviations: Co = unblanched fish; Bl = blanched fish; RM = raw material; M0 = after drying (month 0); M5 = end of storage (month 5)

[*] Different letters (superscript) within fatty acid class (within a row) indicate significantly different values between samples ($p < 0.05$)

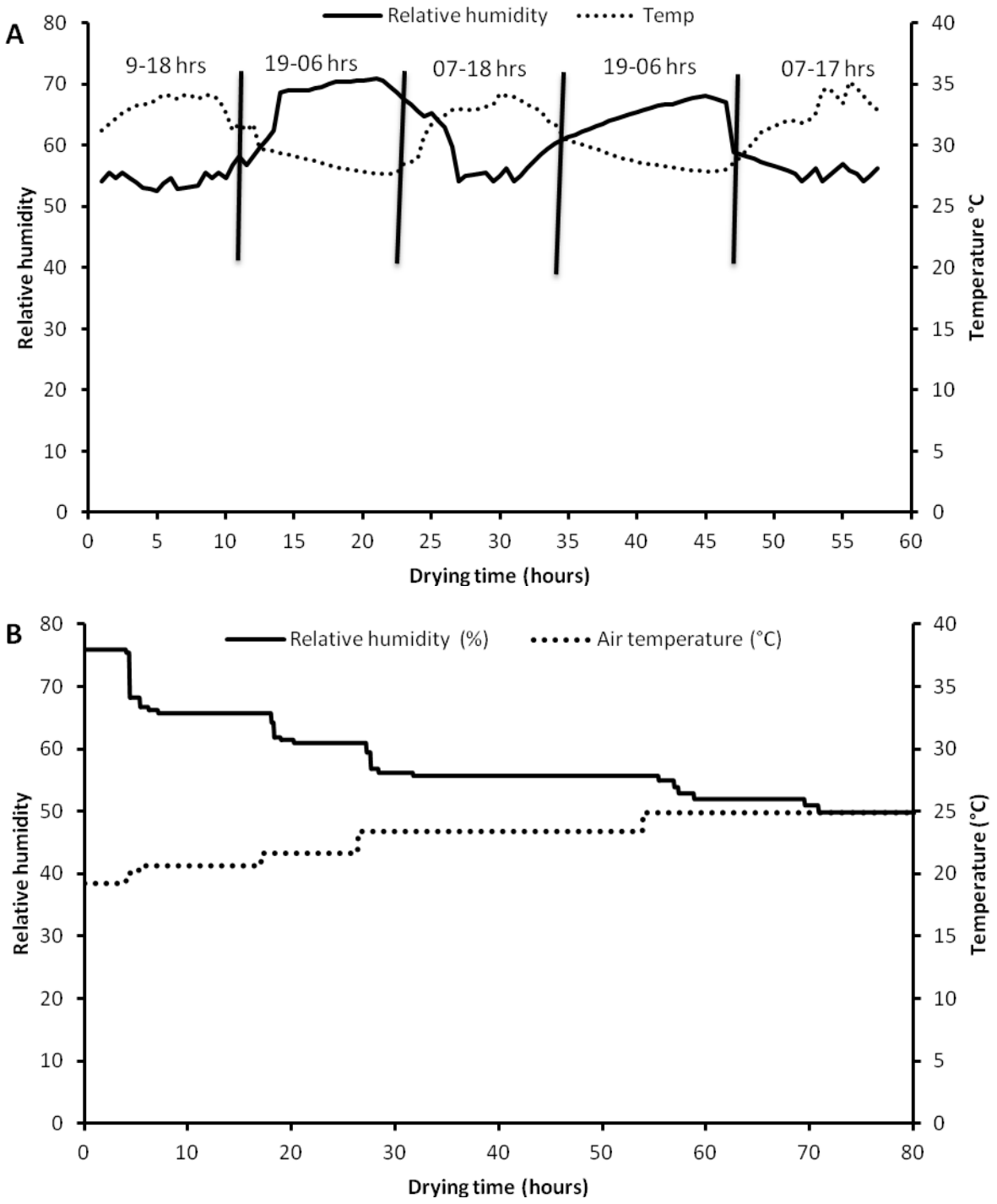

Figure 1 Variation of air temperature and relative humidity during open-sun (A) and indoor (B) sardine drying (n=3).

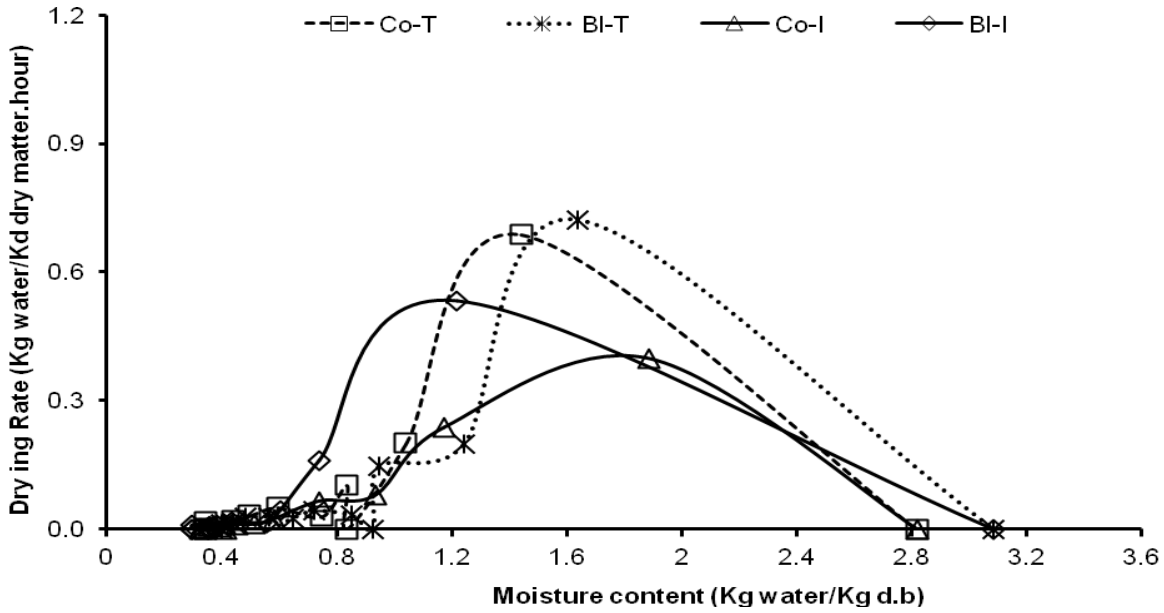

Figure 2 Variation in drying rate with the moisture content in unblanched (Co) and blanched (Bl) sardine during indoor (I) and open sun (T) drying (n=10)

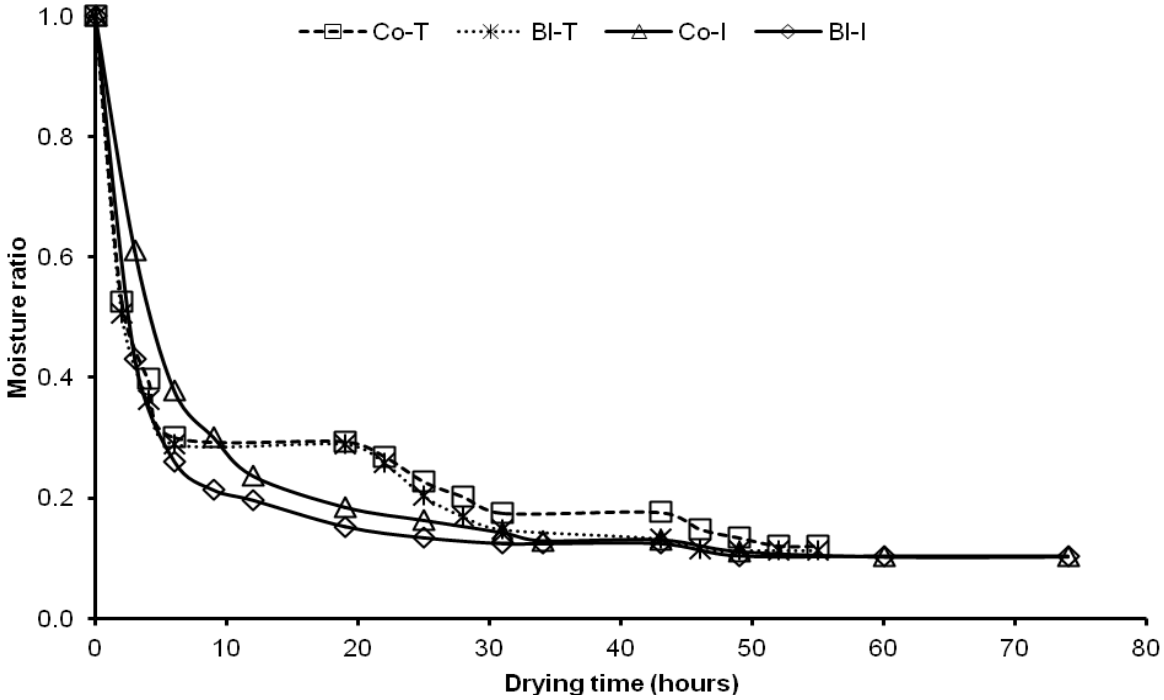

Figure 3 Variation in moisture ratio in unblanched (Co) and blanched (Bl) sardine during indoor (I) and open sun (T) drying (n=10)

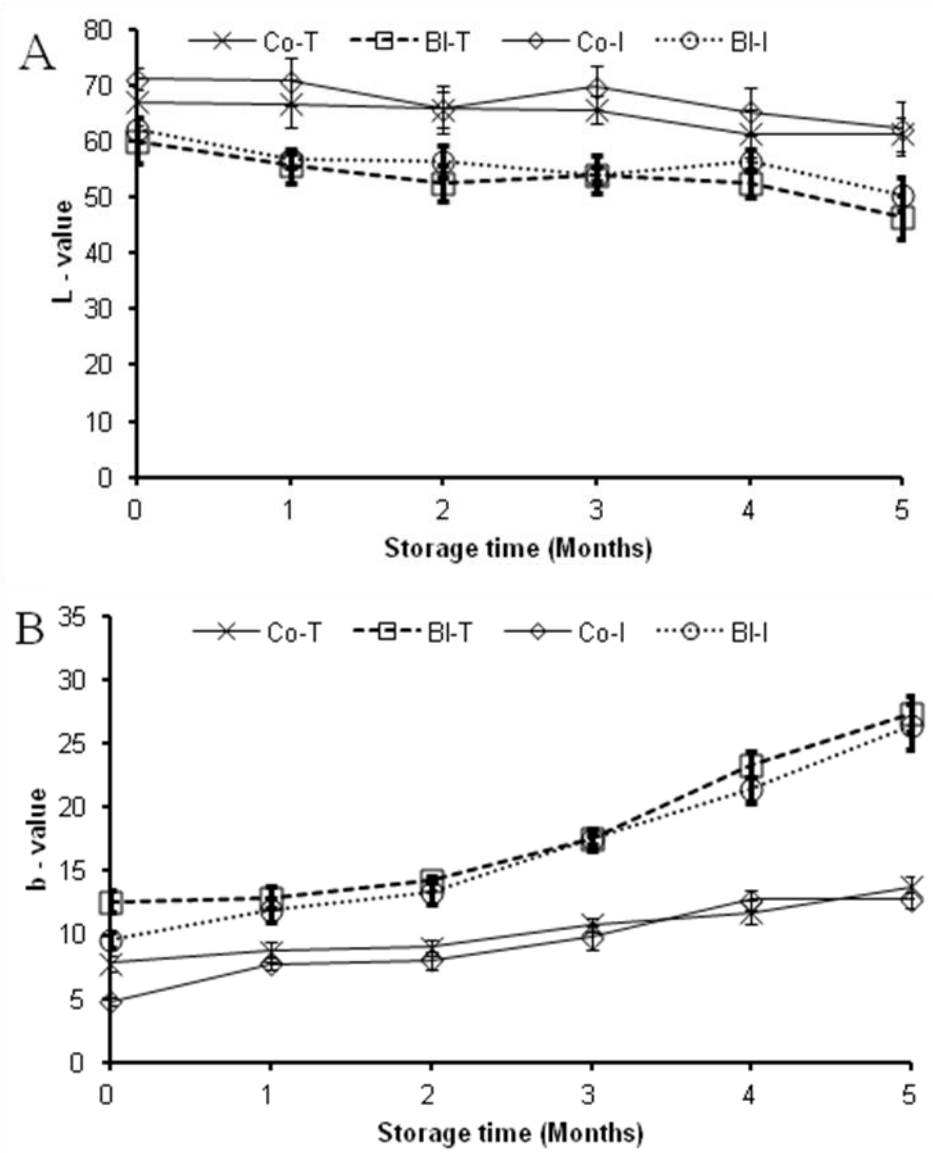

Figure 4 Changes in the colour parameters; lightness (A) and yellowness (B) in unblanched (Co) and blanched (Bl) sardine dried in indoor (I) and open sun (T) conditions as a function of storage time (n=15)

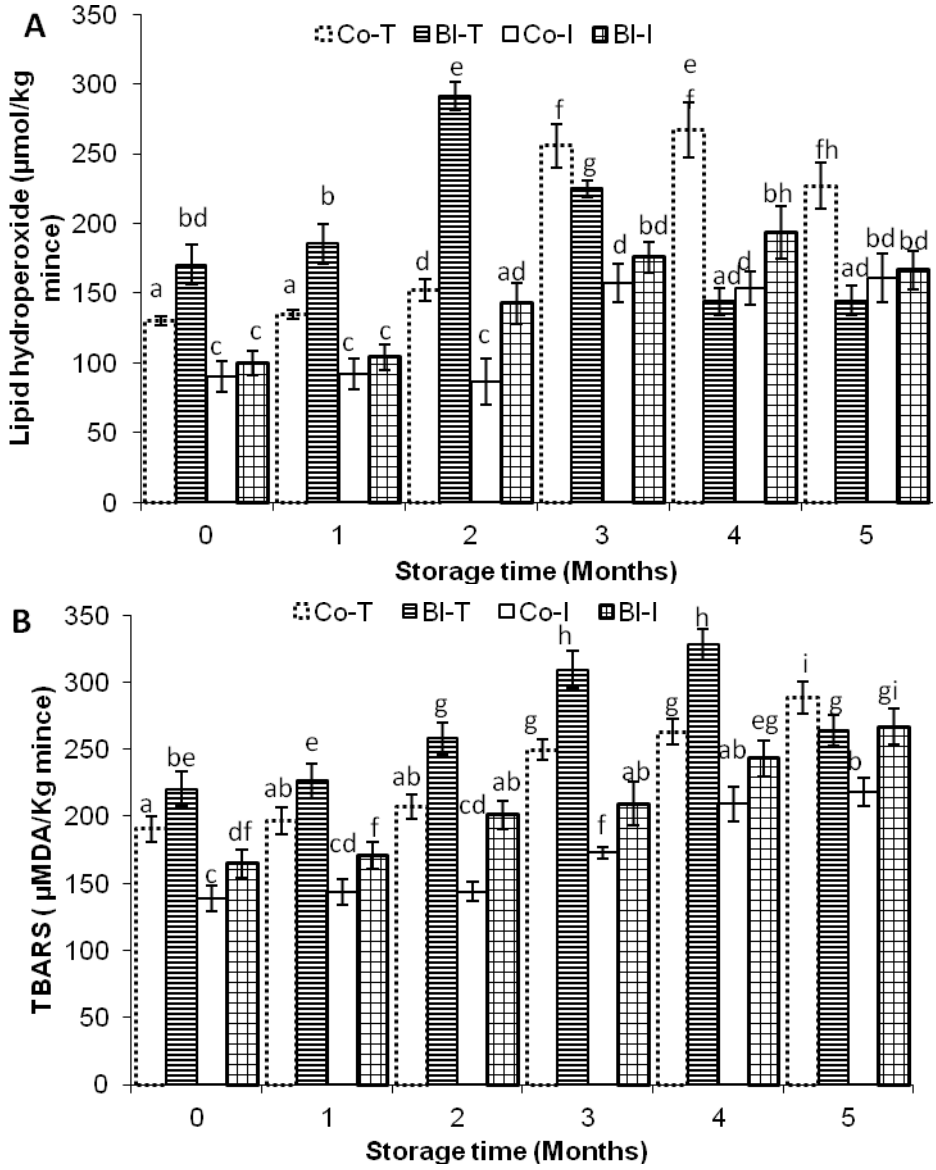

Figure 5 Lipid hydroperoxides (PV) formation (A) and Thiobarbituric acid reactive substances (TBARS) formation (B) in unblanched (Co) and blanched (Bl) sardine dried under indoor (I) and open sun (T) conditions as a function of storage time (n=3)

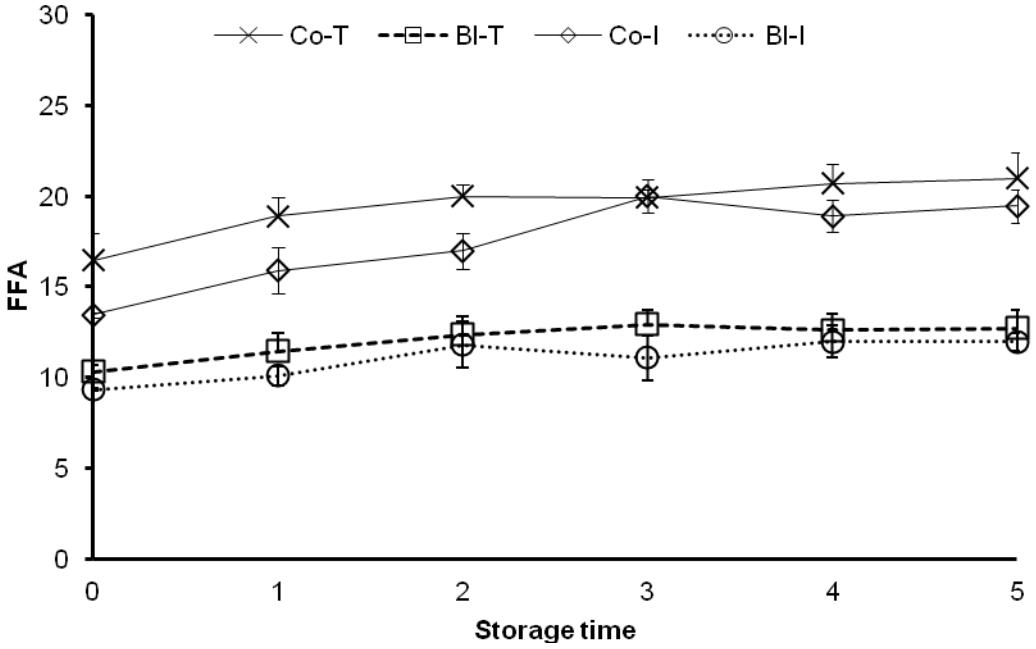

Figure 6: Changes in free fatty acid in unblanched (Co) and blanched (Bl) sardine dried in indoor (I) and open sun (T) conditions as a function of storage time (n=3)

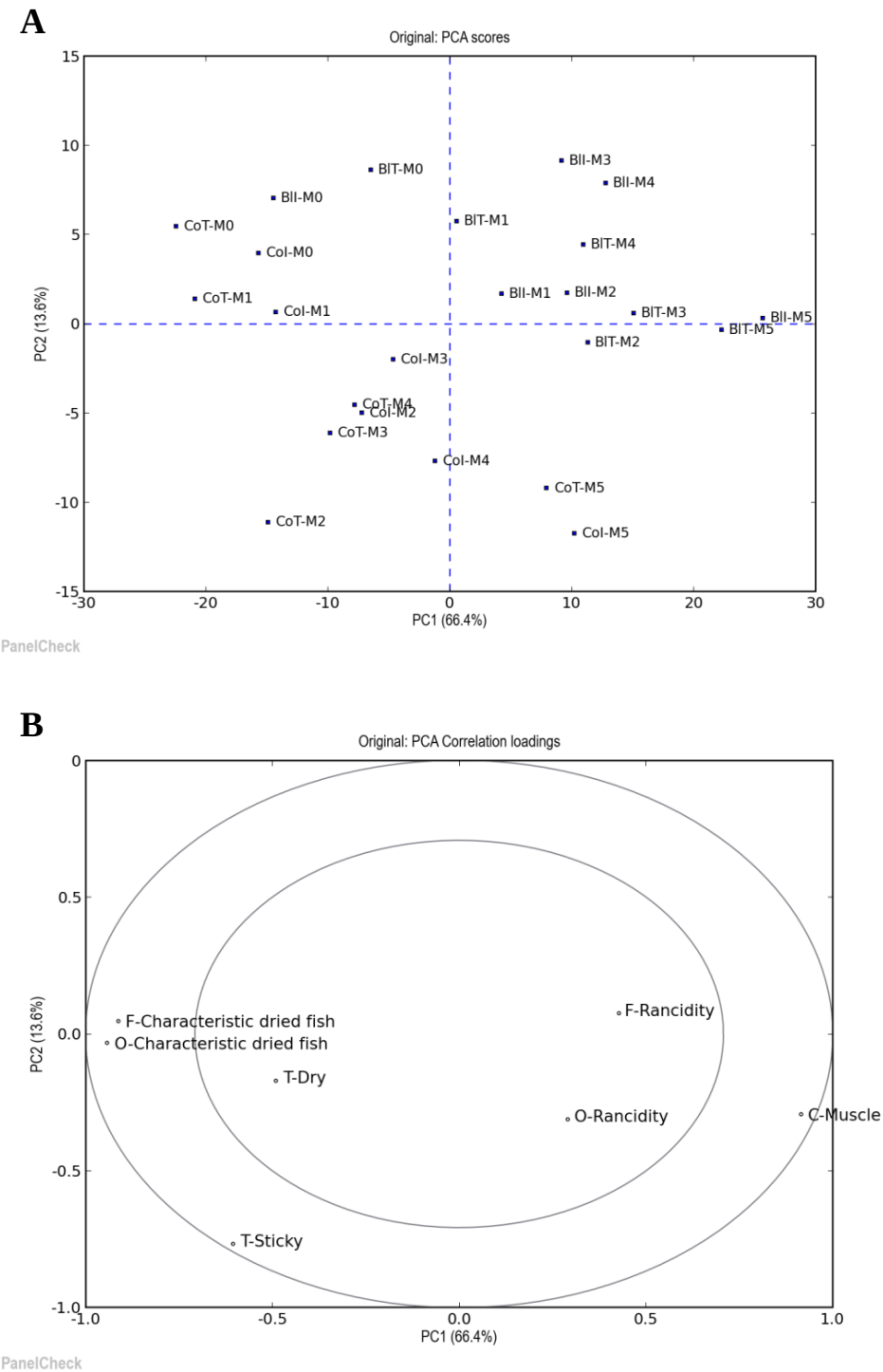

Figure 7 PCA: (A) Scores and (B) correlation loadings describing sensory quality of the dried sardine as evaluated by a trained sensory panel. PC 1 (66.4%) vs. PC 2 (13.6%). M = month of storage; Co = unblanched group; Bl = blanched group; I = indoor dried; T = open sun dried; O = odour; F = flavour; T = texture; C = colour

Influence of lipid content and blanching on capelin (Mallotus villosus) drying rate and lipid oxidation under low temperature drying

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INFLUENCE OF LIPID CONTENT AND BLANCHING ON CAPELIN (*MALLOTUS VILLOSUS*) DRYING RATE AND LIPID OXIDATION UNDER LOW TEMPERATURE DRYING

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ABSTRACT

The drying characteristics and lipid oxidation in brined and blanched whole capelin were studied during controlled low temperature drying to establish the influence of lipid content and blanching. Drying characteristics (moisture content, drying rate and water activity) and lipid oxidation indicators (PV, TBARS and color) were determined. Drying was influenced by blanching as well as the lipid level. Moisture content at equilibrium for brined and blanched high lipid dried capelin was 20 and 13% (a_w, 0.69 and 0.62), whereas counterpart low lipid groups had 19 and 11% (A_w, 0.67 and 0.59) accordingly after 184 h of drying. Changes in PV, TBARS and color were observed as drying progressed with brined capelin exhibiting more stability in the attributes than blanched capelin. Blanched and low lipid capelin dried faster; however, blanching resulted to increased yellowness color making it unpopular for capelin drying.

PRACTICAL APPLICATIONS

Capelin is one of the important pelagic fisheries in northern hemisphere with sustained catch stock; nonetheless, small quantities are used fresh, dried or salted for human food. In this paper, effects of lipid content and blanching on capelin drying characteristics and lipid oxidation are assessed. The study shows that lipid content affected drying characteristics as capelin with low lipid recorded reduced moisture at equilibrium. Blanching as practiced prior to drying of other small fish products in the intended markets for dried capelin affected capelin muscle water holding thereby enhancing drying but led to undesirable yellowness color and oxidation. It was recommended that dried brined capelin with low lipid content is tested for consumer acceptability prior to commercialization.

INTRODUCTION

Fish is considered as a source of high-quality animal protein and its consumption is on the increase worldwide as more information about its health spread out (Ruidavets *et al.* 2007; Abolagba and Melle 2008). Small fish species commonly consumed with bones and shell (chitin) body are a good source of calcium, protein, vitamin B and vitamin B_{12} (Jain and Pathare 2007). Capelin (*Mallotus villosus*) is a small, schooling pelagic fish common in the northern hemisphere (Carscadden, 2002). In Iceland, it is one of the most important pelagic fisheries with sustained catch stock of more than 1 million tons annually (Vilhjálmsson 2002). The catching seasons affect the chemical composition of fish, particularly the lipid content in the muscle (Arason *et al.* 2014). The lipid content in capelin can vary from 3–4% to about 15–20% of their body weight (Vilhjálmsson 2002), with the highest lipid content in late fall and lowest during the summer spawning season (Montevecchi and Piatt 1984).

Fresh fish is highly perishable with a short storage life and once it is not utilized by consumers or converted into finished products, the surplus is discarded. Traditionally, small quantities of capelin have been used fresh, dried or salted for human food. Therefore, capelin is considered as being underutilized as most of it is used for the production of fish meal, pet food and fish oil (Shahidi *et al.* 1995; Arason 2003). Because of general desire to better utilize capelin as human food, it is thought that the value may be enhanced through drying for the product to be consumed at affordable cost in developing countries such as Africa, where small dried fish constitutes a major protein source (Oduor-Odote *et al.* 2010a,b).

Drying is an efficient method for food preservation with the most widespread publicity (Dewi *et al.* 2011). It enhances the resistance of high-humidity products to the degradation by decreasing their water activity. Drying is a process of simultaneous heat and mass transfer. The heat transfer causes the evaporation of water and the mass transfer of the evaporated water from the interior of the fish and subsequently the removal of moisture away from the surface (Jain 2006). Moisture diffusivity takes place due to induced vapor pressure difference between the fish and surrounding medium (Jain 2006; Jain and Pathare 2007). These processes are generally characterized either as constant or falling rate periods and are controlled by the air velocity, temperature and the level of humidity (Bellagha *et al.* 2002; Oduor-Odote *et al.* 2010a). In the falling rate period of drying, moisture is transferred mainly by molecular diffusion. Even though materials moisture diffusivity is influenced majorly by water content and its temperature (Jain and Pathare 2007), chemical composition, particularly the lipid content, has additionally been reported to have an influence (Cardinal *et al.* 2001).

Dried fish is a traditional part of the diet of a large section of the world's population (Chukwu and Shaba 2009; Reza *et al.* 2009). Upon drying, fish meat becomes condensed, saturated with oil, more translucent and acquires an amber color, a typical flavor and dense consistency. Even so, methods of fish drying vary between countries and within the same country depending on the species used and the type of product desired. Fish may be dehydrated to various degrees with moisture levels in the final product ranging from about 10 to 60%. It is common practice in some countries especially in Africa that fish is partially boiled (blanching) before being dried purportedly to add flavor and inhibit microbial and enzymatic activities (Omodara and Olaniyan 2012). Drying processes are conducted either at high temperature and short time or at low temperature and long time (Arason 2003; Lewicki 2006). Low drying temperature has shown to maintain more properties of fresh fish, as it minimizes lipid oxidation and reduces protein denaturation, resulting in lower nutrient degradation (Bellagha *et al.* 2002; Lewicki 2006). However, as fish meat becomes saturated with oil owing to dewatering processes, the long time spent to attain full drying at low drying temperature may adversely influence lipid oxidation.

Although the drying characteristics for fish under different conditions have extensively been studied (Bellagha *et al.* 2002; Lewicki 2006; Jain and Pathare 2007; Djendoubi *et al.* 2009), no documentary was found investigating the effect of lipid content and blanching on drying rate and lipid oxidation in the same species. The aim of this work was to study the effects of lipid content and blanching on capelin drying characteristics and oxidation with intent of introducing dried capelin in markets where blanched dried fish products exist.

MATERIALS AND METHODS

Experimental Setup

The drying experiment was conducted in Vestfirska Hardfisksalan (Reykjavik, Iceland) fish drying company, whereas chemical analyses were done at Matis (Icelandic Food Research and Biotech) both in Reykjavik, Iceland. The dryer used consisted of heating chamber with an electrical heating system connected directly to a centrifugal fan and drying chamber. The heating system was connected to a temperature regulator that controlled the drying temperature (19–25C).

Sample Preparation

The description of sample groups is presented in Table 1. Two batches of capelin (*M. villosus*) frozen 2 days post catch on February 24 (C1) and March 7 (C2) 2013 were received from HB Grandi fishing company in Reykjavik, Iceland. The fish had been graded by size (32 ± 4 g), frozen into blocks weighing 25 kg each and kept for 3 months in frozen state (−25C) until the time of study. Thawing was done overnight in open air at 18–20C, after which each group was equally divided into two subgroups for brining (BR) and blanching (BL) prior to drying. Ten fishes from each subgroup were weighed and tagged for identification during subsequent weighing. The brined (BR) subgroups of C1 and C2 were immersed separately in predetermined brine with 5% fine NaCl-salt solution (ratio 1:1, brine : fish) for 2 h at 2C (to attain about 2% salt content) and thereafter spread on meshed trays to remove excess surface water. The tagged fishes ($n = 10$) were thereafter individually weighed prior to arrangement with others in a single layer on plastic meshed bottom trays in the drying racks. The blanched (BL) subgroups of C1 and C2 were put in perforated metallic pans and immersed respectively into boiling water (with 5% fine NaCl-salt solution) for 2 min before removing and

TABLE 1. DEFINITION OF CAPELIN GROUPS STUDIED AND DRYING TIME

Group codes	Lipid level/harvesting time	Pre-drying treatment (5% salt)	Treatment time (min)	Drying time to W_e (h)
C1	High (February 24)	None	0	0
C2	Low (March 7)	None	0	0
C1-BR	High (February 24)	Brining	60	184
C1-BL	High (February 24)	Blanching	2	184
C2-BR	Low (March 7)	Brining	60	184
C2-BL	Low (March 7)	Blanching	2	184

W_e, weight of fish at equilibrium state.

spreading on meshed trays to cool and remove the excess surface water. Weighing and arrangement were done as in brined groups.

EXPERIMENTAL PROCEDURE

Stacked dying racks were positioned at the middle of the chamber with temperature and humidity data loggers placed in the upper, middle and lower sections. The heating system and fan were switched on and drying continued until the samples reached a constant weight to obtain the hygroscopic equilibrium moisture. During drying, various parameters were measured as elaborated below.

(1) Temperature: Temperature was measured using temperature data logger iButton type DS1922L (Maxim Integrated Products, Sunnyvale, CA, USA) with an accuracy of ±0.5C and an operating range of −40 up to +85C. The readings were automatically recorded after every 15 min ($n = 3$).

(2) Humidity: Relative humidity values were measured using humidity data logger model digital infrared humidity meter with reading accuracy of ± 0.01%. As with temperature, readings were automatically recorded after every 15 min ($n = 3$).

(3) Air velocity: Airflow automatic digital thermo anemometer (TA-2 model) with reading accuracy of ± 0.01 m/s was used to measure air velocity at three points in the drying chamber ($n = 3$). The air velocity readings in drier were taken in parallel to weight reduction measurements and sampling for chemical analyses.

(4) Weight reduction: Tagged samples were carefully weighed using a Sinbo SMX 4507 model sensitive digital with reading accuracy of ± 0.01 g. Weight changes were converted to weight loss based on initial sample weight ($n = 10$).

Mathematical Formulation

The moisture content on wet basis is the weight of moisture present in the product per unit weight of the product. Instantaneous moisture content "M_t" at any given time "t" on wet basis was computed using Eq. (1) (Shanmugam and Natarajan 2006):

$$M_t = \left[\frac{(M_o + 1)W_t}{W_o} - 1\right] \quad (1)$$

where M_t is the moisture content at any time of drying (wet basis); M_o the initial moisture content (wet basis); W_t is the weight of sample at any time t (g); and W_o is the weight of sample at $t = 0$ (g).

The main characteristic used for estimating drying performance is the drying rate. The drying rate should be proportional to the difference in moisture content between the material to be dried and the equilibrium moisture content. Mathematically, it can be expressed using thin layer (Eq. 2) (Kilic 2008):

$$R = \frac{dM}{dt} = \frac{M_{t+\Delta t} - M_t}{\Delta t} \quad (2)$$

where R is the drying rate at any time of drying; dM the change in mass (g); dt the change in time; $M_{t+\Delta t}$ the moisture content at $t = \underline{t} + \Delta t$; and M_t is moisture content at $t = t$.

Color Measurements

The color intensity of whole capelin was measured on samples collected during drying using the Minolta CR-300 Chroma Meter (Minolta Camera Co., Osaka, Japan) in *Lab** system with CIE IlluminantC. The instrument recorded the L^*, a^* and b^* (values on CIELAB color scale). The L^* value describes the intensity in white color and in black color, the a^* value describes the intensity in red color and in green color, and the b^* value describes the intensity in yellow color and in blue color. Three positions (between the head and the tail) along the lateral line area of the fish were measured. The average L^*, a^* and b^* values of five sample measurements were used to calculate the mean ($n = 15$).

Chemical Determinations

Water Content, Water Activity and Salt Measurements. Actual water content and water activity were analyzed on samples collected during drying. Water content was calculated as the loss in weight during minced sample drying at 105C for 4 h (ISO 1993). Results were expressed as percentage of wet weight ($n = 3$). Water activity (A_w, $n = 3$) values of the minced samples were measured using an Aqua Lab Model cx2 (sensitivity, ±0.003). The salt content (NaCl) of the samples was determined based on AOAC (2000) and expressed as g salt/100 g muscle ($n = 3$).

Total Lipid. Total lipid (TL) was analyzed for raw material (fresh), brined/blanched and dried fishes. TL was extracted from 25 g samples (80 ± 1% water) with methanol/chloroform/0.88% $KCl_{(aq)}$ (at 1/1/0.5, v/v/v) according to Bligh and Dyer's (1959) method. The lipid content was determined gravimetrically and the results were expressed as % wet muscle basis.

Lipid Oxidation Measurements. Lipid hydroperoxides (PV) and thiobarbituric acid-reactive substances (TBARS) were done on samples collected during drying. PV was determined using the ferric thiocyanate method described by Santha and Decker (1994) with modifications according to Karlsdottir *et al.* (2014), except that 3 ± 0.5 g of sample was used instead of 5 g, and subsequent to extraction and centrifuging at 5,100 rpm for 5 min (at 4C), 200 μL of the chloroform layer was collected and mixed with 800 μL of chloroform: methanol solution. The results were expressed as μmol lipid hydroperoxides per kg dry weight.

TBARS were measured as described by Lemon (1975) with modifications. A 3 ± 0.5 g muscle sample was homogenized with 10 mL of trichloroacetic acid (TCA) solution (7.5% TCA, 0.1% propyl gallate and 0.1% Ethylenediaminetetraacetic acid (EDTA) mixture prepared in distilled water) using an Ultra-Turrax homogenizer (Ika Labortechnik, T25 Basic, Staufen, Germany) at 8,000 rpm for 10 s. The homogenate was then centrifuged at 5,100 rpm for 20 min at 4C (TJ-25 Rotor Centrifuge Beckmann, California, USA). A 100 μL of supernatant was collected and mixed with 900 μL of 0.02 M thiobarbituric acid solution in 1.5 mL eppendorfs and heated in a water bath for 40 min at 95C. The samples were cooled down on ice after which 200 μL was placed in duplicate into a 96-well microplate reader (NUNC A/S Thermo Fisher Scientific, Roskilde, Denmark) for absorbance reading at 530 nm (Sunrise Micro Plate Reader, Tecan GmbH, A-5082 Grödig, Austria). The results were expressed as μmol of malomaldehyde diethylacetal/kg dry weight, calculated based on a standard curve prepared using tetraethoxypropane.

Statistical Analysis

Analysis of variance (ANOVA) of the results was performed in the statistical program NCSS 2000 (Number Cruncher Statistical System (NCSS) Utah, USA). The program (ANOVA) calculates multiple comparisons using Duncan's test to determine if sample groups are different. Significance of difference was defined at the 5% level.

RESULTS AND DISCUSSION

Drying Characteristics

In describing the low temperature drying behavior of capelin fish samples during the single layer drying process, the following drying characteristics were taken into consideration: drying air temperature, relative humidity, moisture content, water activity and drying time.

The average air temperature and humidity readings were varied during drying (Fig. 1) using a predetermined program used for commercial fish drying (Vestfirska Hardfisksalan). At the beginning of the drying process, the air temperature was about 19.00 ± 0.5C and periodically increased attaining 25.00 ± 0.5C toward the end of drying. Conversely, air humidity was 76% at the beginning with a declining trend as drying time progressed, corresponding to increase in temperature, until the end of drying when it was about 45%. The low temperature and high humidity at drying onset were ensued to ostensibly prevent the formation of dry surface layer, trapping the moisture interior and causing case hardening. This is because case hardening caused by quick drying during constant rate period could be prevented by slowing down the drying rate since samples were whole with skin on. The maximum temperature of 25.00 ± 0.5C reached during drying was decided upon to prevent protein denaturation that could result in nutrient degradation (Bellagha *et al.* 2002; Lewicki 2006). The relative humidity was however decreased with drying time to continue dewatering process to the desired moisture level. Low relative humidity enhances water evaporation from the surface and in return increases diffusivity from the interior of the fishes that are becoming drier because of the higher sensible heat of the drying air. It is important to note that during drying, the air velocity in the dryer was kept constant, averaged at 3.6 m/s.

It took 184 h to dry capelin to equilibrium moisture/weight under the aforementioned temperature, humidity and air velocity (Fig. 3). The moisture content of capelin groups was high (>75%) during the initial phase of the drying. Specifically, the groups had different initial moisture contents that varied considerably during drying and at equilibrium (Fig. 3). The reported disparity in moisture content was a function of lipid content (C1 and C2) as well as blanching. The C1-BR and C1-BL groups had an initial

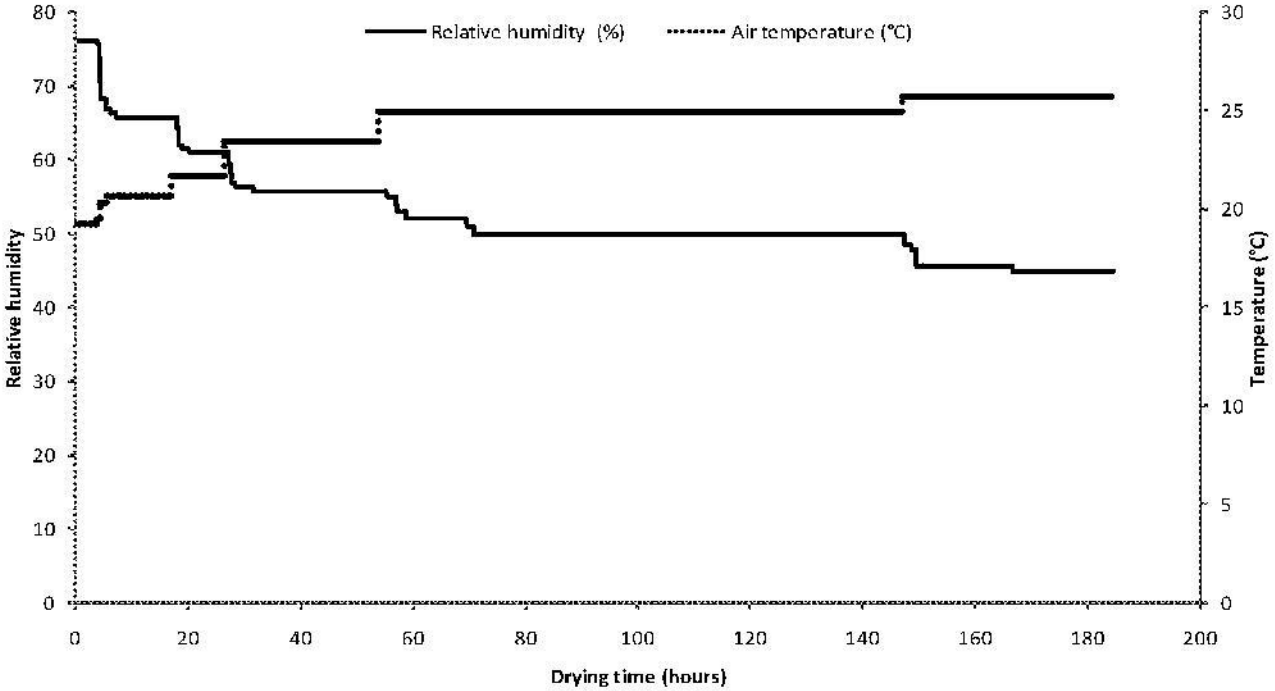

FIG. 1. VARIATION OF AIR TEMPERATURE AND RELATIVE HUMIDITY IN THE DRIER VERSUS TIME IN HOURS DURING DRYING OF BRINED AND BLANCHED CAPELIN ($N = 3$)

moisture content of approximately 76 and 74%, and the moisture at equilibrium of 20 and 13% accordingly. On the other hand, the C2-BR and C2-BL groups had an initial moisture content of approximately 79 and 76%, and the moisture at equilibrium of 19 and 11%, respectively. In this regard, it is obtained that the moisture loss of fish with high lipid content, C1-BR and C1-BL, was slower than in counterpart groups that had lower lipid content (C2). As a consequence, it can be said that fish with lower lipid content (C2 groups) lost moisture faster during drying even when the groups' moisture content was evidently less than that of high lipid groups at a later drying time. More so, it can be understood that particularly lipids played an important role as a limiting factor in the drying steps either by replacing the aqueous phase that serves as vector for transfers or acting as a physical barrier to heat transfer that causes the evaporation of water and/or the moisture diffusivity from capelin interior to the surface.

On the other hand, blanched (BL) groups recorded higher moisture reduction than brined (BR) groups. After 17 h of drying, a clear grouping based on drying pretreatments was evidenced with blanched groups recording significantly lower moisture contents. This was attributed to denaturation/aggregation and possibly hydrolysis of myofibrillar proteins that might have occurred during blanching, hence affecting muscle water holding. More so, high temperature encountered in blanched groups (BL) may have increased the activity of the water molecules leading to higher moisture diffusivity in addition to the lipid leached out during cooling, compared to capelin brined at 5% salt for 2 h. At the end of drying, the moisture content was different in all groups, but significantly ($P < 0.05$) between blanched (C1-BL and C2-BL) and brined (C1-BR and C2-BR) groups. The difference in moisture at equilibrium between the groups can be reinforced by the fact that the equilibrium vapor pressure on the surface of a food is determined not only by the temperature and the water content in food but also by the interactions of water molecules with solutes.

Corresponding to water content, water activity (A_w) values reduced as drying time progressed but differently by groups (Fig. 3). At drying onset, A_w values were not significantly different ($P > 0.05$). However, during drying blanched (BL) groups that had high moisture reductions and high salt content (Fig. 1) recorded low A_w. Furthermore, it was conspicuous toward the end of drying (moisture at equilibrium, 184 h) that groups had significantly different A_w, even in those under similar drying pretreatment. Here, it can be said that particularly salt content (Fig. 2) contributed to the differences within the same drying pretreatment because salt as a solute is known to decrease the A_w of foods due to its binding effects on free water (Grummer and Schoenfuss 2011). The water activity (A_w) was on average between 0.7 and 0.6 in all experimental

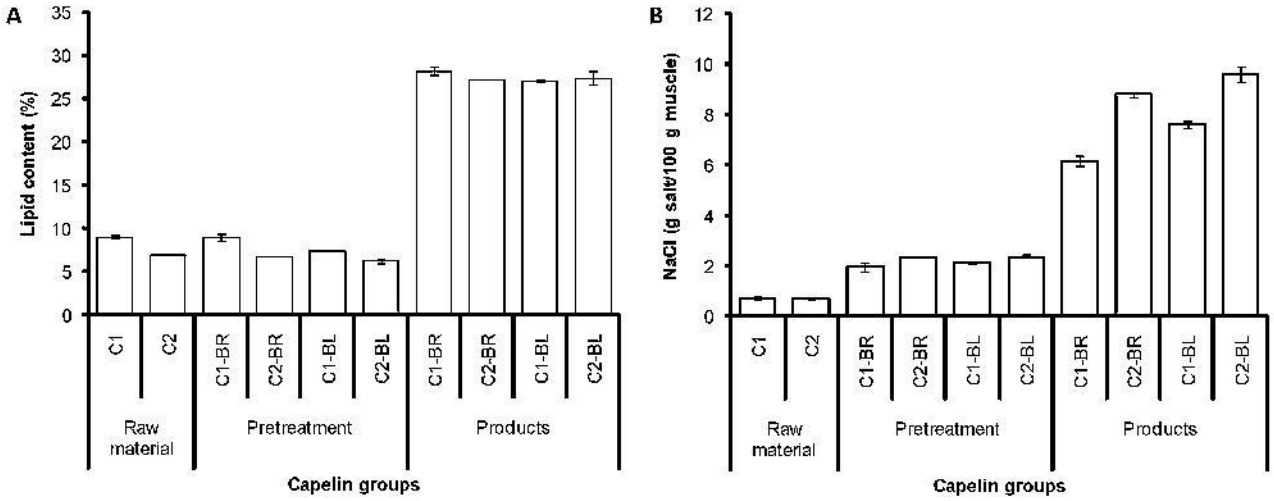

FIG. 2. VARIATION OF LIPID CONTENT (A) AND SALT CONTENT (B) IN BRINED (BR) AND BLANCHED (BL) CAPELIN FISH AS A FUNCTION OF DRYING TIME ($N = 3$)
C1, capelin with high lipid level; C2, capelin with low lipid level.

groups. It is well known that the intermediate moisture foods contain moisture content of 15–50% with A_w of 0.60–0.85 (Kilic 2008).

The instantaneous moisture content (M_t) and drying rate during cold air capelin drying were calculated using Eqs. (1) and (2) based on the weight reduction measurements. The drying rates were calculated from drying data by estimating the change in moisture content, which occurred at each successive time interval and was expressed as g water/h (Table 2). The drying rate was high at the beginning but slowly decreased with the reduction in moisture content. The trends were similar but occurred differently by groups, with blanched (BL) and low lipid (C2) groups recording higher drying rates compared to counterpart brined (BR) and high lipid (C1) in early drying times. After 17 h, the drying rate was not significantly different ($P > 0.05$) between the groups except in low lipid blanched (C2-BL) that differed from others at 72 h. Toward the end of drying (160–184 h), the drying rate had reduced to ≤0.05 g water/h 100 g fish in all groups. The findings portray more rapid dewatering took place in the first 48 h, but intense in blanched and low lipid groups compared to brined and high lipid counterparts. This was due to high moisture content and corresponding lower binding energy of water at the beginning of drying and also blanching effects that resulted in higher drying rates owing to the higher moisture diffusion. However, in the late period of drying generally characterized by a slow decreasing drying rate, moisture transfer takes place largely by diffusion that is slower due to the fact that the drier the product, the further the water must diffuse to reach the surface. The drying time of 184 h (moisture at equilibrium) obtained is long compared to results reported elsewhere though on dissimilar fish (Jain and Pathare 2007; Kilic 2008; Reza *et al.* 2009; Oduor-Odote *et al.* 2010a; Omodara and Olaniyan 2012). This can be assumed to be largely due to low drying temperature and humidity used in the present study, in addition to difference in fish chemical composition as fish species were different.

TABLE 2. VARIATION IN DRYING RATE (G WATER/H) IN 100 G BRINED AND BLANCHED CAPELIN FISH AS A FUNCTION OF DRYING TIME ($N = 10$)

	Sampling time interval (Δt) during drying						
Group	17	7	24	24	40	48	24
C1-BR	1.58 ± 0.14^{a}	0.77 ± 0.08	0.43 ± 0.03	0.15 ± 0.02^{a}	0.12 ± 0.03	0.07 ± 0.03	0.04 ± 0.02
C1-BL	1.71 ± 0.14^{ab}	0.74 ± 0.04	0.33 ± 0.04	0.15 ± 0.02^{a}	0.08 ± 0.03	0.05 ± 0.04	0.01 ± 0.00
C2-BR	1.72 ± 0.11^{ab}	0.73 ± 0.08	0.38 ± 0.02	0.16 ± 0.03^{a}	0.14 ± 0.04	0.09 ± 0.03	0.03 ± 0.01
C2-BL	1.86 ± 0.13^{b}	0.67 ± 0.01	0.35 ± 0.04	0.22 ± 0.03^{b}	0.11 ± 0.02	0.04 ± 0.03	0.01 ± 0.00
***P* value**	**0.046**	0.862	0.079	**0.045**	0.065	0.095	0.575

Different letters (superscript) indicate significantly different values between samples within column.
Numbers in bold indicate significant values at $P < 0.05$.

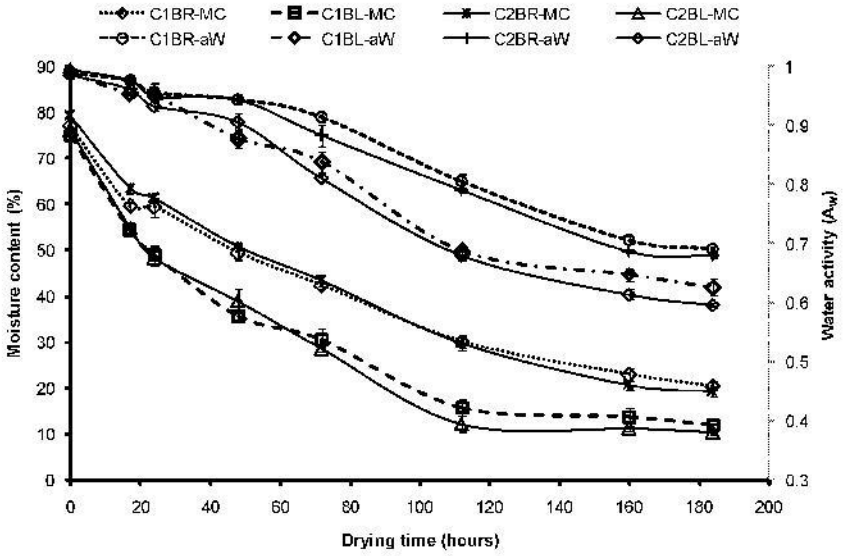

FIG. 3. VARIATION IN MOISTURE CONTENT (M_c, $N = 3$) AND WATER ACTIVITY (A_w, $N = 3$) IN BRINED (BR) AND BLANCHED (BL) CAPELIN FISH AS A FUNCTION OF DRYING TIME
C1, capelin with high lipid level; C2, capelin with low lipid level.

TL and Salt Content

The lipid content for raw fish was significantly ($P < 0.05$) different between the groups with high lipid group (C1) constituting 9% muscle lipid, whereas low lipid group (C2) had 7% (Fig. 2A). As understood from these data, the difference of about 2 weeks between catch periods for capelin groups led to 2% lipid difference, which is supported by Montevecchi and Piatt (1984) who reported lipid levels in capelin to vary considerably within and between the seasons. Upon drying pretreatments, the lipid content for high lipid blanched group (C1-BL) differed significantly ($P < 0.05$) from the raw material as well as the counterpart brined (C1-BR), whereas low lipid blanched group (C2-BL) did not differ ($P > 0.05$) from the group's raw material and brined counterpart (C2-BR) even though it recorded a low value. This indicates the lipid loss phenomenon was intense with blanching and more so in C1-BL than in brined groups. As a consequence, it can be said that some lipids were exuded during blanching and/or with the drip water while cooling prior to drying onset. However, in the end products lipid content did not differ significantly ($P > 0.05$) between the groups, with values ranging between 27 and 28% (Fig. 2A). This can be explained by the extent of dryness by the groups, as low lipid groups (C2) as well as blanched (BL) groups that recorded low lipid after pretreatment were more dewatered at the end of drying as observed with the groups moisture content (Fig. 3).

The salt content in raw capelin groups was not significantly different ($P > 0.05$) with values of 0.72 and 0.68 g salt/100 g muscle in high lipid (C1) and low lipid (C2) accordingly (Fig. 2B). However, after pretreatments, an increase in salt content was observed, with higher increments in low lipid groups than high lipid counterparts. This could be due to the difference in groups' initial moisture and salt content as high lipid (C1) had low moisture content of 76% against 78% for low lipid (C2) group, given the moisture content in capelin muscle is inversely related to the lipid (Henderson *et al.*, 1984). This suggests the muscle fluid in low lipid (C2) group had less solute concentration than high lipid (C1) resulting in more salt ion diffusion into C2 than C1 under 5% fine NaCl-salt solution for 2 h. On the other hand, dried capelin recorded salt contents that were significantly different ($P < 0.05$) between the groups based on the lipid content as well as drying pretreatment. Low lipid (C2) and blanched (BL) groups had higher salt content than counterparts' C1 and BR that was mainly attributed to the extent of dryness as reflected in groups' moisture content. The disparity in moisture content (dryness) observed between brined and blanched capelin fishes may be attributed to denaturation/aggregation and possibly hydrolysis of myofibrillar proteins that could have occurred during blanching, hence affecting muscle water holding.

Lipid Oxidation and Color Changes

Lipid peroxidation, corresponding to the oxidative deterioration in capelin, had similar trends of decreasing at drying onset followed by an increasing and subsequently decreasing values (Fig. 4A). These changes occurred at different times by the group based on drying pretreatment and lipid content. At early drying time (17 h), when moisture content and drying rates were high, PV was observed to decrease in all groups. However, at 24 h of drying when moisture content was about 48% and A_w was 0.95 (reduced drying rate) in blanched (BL) groups, the PV increased rapidly to maximum values of 370 and 281 μmol/kg in C1 and C2 accordingly. However, in brined (BR) groups it attained maximum values at 48 h for

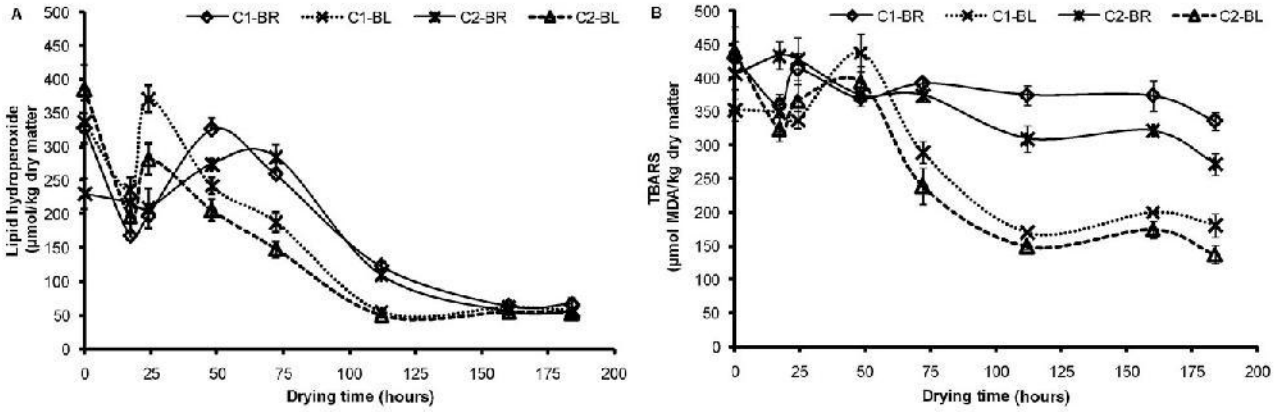

FIG. 4. LIPID HYDROPEROXIDE FORMATION (A) AND THIOBARBITURIC ACID REACTIVE SUBSTANCE FORMATION (B) IN BRINED (BR) AND BLANCHED (BL) CAPELIN FISH AS A FUNCTION OF DRYING TIME ($N = 3$)
C1, capelin with high lipid level; C2, capelin with low lipid level.

C1 and 48–72 h of drying for C2 (325 and 285 μmol/kg) when the groups' moisture content and A_w were 50–45% and 0.944–0.92 accordingly. The result suggests that there could be major changes in the capelin muscle structure at that moisture content, leaving the lipids more accessible to oxygen and catalysts than at neither higher nor lower water content and/or drying rates instigating the formation of hydroperoxides. It can also be said that PV accumulation as well as decomposition was faster in blanched (BL) and high lipid groups (C1). After the maximum values (below 45% moisture content), the PV decreased gradually in all groups to about 53 μmol/kg at the end of drying. The observed decrease in PV is due to hydroperoxides that are readily decomposed into a wide range of carbonyl compounds, hydrocarbons, ketones and other materials that contribute to off-flavor of foods (Undeland *et al.* 1999). As lipid hydroperoxides together with the free radicals have been reported to have no impact on the sensory properties (Stapelfeldt *et al.* 1997), the observed PV changes could be of concern only when considering its decomposition to secondary products of oxidation.

Unlike with PV, TBARS tended to reduce as drying time progressed except in blanched groups (BL) that recorded an increase at 24 h of drying (Fig. 4B). After 24 h drying, the reductions were rapid in blanched than brined groups. TBARS reduction may be explained by the malomaldehyde capability of cross-linking amino acids to form amidine linkages and/or its interactions with other components of fish which are the end products of lipid oxidation (Undeland *et al.* 1999). The secondary lipid oxidation products that were measured as TBARS have been reported to have a profound impact on both sensory and functional properties of foods (Stapelfeldt *et al.* 1997). This indicates the brined groups that showed less change in TBARS during drying could have maintained the sensory properties of dried fish than blanched groups.

Changes in the color (L^* and b^* values) of brined (BR) and blanched (BL) capelin during drying are shown in Fig. 5A,B. Generally, capelin color was significantly ($P < 0.05$) affected upon blanching and during drying. At the beginning of drying, L^* value (lightness) was significantly ($P < 0.05$) different between groups based on pretreatment, with blanched groups obtaining low values. During drying, lightness showed a tendency of decreasing in all groups up to 112 h which after it tended to increase, while steadying toward the moisture at equilibrium. As with lightness, the b^* value (yellowness) was significantly ($P < 0.05$) affected by blanching and exhibited similar trends to lightness during drying. The difference between groups upon treatment was mainly due to decreased lightness and increased yellowness that occurred during blanching resulting from lipid degradation observed in Fig. 2 as well as PV and TBARS values.

On the other hand, the decrease in lightness that occurred during drying in all groups might have resulted from the discoloration of the flesh surface due to the decrease in water content as the changes were greater during rapid dewatering (high drying rate, 17 h). Nguyen *et al.* (2012) associated the decrease in lightness in salted cod with brownish discoloration of the flesh surface and the decrease in water content affecting the light refraction of the muscle. Yellowness associated with lipid oxidation and enzymatic browning differed significantly during drying based on treatment, with blanched groups recording stronger color that is in agreement with accelerated PV and TBARS formation and decomposition in the group. However, as regards the lipid content, samples within the same treatment did not differ significantly ($P > 0.05$), even though high lipid groups showed a tendency of being more yellow. This suggests that during drying the yellowness might have been caused mainly by enzymatic browning since a weak correlation between yellowness and PV ($r \leq 0.02$), and TBARS

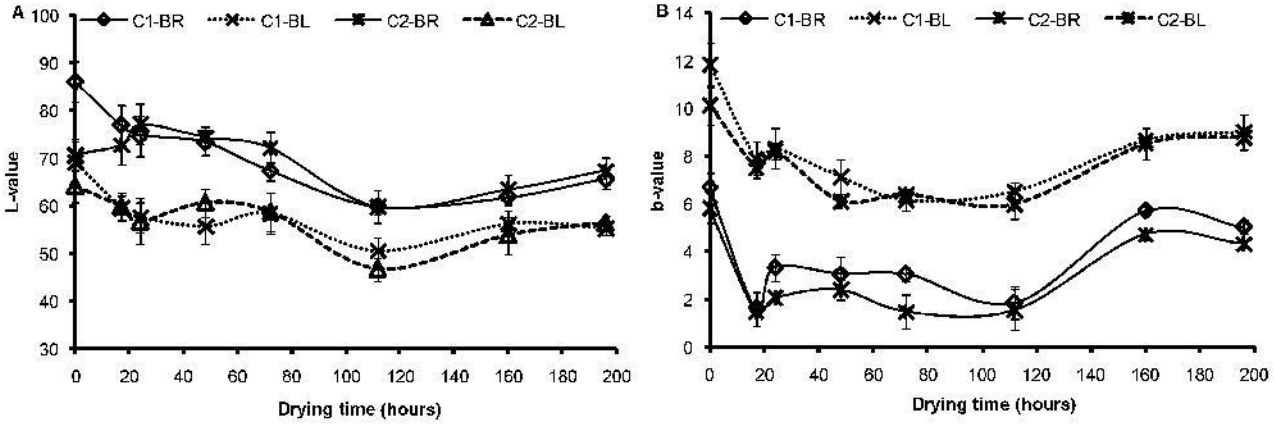

FIG. 5. CHANGES IN THE COLOR PARAMETERS; LIGHTNESS (A) AND YELLOWNESS (B) IN BRINED (BR) AND BLANCHED (BL) CAPELIN FISH AS A FUNCTION OF DRYING TIME ($N = 15$)
C1, capelin with high lipid level; C2, capelin with low lipid level.

($r \leq 0.05$) was observed in all groups. More so, the 2 min blanching time employed might have left majority of enzymes responsible for browning indentured. The red-green color (a^* value not presented) changes were not statistically different between the groups and during drying. Color has been an important quality attribute of food products that influences consumer choices (Murat and Onur 2000); blanching of capelin for drying as practiced with small fish products sold in intended markets resulted in undesirable color and is therefore not recommended.

CONCLUSIONS

Lipid content significantly affected the drying rate and product moisture at equilibrium, with low lipid capelin exhibiting faster drying and lower moisture at equilibrium. Furthermore, blanching accelerated moisture loss because of the decreased lipid and protein denaturation that affected muscle water holding, but the treatment resulted to undesirable yellowness (higher b^* values) and faster lipid oxidation. As a result, blanching as practiced prior to drying of other small fish dried products in the intended markets is not recommended for capelin drying. Although all dried capelin groups attained water activity (<0.7) sufficient to inhibit spoilage bacteria, brined low lipid capelin demonstrated more stability in quality during drying. In continuation of the findings of this research, low lipid dried capelin acceptability and quality during storage should be evaluated.

NOMENCLATURE

dM	change in mass (g)
dt	change in time (h)
Δt	sampling time interval (h)
t	drying time (h)
M_c	Moisture content (wb)
M_o	initial moisture content (wb)
M_t	moisture content at any time of drying (wb)
R	drying rate in g/h.
W_e	weight of fish at equilibrium state (g)
W_o	weight of sample at $t = 0$ (g)
W_t	weight of sample at any time t (g)
$M_{t+\Delta}$	moisture content at time $t + \Delta t$ (wb)

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Conformational changes in Capelin (*Mallotus villosus*) proteins during smoking and drying

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Food Chemistry

Under review

Conformational changes in Capelin (Mallotus villosus) proteins during smoking and drying

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Abstract

Effects of lipid content and blanching in brine and brining on conformational changes in capelin proteins during drying and smoking were studied. Changes in salt soluble proteins (SSP), sulfhydryl groups (SH) content and disulfide bonds (S-S) were monitored in soluble protein fractions. Salt and water content was also measured. Significant ($p<0.05$) changes in protein conformation occurred during blanching and early drying when water content and dehydration rates were relatively high. SSP and total SH content were reduced while available SH and disulfide content increased. These changes can be explained by protein denaturation and aggregation due to high temperature and dehydration. Protein denaturation resulted in reduced protein solubility thereby compromising nutritional value due to loss of thermolabile compounds and yield during hot smoking. Protein aggregates that were highest in blanched dried fish might have reduced dried fish eating quality. Higher lipid content had a protective effect on protein changes.

Key words: Capelin, heating, drying, protein conformation

1 Introduction

Drying and smoking are widely used to preserve fish in developing countries (Akintola, Brown, Bakare, Osowo, & Omolola, 2013; Darvishi, Azadbakht, Rezaeiasl, & Farhang, 2013; Bellagha, Sahli, Farhat, Kechaou, & Glenza, 2007). Small fish species are mostly consumed dried (Oduor-odote, Shitanda, Obiero, & Kituu, 2010; Darvishi et al., 2013). Dried fish is also used in the production of weaning foods for children (IOC, 2012). In some countries fish is partially boiled in brine before drying. Blanching of fish results in the denaturation of proteins and speeds up the drying process (Cyprian et al., 2015a), but it also influences flavor and inhibits microbial and enzymatic activity (Omodara & Olaniyan, 2012).

Capelin (*Mallotus* v*illosus*) a small arctic fish, supports one of the most important pelagic fisheries in Iceland with annual catches exceeding half a million tons in recent years (Statistics Iceland, 2015). About 80% of the catch is reduced to fish meal and fish oil (Statistics Iceland, 2015). The proportion of capelin used for human consumption could be increased by drying and smoking, and marketing in countries where such fish products are traditionally consumed (Cyprian et al., 2015a; Cyprian et al., 2015b).

Fish constitutes an important source of high quality and nutritious protein. Fish proteins have also been identified as a rich source of bioactive peptides that differ in size, composition and sequence of amino acids, whose quality depend on stability (Ren et al., 2008). Fish proteins are less stable than those derived from mammals (Baylan et al., 2015; Poulter Ledward, Godber, Hall, & Rowlands, 1985) and processing methods can affect their denaturation and aggregation, particularly if it involves heating (Ghelichpour & Shabanpour, 2011). Fish protein denaturing temperature mainly depends on the species, but also on water content as protein stability increases at low water content (Rustad & Nesse, 1983). Tropical fish species contain proteins that are thermally more stable than proteins of temperate species (Poulter et al., 1985) (Meneshi Harwood, & Grant, 1976). Murueta, Toro, & Carreño (2007) reported about 90% of tropical fish proteins to be denatured at temperatures between 60-65°C.

Protein denaturation and aggregation is associated with conformational changes and the formation of disulfide bonds. These include changes in the reactive groups, mainly loss of hydrophilic surface, exposure of hydrophobic areas and sulfhydryl groups that are buried or

blocked in native proteins (Nguyen, Thorarinsdottir, Gudmundsdottir, Thorkelsson, & Arason, 2011; Baylan et al., 2015; Raman & Mathew, 2014). Secondary and tertiary structures of proteins can be lost during heating due to the split in hydrogen bonds, resulting in unfolding of native conformation (Baylan et al., 2015; Raman & Mathew, 2014). The conformation of proteins plays an important role in defining their functional properties (Ghelichpour & Shabanpour, 2011). Therefore, changes in protein conformation can lead to the loss of some of their physical and chemical properties (Skipnes, Plancken, Loey, & Hendrick, 2008; Rustad & Nesse, 1983), affecting nutritional value (for instance loss of amino acids) and water holding capacity that results in a change in texture of the product (Raman & Mathew, 2014).

The effect of partial boiling (blanching) and lipid content on proteins during drying and smoking is poorly understood. Moreover, dried and smoked small pelagic species traded in developing countries are unlike capelin, commonly warm water species. The effect of blanching, drying and smoking on the fish eating quality specifically texture, nutritional value and yield that are affected by changes in proteins might be greater in capelin than warm water species. The objective of this study was to evaluate the effects of brining and blanching in brine on changes in the capelin proteins during drying and smoking and how the changes are affected by variation in lipid content. Conformational changes in proteins were evaluated through measuring of salt soluble proteins (SSP), sulfhydryl (SH) groups and disulfide bonds (S-S) during drying and smoking. Knowledge of the influence of blanching in brine and brining and lipid content on changes protein would be useful in determining appropriate pre-treatment and lipid level for preserving thermolabile compounds while maintaining yield and eating quality during smoking and eating quality of dried fish.

2 Materials and methods

2.1 Raw material, processing and sampling

Two batches of 32± 4g capelin (*Mallotus* v*illosus*) were obtained from HB Grandi fishing company, Reykjavik, Iceland. Fish were caught on 13th February and 7th March, 2013 and kept in chilled seawater for two days prior to grading and freezing in 25 kg blocks. The blocks were kept frozen for three months at -25°C until the time of study. At that time, fish were thawed overnight in open air at 18-20°C, after which each batch was divided into four equal portions. Two of the

portions were pre-treated either by brining or blanching in brine before drying. The other two portions were both brined and either cold or hot smoked. Portions for brining were immersed separately in 5% NaCl-salt solution for 2 hours at 2°C to attain about 2% salt content in the fish muscle and thereafter arranged in a single layer on meshed trays to drip dry. The trays were then stacked on drying and smoking racks. Portions to be blanched were put in perforated metallic pans and immersed in boiling 5% fine NaCl-salt solution for 2 minutes before removing and spreading on meshed trays to cool and drip dry.

Drying was done in a tunnel drier under controlled temperature (22±2°C), relative humidity (40±5%) and air speed (3±0.5 m/s) at Vestfirska Hardfisksalan fish drying company in Reykjavik, Iceland. Sixteen capelin pieces (8 pooled together, in 2 bags) per group were taken each time during drying (0, 17, 24, 48, 72, 112, 160 & 184 hours). Smoking was done in a conventional smoking kiln equipped with an automatic control for temperature, humidity and density of wood smoke. Sixteen capelin pieces (8 pooled together, in 2 bags) per group were taken at refreshing time after the second cycle (55-60 minutes) in the first and second stages, and at the end of the third stage during hot smoking (0, 1, 2, 3, 4 & 4.5 hours) as describe by Cyprian et al. (2015b). During cold smoking, samples were taken at refreshing time after the second, fourth and sixth cycles, and at the end of smoking (0, 1, 2, 3 & 4 hours). Samples were packed in polyethylene bags, frozen and stored at -80 °C until analysed.

2.2 Water, salt and lipid content

Water content of raw materials and products was determined as the difference in weight of minced samples before and after drying for 4 hours at 103±1 °C (ISO, 1993a). The results were expressed as g water/100 g mince. The salt content (NaCl) in the samples was determined based on AOAC (2000) and expressed as g salt/100 g mince. Lipid was extracted from 25 g of mince (80±1% water) with methanol/chloroform/0.88% $KCl_{(aq)}$ (at 1/1/0.5, v/v/v) according to the Bligh & Dyer (1959) method. The lipid content was determined gravimetrically and the results were expressed as g lipid per 100 g mince.

2.3 Extraction of salt soluble proteins (SSP)

Salt soluble proteins (SSP) were extracted by the method of Kelleher & Hultin (1991) with a modification of a buffer. Capelin was minced in a Braun Mixer (Type 4262, Germany) for 2 to 3 minutes and 10 g of the mince added to 190 ml of salt solutions (1 M NaCl and 0.02 M Na_2CO_3, pH 7.0). The mixture was homogenized in an Ultra-Turrax homogenizer (Ika Labortechnik, T25 basic, Staufen, Germany) at 6000 rpm for 1 minute. The homogenate was kept on ice for 1 hour and centrifuged at 0–5 °C for 15 minutes at 10,000 rpm using an Avanti Centrifuge J-20 XPI (Beckmann Coulter, Fullerton, California, USA). Protein solubility was evaluated by quantifying the amount of solubilized protein in the supernatant. The Bradford (1976) method, adapted to micro-assays using bovine serum albumin as a standard was used. The diluted supernatant and the Bradford reactive were placed in a 96-well micro-plate and absorbance measured at 595 nm in a Sunrise Micro plate Reader (Tecan GmbH, A-5082 Grödig, Austria).

2.4 Disulfide bond content of protein

The disulfide bond content was assayed using 2-nitro-5-thiosulfobenzoate (NTSB), according to the method of Thannhauser, Konishi, & Scheraga (1984). The supernatants from NaCl protein extractions were diluted to protein concentration of 0.1 mg/ml. Three ml of freshly prepared NTSB assay solution, pH 9.5 (adjusted with 0.1M HCl or 0.1M NaOH) were added to 0.25 ml protein solution (0.1 mg/ml) in a 4.5 ml cuvette. The mixture was incubated in a dark place at room temperature for 25 minutes before reading the absorbance at 412 nm (UV- 1800 spectrophotometer, Shimadzu, Kyoto, Japan). The disulfide content was calculated using a molar extinction coefficient of 13,900 M^{-1} cm^{-1} and results expressed as µmol SS/g protein.

2.5 Sulfhydryl group content of protein

The sulfhydryl groups contents were determined using 5,5'-dithiobis-(2-Nitrobenzoic acid) (DTNB), according to the method of Beveridge, Toma, & Nakai (1974). The supernatant from NaCl protein extractions were diluted to the concentration of 0.1 mg/ml that were used in analyzing total sulfhydryl (SH) groups and available sulfhydryl groups. For the total SH content, 0.5 ml of the protein solution were added to 2.5 ml of Tris–SDS buffer pH 8.0 (0.1 M Tris, 3 mM EDTA, 3 % sodium dodecyl sulphate (SDS), 0.1 M glycine and 8 M urea). To the mixture, 0.1 ml of Ellman´s reagent (2 mM 5,50-dithio-bis-(2-nitrobenzoic acid)) were added and mixed

before incubating in a dark place at room temperature for 30 min. After incubation, the solutions absorbance was measured at 412 nm (UV- 1800 spectrophotometer, Shimadzu, Kyoto, Japan). Total SH content was calculated using a molar extinction coefficient of 13,900 M^{-1} cm^{-1}. The available SH content was determined as with total SH content except that 0.5 ml of protein solution was added to 2.5 ml of Tris buffer pH 8.0 (0.1 M Tris, 3 mM EDTA, 0.1 M glycine and 8 M urea). The SH content was expressed as µmol SH/g protein.

2.6 Data analysis

Results are expressed as means ± standard deviation. Statistical differences between treatments were analyzed using one way analysis of variance (ANOVA) with Duncan's Post-hoc test using statistical program NCSS 2000 (NCSS, Utah, USA). Pearson correlation analysis was performed on means of the variable values. P values of <0.05 were considered significant.

3 Results and discussion

3.1 Lipid, water and salt content

Capelin caught in mid February (13^{th}) had a lipid content of 9.1% and declined to 7% in early March (7^{th}) hereafter being referred to as fatty and less fatty batches. The corresponding water content increased from 76.8% to 78.2% (Table 1). Generally, there is an inverse relationship between lipid and water contents in fish. Blanching in brine significantly reduced the lipid content (Table 1). This might be due to exudation of lipid during blanching and drip drying (Cyprian et al., 2015a). As with the lipid, water content was significantly reduced after blanching, a phenomenon attributed to reduced water holding capacity due to denaturation and possible hydrolysis of myofibrillar proteins that might have occurred during blanching. Salt content of raw capelin was not significantly ($p > 0.05$) different between batches with values of 0.72 and 0.68 g salt/100 g mince in fatty (C1) and less fatty (C2) capelin respectively (Table 1). After brining and blanching in brine, salt content increased significantly ($p < 0.05$), but higher increase was observed in less fatty and blanched brined capelin. This could be due to differences in raw material water and salt content. Less fatty capelin had higher water content suggesting that muscle fluid had less solutes concentration resulting in more diffusion of salt ions than in fatty capelin under similar brine concentration (5% NaCl). Higher salt content in less fatty

capelin after brining and blanching in brine could also imply that lipids act as a barrier to salt uptake since the uptake was slower in fatty capelin.

Drying trials were carried out using both brined and blanched brined capelin, whereas, only brined capelin was used in smoking. After both drying and smoking, lipid and salt content increased while water content was reduced (Table 1). Lipid and salt content increased more in dried than in smoked capelin, a fact ascribed to the dehydration level as smoked capelin retained more water than dried capelin. There were also differences between fatty and less fatty capelin within the same processing method. Less fatty capelin became drier than fatty capelin and blanched brined capelin became drier than brined capelin. Hot smoked capelin lost more water than cold smoked capelin. It's worth noting that when dried, brined and blanched brined capelin had similar lipid content (not significant $p > 0.05$), but differed in water and salt contents. Similar lipid content might be explained by the dehydration level and loss of lipid during blanching and drip drying. The relative amount of lipid increased as the fish became drier. Blanched brined capelin lost more water ending up with similar lipid content as brined capelin. Salt content differed significantly ($p < 0.05$) between the treatments. The drier capelin became the higher the salt content.

3.2 Changes in protein solubility (salt soluble proteins)

Lipid content, pre-treatment and smoking methods affected protein solubility in capelin but the occurrence differed (Figure 1 A, B). Protein solubility measured as salt soluble protein (SSP) was reduced in all groups as drying progressed and was lowest in blanched brined less fatty capelin (Figure 1 A). SSP were significantly ($p < 0.05$) reduced by blanching in brine, while SSP of brined capelin were not significantly ($p > 0.05$) different from the raw material. The decrease in SSP after blanching in brine and during drying is thought to be due to conformational changes in the proteins. Denaturation and hydrolysis of myofibrillar proteins result in increased hydrophobicity. This might have occurred during heat processing thereby reducing the content of thermolabile compounds in blanched brined capelin (Ghelichpour and Shabanpour, 2011; Finot, 1997). Even though blanched brined capelin contained more salt than brined capelin (Table 1), salt content was not significantly ($p > 0.05$) different between groups. Therefore influence of salt on capelin protein solubility was not detected mainly due to low salt content after treatments (<

2.5%), as SSP in brined capelin were not significantly different from the raw materials. Similar results were obtained prior to smoking where only brined capelin batches were used (Figure 1 B).

Drying may have altered protein-water interactions, exposing protein molecules to an environment that is less polar than water. When water content is reduced, the distance between protein chains diminishes thereby increasing the formation of cross-linkages between them (Rustad and Nesse, 1983). These cross-linkages can result in a tighter network of proteins perturbing their structure and integrity leading to the formation of intrinsic protein particles or aggregates (Wang, Nema, & Teagarden, 2010; Fennema, 1977) reducing solubility. Reduced protein solubility may also be linked to the lipid oxidative and degradative products such malonaldehyde that form complex interactions through cross-linking with protein components mainly peptides and acid amines (Underland et al., 1999) resulting in protein denaturation and deterioration. According to Cyprian et al. (2015a) secondary lipid oxidation products such as malonaldehyde were higher at the start of drying but decreased with time. The authors associated the decrease in secondary lipid oxidation products to their interactions with protein components giving rise to tertiary lipid oxidation products.

Hot smoking was divided into three stages of 2 hours preliminary smoking at 30°C, followed by 2 hours smoking at 40°C and 30 minutes smoking at 75°C (Cyprian et al., 2015b). Cold smoking was conducted for 4 hours at 24°C. Protein solubility was less influenced by brining and was apparently lower in less fatty than fatty capelin (Figure 1 B). Protein solubility remained stable during smoking in both treatments except for slight increase during the first two hours of smoking and significantly lower values obtained in the batches towards the end of hot smoking. The slight increase in SSP during the first two hours of smoking might be due to protein hydrolysis (caused by enzymatic activity) into components of less molecular weight (Stoknes et al., 2005). In general, the results indicate that during early smoking when the temperature in both cold and hot smoking was equal to or less than 30°C, slight protein denaturation occurred and the difference in protein solubility could mainly be accounted for by differences in lipid content. Lipids seem to have a protective effect on protein as protein solubility was highest in fatty capelin. This may be due to the low heat transfer coefficient of lipids, leading to protection of the proteins during drying and smoking. Dyer and Dingle (1961)

reported lower protein extractability in smoked lean fish (< 1% fat) than fatty fish (3-10% fat). Lipid acted as a barrier to salt uptake in the current study with fatty fish obtaining lower salt content than less fatty fish. Blanching in brine accelerated fish salt uptake when compared to brining. Salt is known to have a major effect on fish protein solubility and water holding capacity (Nguyen, Thorarinsdottir, et al., 2011) and could have contributed to low protein solubility especially as drying progressed in blanched brined capelin.

Protein solubility decreased in both batches towards the end of hot smoking, in particular during the last 30 minutes when temperature was increased from 40°C to 70°C (Figure 1 B). Murueta et al. (2007) reported about 90% of fish proteins to be denatured between 60-65°C. In the current study, protein denaturation appeared to have started at 40°C but the rate of denaturation increased significantly at 75°C. Protein denaturing temperature in fish is mainly influenced by protein type but also the species. Proteins of tropical fish species are more thermally stable than proteins in temperate species (Poulter et al., 1985) and generally the stability of tissue proteins increases with increasing habitat temperature (Meneshi et al., 1976). Capelin is a high latitude species which may explain why protein denaturation occurred from a temperature of 40°C in the present study. Protein solubility was stable during cold smoking implying the changes in solubility observed during hot smoking were primarily influenced by temperature rather than salt content which was less than 5% in all groups. A significant positive correlation was obtained between SSP and water content (Table 2) during drying and smoking emphasizing that protein solubility decreased with decreasing water content, with stronger correlation during drying ($r = 0.71$) than smoking ($r = 0.61$). SSP had the strongest correlation with temperature during smoking ($r = -0.82$) indicating that protein denaturation during smoking was primarily influenced by temperature.

3.3 Changes in sulfhydryl (SH) and disulfide bond content

Total sulfhydryl (SH) content of the soluble proteins generally decreased during drying and smoking, except during cold smoking where it remained relatively stable (Figure 2 A, B). The decrease in total SH content during drying and hot smoking was understood to be due to the exposure of sulfhydryl groups buried in native proteins making them vulnerable to oxidation (Nguyen et al., 2011; Rawdkuen, Jongjareonrak, Phatcharat, & Benjakul, 2010; Zuazaga,

Steinacker, & Castillo, 1984), even though masking of sulfhydryl groups by protein particles or aggregates due to dehydration could have also contributed. Total SH content of brined capelin was higher than in blanched brined capelin, a tendency maintained throughout the drying process (Figure 2 A). Brined capelin showed a brief increase in total SH content at the start of drying. After about 24 hours of drying, the total SH content decreased as was the case in blanched brined capelin from the onset of drying, with both groups obtaining fairly stable total SH contents towards the end of drying (Figure 2 A).

During drying, brined fatty capelin obtained the highest total SH content than the other groups while blanched less fatty capelin had the lowest total SH. The higher total SH content in brined fatty capelin was contrary to disulfide bond content obtained in the group when compared to blanched less fatty fish (Figure 4 A). Blanching exposes sulfhydryl groups buried in native proteins to reactive oxygen or secondary oxidative by-products, whereas lipid offers protection against denaturation caused by exposure of sulfhydryl groups to reactive oxygen or by-products of lipid oxidation. Blanching then triggers oxidation of sulfhydryl groups with the formation of hydrogen and hydrophobic bonds (disulfide interchanges) (Baylan et al., 2015; Raman & Mathew, 2014; Hsu, Hwang, Yu, & Jao, 2007). The slight increase in total SH content in brined capelin might have been caused by modifications of chemical groups, especially SH due to initial alteration of protein-water interactions. During the early stages of drying surface water evaporated but later on further drying water loss took place by diffusion from the interior to the surface of the fish (Cyprian et al., 2015a) resulting in protein dehydration.

Smoke contains phenolic compounds that have antioxidant properties (Guillen & Errecalde, 2002), implying that changes in sulfhydryl content during hot smoking (Figure 5.6) may have been caused by aggregation of proteins rather than oxidation factors. After hot and cold smoking, capelin showed reduced content in lipid oxidation indicators (Cyprian et al., 2015b). It can therefore be postulated that masking of sulfhydryl groups by intrinsic protein aggregates could have decreased the total sulfhydryl content during hot smoking, since it was relatively stable in the capelin proteins during cold smoking. Significant positive correlation was obtained between SH and water content during drying and smoking, and also with temperature during smoking (Table 2). This supports the explanation that sulfhydryl groups might have been masked by intrinsic protein aggregates. The influence of lipid was not pronounced as the groups

differing in lipid content within the same drying pre-treatment or smoking method had similar total SH content.

The available SH content increased after blanching, during early drying of brined capelin and during smoking, but rose most after blanching and towards the end of hot smoking (Figure 3 A, B). Blanched brined capelin that had higher available SH content than brined at onset of drying, showed a tendency of decreasing values during early drying inversely relating to with total SH content (Table 2, $r = -0.40$). Available SH content was generally higher in blanched brined than brined capelin. Proteins were denatured during blanching exposing the sulfhydryl groups that are buried or blocked in native proteins thereby increasing the proportion of available SH in the total SH content. The increase in available SH content in brined capelin during early drying may be related to proteins hydrolysis caused by enzyme activity and or the groups increased salt content in the fish muscle resulting in protein swelling (salting-in effect), exposing SH groups (Nguyen et al., 2011; Baylan et al., 2015). It may also be due to changes in the reactive groups, mainly loss of hydrophilic surface and exposure of hydrophobic areas (Raman & Mathew, 2014). Available SH content in blanched brined capelin decreased during early drying, stabilizing towards end of drying in all groups. The decrease in available SH was related to the masking of sulfhydryl groups by protein aggregates that were formed because of the tight proteins network due to dehydration. Changes in available SH were in agreement with the changes in disulfide bond content obtaining a significant correlation (Table 2, $r = 0.80$).

During smoking, available SH increased significantly ($p < 0.05$) during hot smoking when temperature was higher than 40 °C. High temperature during smoking resulted in protein denaturation, exposing SH groups that were buried in native proteins. However, due to the antioxidant properties of the smoke (Guillen & Errecalde, 2002), the reactive SH groups were less oxidized and therefore underwent minimal disulfide interchanges as revealed by disulfide content changes that were not significantly ($p > 0.05$) different during smoking (Figure 4 B & Table 2, $r = 0.32$). Generally available SH content was negatively correlated with water content (Table 2) and only correlated strongly with disulfide content during drying. Disulfide bond content of blanched brined capelin was significantly higher ($p < 0.05$) after treatment and throughout the drying process than for any other group (Figure 4 A). Brining alone appeared not to affect disulphide interchanges as only slight increase was observed during early drying, after

which disulphide content remained stable with minor variations in groups under both treatments. The results are in agreement with total SH content in that higher disulphide content was obtained in blanched brined capelin, that had lower total SH content and vice versa in brined capelin. This is because sulfhydryl groups get oxidized when they are exposed to reactive oxygen or secondary oxidative by-products and subsequently undergo disulfide interchanges (Baylan et al., 2015; Raman & Mathew, 2014; Hsu, Hwang, Yu & Jao, 2007), resulting in reduced total SH content and increased disulphide content. The results suggest that proteins aggregate after blanching, resulting in increased disulfide bonds and reduced total SH content. During later stages of drying disulfide bond content was stable and corresponded with the stability in SH groups. Lipids did not significantly affect the available SH and disulfide content during capelin drying and smoking even though fatty capelin had tended to have higher SH content and lower available SH content than less fatty capelin.

4 Conclusions

The results demonstrate that brining and blanching in brine, the drying process, smoking methods and also the lipid content affect the conformational changes in capelin proteins. SSP and total SH content were reduced while available SH and disulfide content increased during drying and smoking. Protein conformational changes occurred mainly during early drying when water content and dehydration rate were relatively high and became stable later when water content was lower. Blanching of capelin before drying reduced protein solubility and SH content that resulted in high protein aggregation during drying which could have affected the eating quality of the product mainly texture (i.e. sticky texture on biting) and reduced nutritional value due to loss of thermolabile compounds for instance amino acids. Conformational changes that occurred during hot smoking were ascribed to protein denaturation, that in addition to loss of nutritional value, reduced muscle water holding capacity thereby affecting the yield which is of importance to processors. As expected less changes in protein structure were observed during cold smoking.

Lipid content seems to have a protective effect on capelin proteins during drying and smoking as there were less changes (SSP and SH groups) in fatty capelin. Blanching and drying in general had more influence on protein conformational changes than brining and smoking.

Blanching is not suitable for commercial capelin drying, and drying should be conducted under low temperature conditions to maintain protein quality and products textural properties. We recommend a study to be conducted to assess the effect of fish blanching on texture and product acceptability for tropical fish that are blanched prior to drying.

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Table 1: Lipid, salt and content of raw and processed (dried and smoked) capelin differing in lipid content (n=4; Mean±SD)

Capelin batch	Variable	Raw material	Pre-treatment		Dried products		Smoked products	
			Brined	Blanched-Brined *	Brined	Blanched-Brined	Brined-Cold	Brined-Hot
High lipid (C1)	Lipid[a]	9.05±0.2[a]	8.98±0.44[a]	7.39±0.02[b]	28.13±0.50[c]	27.06±0.73[c]	-	-
	Salt	0.72±0.04[a]	1.95±0.18[b]	2.15±0.02[b]	6.2±0.2[c]	7.6±0.13[d]	-	-
	Water	76.8±0.08[a]	76.91±1.05[a]	74.83±0.85[b]	20.43±1.01[c]	12.03±0.5[d]	-	-
Low lipid (C2)	Lipid	6.99±0.25[a]	6.75±0.12[a]	6.28±0.22[b]	27.18±0.11[c]	27.41±0.77[c]	-	-
	Salt	0.68±0.02[a]	2.36±0.02[b]	2.41±0.07[b]	8.8±0.1[c]	9.6±0.28[d]	-	-
	Water	78.18±1.05[ab]	79.13±1.55[a]	76.06±0.95[b]	19.11±0.55[c]	10.27±0.74[d]	-	-
High lipid (C1)	Lipid	9.05±0.2[a]	8.98±0.44[a]	-	-	-	15.95±0.52[b]	17.75±60[c]
	Salt	0.72±0.04[a]	1.95±0.18[b]	-	-	-	3.45±0.44[c]	3.81±0.35[c]
	Water	76.8±0.08[a]	76.91±1.05[a]	-	-	-	59.14±1.52[b]	54.52±0.75[c]
Low lipid (C2)	Lipid	6.99±0.25[a]	6.75±0.12[a]	-	-	-	13.24±0.66[b]	15.21±1.00[c]
	Salt	0.68±0.02[a]	2.36±0.02[b]	-	-	-	4.25±0.25[c]	4.7±0.23[c]
	Water	78.18±1.05[a]	79.13±1.55[a]	-	-	-	57.8±1.22[b]	53.67±0.78[c]

[a]Abbreviations: Lipid (% lipid content), Salt (% lipid content), water (% content).

*Pre-treatment done only for drying trials.

-Reported earlier/later.

[a-d]Different letters within a row indicate significantly different values between samples ($p < 0.05$).

Table 2: Correlation (Pearson) matrix for several parameters[a] evaluated for dried and smoked capelin differing in lipid content

A: Drying (p = 0.38)	Temp	MC	SSP	Tot-SH	Av-SH	Disul
DT[a]		**-0.91**[b]	**-0.53**	**-0.63**	-0.11	**-0.44**
MC			**0.71**	**0.80**	-0.08	0.29
SSP				**0.90**	**-0.59**	-0.23
Tot-SH					**-0.40**	**-0.07**
Av-SH						**0.80**
B: Smoking (p = 0.38)	Temp	MC	SSP	Tot-SH	Av-SH	Disul
ST	**0.78**	**-0.97**	**-0.61**	**-0.76**	**0.66**	**0.53**
Temp		**-0.83**	**-0.82**	**-0.89**	**0.46**	**0.78**
MC			**0.67**	**0.78**	**-0.54**	**-0.59**
SSP				**0.94**	-0.23	**-0.78**
Tot-SH					**-0.46**	**-0.78**
Av-SH						0.32

[a] Abbreviations: DT (drying time), MC (content), SSP (salt soluble proteins), Tot-SH (total sulfhydryl content), Av-SH (available sulfhydryl content), ST (smoking time) and Temp (temperature).

[b] Bold font denotes statistically significance

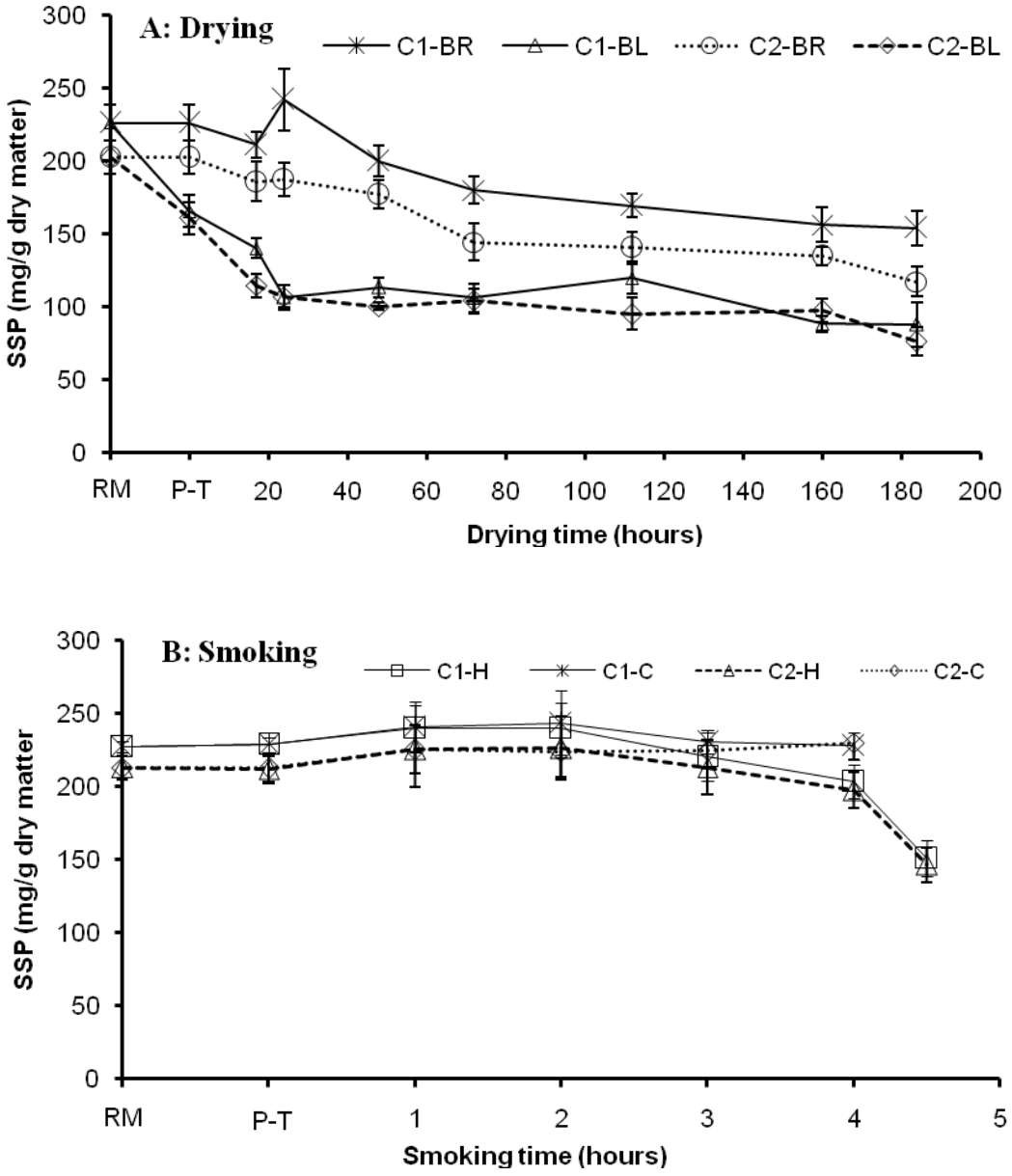

Figure 1: Changes in salt soluble proteins (SSP) in high lipid (C1) and low lipid (C2) capelin during drying and smoking (n=3; Mean±SD). RM = raw material; P-T = pre-treated; –BR = brined; -BL = blanched; -H = hot smoking; -C = cold smoking. Extraction of the protein fraction was performed in 1M salt solutions.

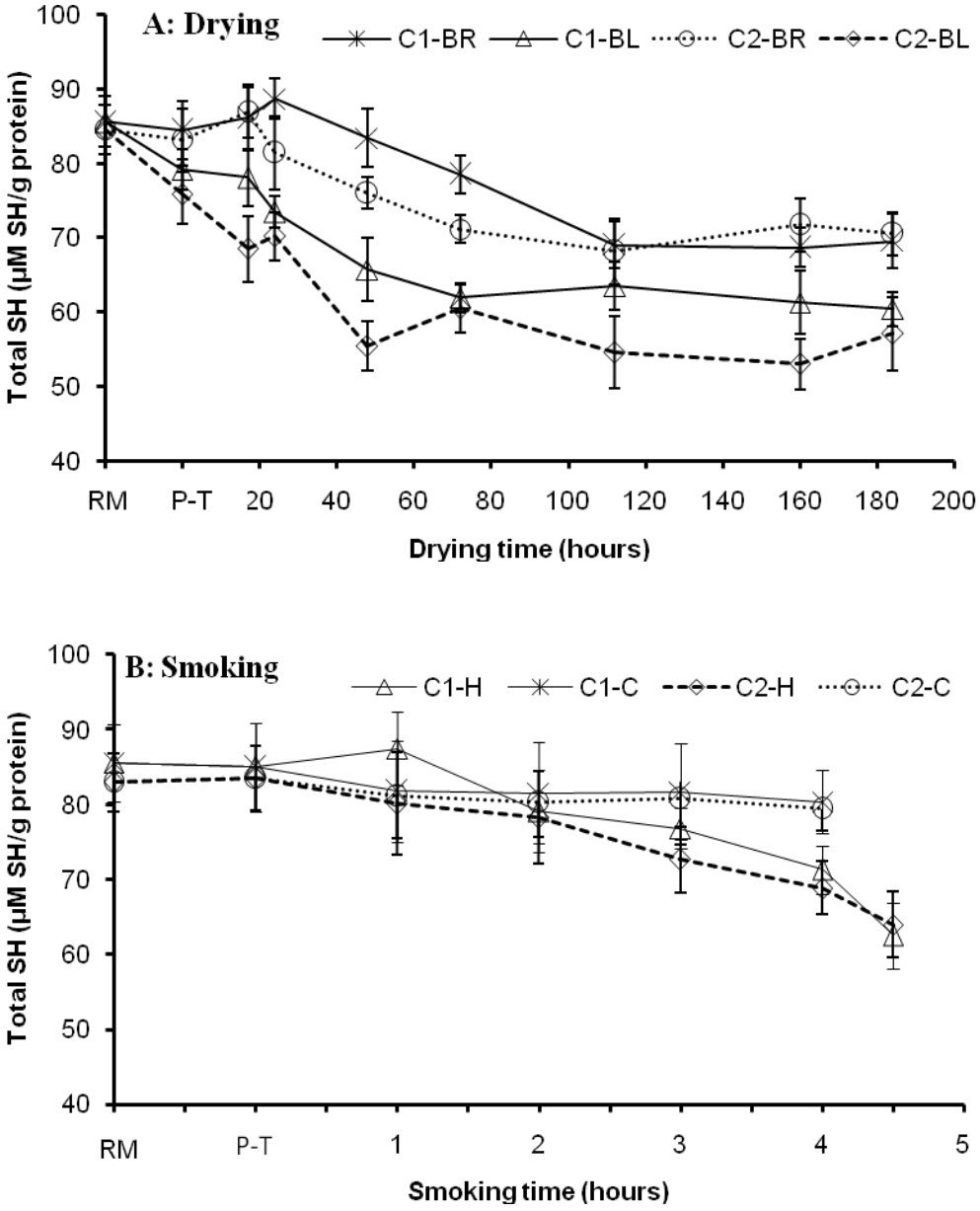

Figure 2: Changes in total sulfhydryl content in high lipid (C1) and low lipid (C2) capelin during drying and smoking (n=3; Mean±SD). RM = raw material; P-T = pre-treated; –BR = brined; -BL = blanched; -H = hot smoking; -C = cold smoking. Extraction of the protein fraction was performed in 1M salt solutions.

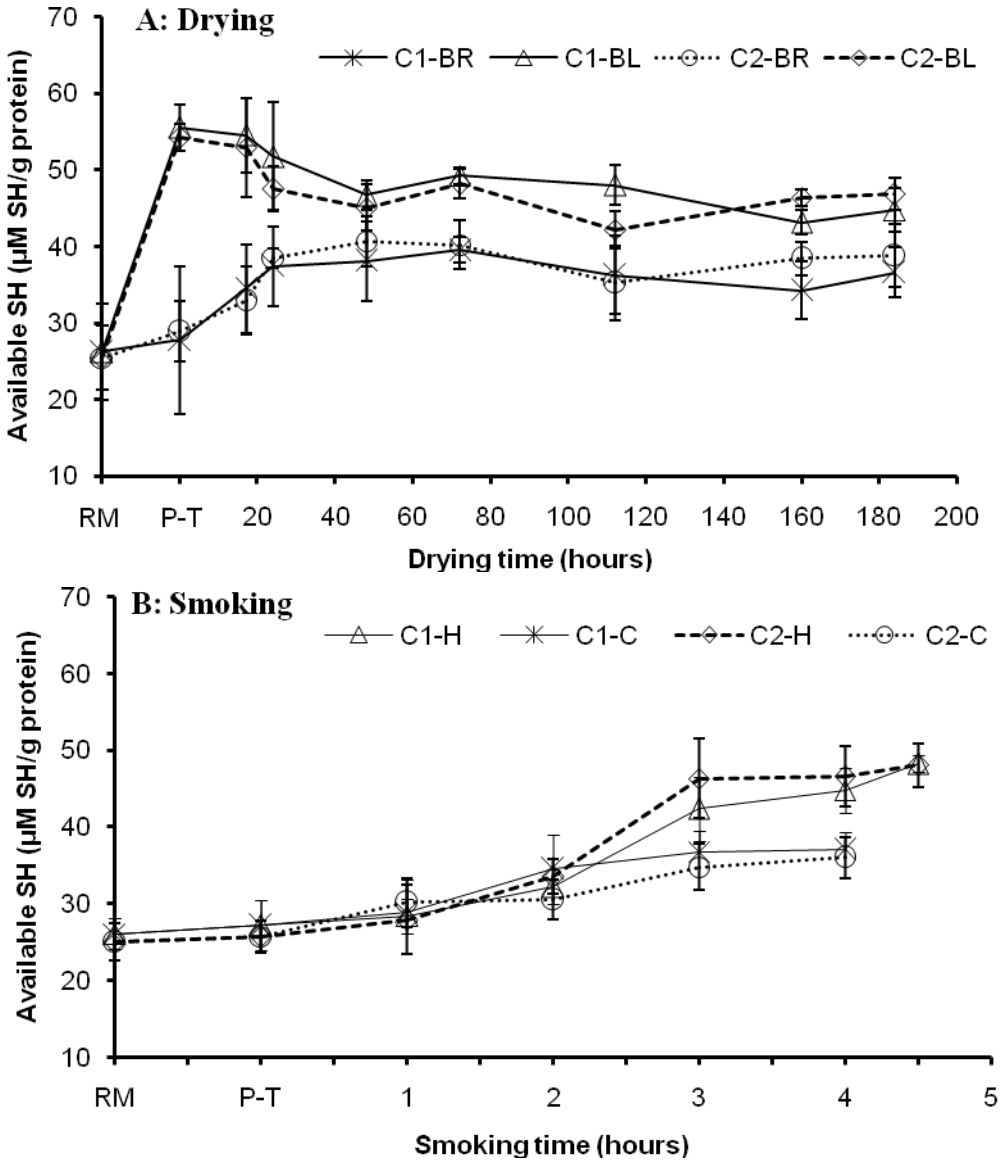

Figure 3: Changes in available sulfhydryl content in high lipid (C1) and low lipid (C2) capelin during drying and smoking (n=3; Mean±SD). RM = raw material; P-T = pre-treated; –BR = brined; -BL = blanched; -H = hot smoking; -C = cold smoking. Extraction of the protein fraction was performed in 1M salt solutions.

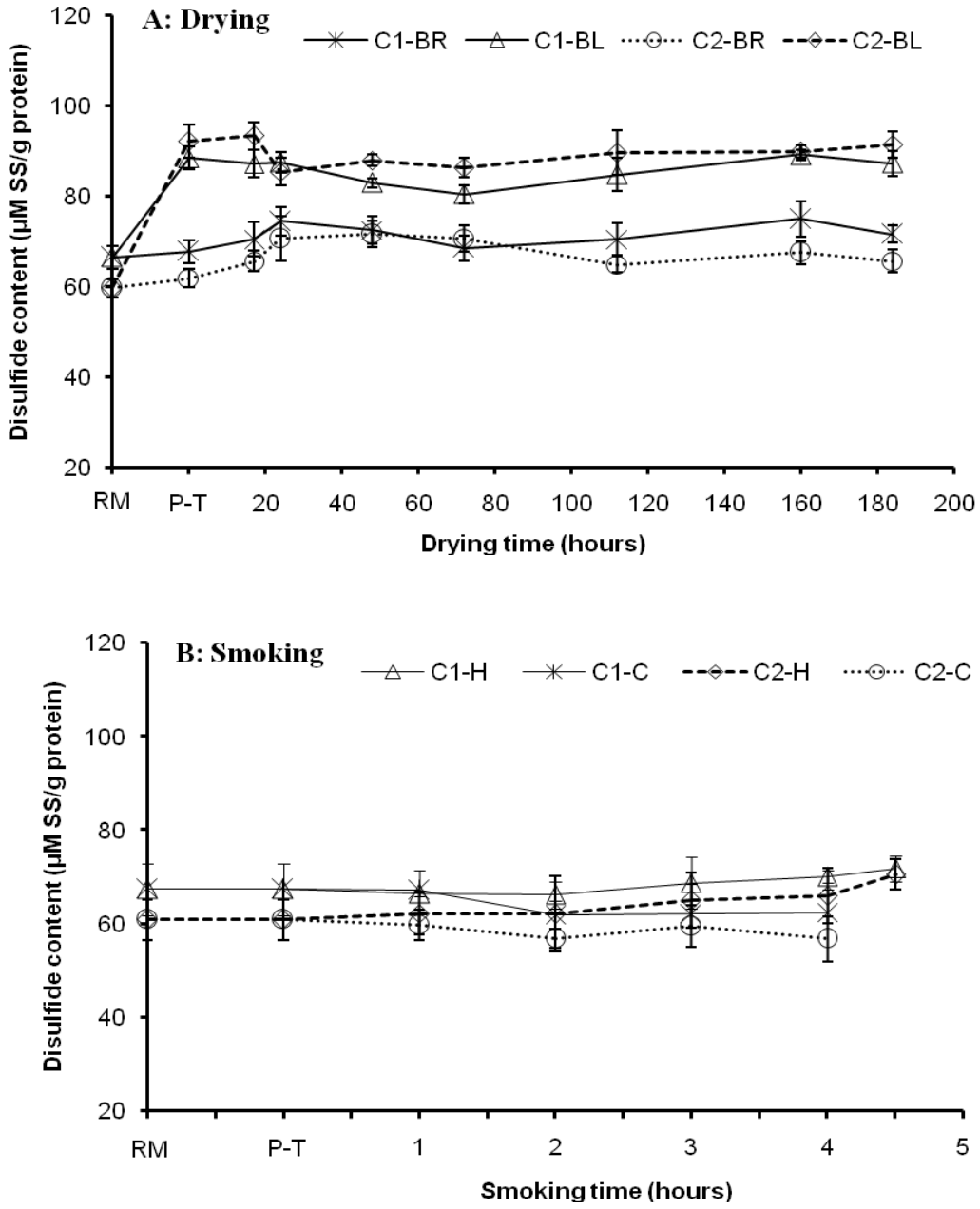

Figure 4: Changes in disulfide content in high lipid (C1) and low lipid (C2) capelin during drying and smoking (n=3; Mean±SD). RM = raw material; P-T = pre-treated; –BR = brined; -BL = blanched; -H = hot smoking; -C = cold smoking. Extraction of the protein fraction was performed in 1M salt solutions.

Influence of smoking and packaging methods on lipid stability and microbial quality of Capelin (*Mallotus villosus*) and Sardine (*Sardinella gibossa*)

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ORIGINAL RESEARCH

Influence of smoking and packaging methods on lipid stability and microbial quality of *Capelin* (*Mallotus villosus*) and Sardine (*Sardinella gibossa*)

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Keywords
Capelin, fatty acids, lipid, sardine, smoked

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Abstract

Lipid and microbial quality of smoked capelin (two groups differing in lipid content) and sardine was studied, with the aim of introducing capelin in the smoked sardine markets. Lipid hydrolysis (phospholipid and free fatty acids) and oxidation index (hydroperoxides and thiobarbituric acid-reactive substances), fatty acid composition, and total viable count were measured in raw and packaged smoked fish during chilled storage (day 2, 10, 16, 22, 28). Lipid hydrolysis was more pronounced in low lipid capelin, whereas accelerated lipid oxidation occurred in high lipid capelin. Muscle lipid was less stable in sardine than capelin. Essential polyunsaturated fatty acids (eicosapentaenoic acid and docosahexaenoic acid) constituted 12% of fatty acids in capelin and 19% in sardine. Vacuum packaging as well as hot smoking retarded bacterial growth, recording counts of ≤log 5 CFU/g compared to ≥log 7CFU/g in cold smoked air packaged. Smoked low lipid capelin was considered an alternative for introduction in smoked sardine markets.

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Introduction

Fresh fish is highly perishable and various preservation techniques such as chilling, freezing, drying, salting, and smoking have been used universally to extend shelf life. In developing countries, the most affordable and widely used fish preservation methods are drying and smoking (Oduor-Odote et al. 2010; Darvishi et al. 2013). Smoking is carried out in two forms, hot and cold smoking. Hot smoking can be considered mild (30–50°C) or high temperature (50–80°C) (Marc et al. 1997), but it is commonly carried out at temperatures of 70–80°C (Erkan et al. 2011). In contrast, cold smoking is achieved without thermal treatment usually at temperatures ≤30°C (Goulas and Kontominas 2005). Smoked fish products are commonly salted. The use of salt is essential to complement the bacterial inhibitory effect of smoke by reducing water activity. For health and acceptability reasons, the practice is to have products with low salt content.

Consumption of smoked fish is increasing. In Europe, cold- and hot-smoked fish products constitute about 15% of the total fish consumption (Stołyhwo and Sikorski 2005; Huda and Dewi, 2010). The consumer preference for these products is not only for their traditionally desirable flavor but also the preservation of nutritional quality such as the highly polyunsaturated fatty acids (PUFAs) and essential

amino acids (Stołyhwo et al. 2006; Bilgin et al. 2008). The long chain PUFAs, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) present in marine lipids have beneficial health effects (Stołyhwo et al. 2006; Karlsdottir et al. 2014). PUFAs are highly susceptible to oxidation, and factors such as the smoking process and storage conditions have been reported to influence their composition (Stołyhwo et al. 2006).

Smoking of small pelagic fish could be a promising processing method for species such as capelin as existing fresh markets cannot absorb the large catches for human consumption. In Iceland, capelin is one of the most important pelagic fisheries with the catch exceeding 1 million tons in some years (Hjalmarsson et al. 2007). Although small pelagic fish are mainly consumed in the dried form (Oduor-Odote et al. 2010; Darvishi et al. 2013), especially in developing countries, smoked products have also been reported (Mhongole and Mhina 2012). In Kenya, small pelagic fish are mainly used for human consumption, especially by low-income fish consumers. The demand for small pelagic fish mainly fresh water 'dagaa' (Rastrineobola argentea) has continued to rise, with declining stocks of table fish (IOC 2012). Along the Kenyan coast, the landing of marine sardines cannot meet the existing demand. At the same time, capelin is under-utilized as a food product for human consumption as most of it is used in the production of fish meal, fish oil, and pet food (Arason 2003). Supplying the market with smoked capelin could increase the direct human consumption of this small pelagic fish. Unlike in tropical sardine, the lipid content in capelin varies considerably from 3 to 4% to about 15 to 20% of their body weight with the highest lipid content in late fall (November–December) and lowest during the spawning season in March–April (Vilhjálmsson 2002). It is therefore important to determine the influence of muscle lipid content on the stability of smoked capelin with the aim of establishing appropriate harvest time for raw material.

Fish processing certainly entails a storage period for the products before consumption. Given that fish products are perishable, adequate storage conditions should be provided to slow down deteriorative changes occurring through oxidation and microbial growth (Erkan 2012). Vacuum packaging is known to inhibit oxidation and aerobic microbial growth. However, due to market demand for smoked fish that contain low quantities of smoke and salt (Beltrin et al. 1989), vacuum packaging may provide suitable conditions for Clostridium botulinum growth in products.

It is important for producers to have knowledge of the raw material and the effects of the processes used, so that characteristics of the finished products are consistent with market demand. The present study was aimed at determining the influence of smoking and the method of packaging on lipid hydrolysis and oxidation during refrigerated storage. A comparison between capelin caught at different times differing in lipid content and sardine was made to assess the influence of lipid content on the stability of smoked capelin, taking into consideration the possibility of its introduction as a new product into the market for smoked sardines.

Materials and Methods

Raw materials

Capelin samples were obtained from HB Grandi fishing company, Reykjavik, Iceland. Fish was caught on 1st (C1) and 28th (C2) February 2013 and kept in chilled seawater for 2 days prior to freezing in 25 kg blocks. One batch of sardine (S) caught on 21st March, 2013 was obtained from artisanal fisherman in Mombasa, Kenya (8 h post catch) and frozen in blocks weighing 25 kg before transportation by air freight to Iceland. Samples were kept frozen at −25°C until the time of the study.

Thawing was done overnight in open air at 18–20°C, after which twenty fish from each group were individually weighed and tagged for identification during subsequent weighing. Each group was subjected independently to strong brine infusion (brine concentration of 24% NaCl at 2°C, fish: brine ratio 1:1, w/w) for 1 h and thereafter spread on meshed trays to remove excess surface water. Each group was equally divided into two subgroups, each including 10 tagged fish for hot (H) and cold (C) smoking. The tagged samples were individually weighed prior to arranging the fish in a single layer on smoking racks.

Smoking process

Smoking was done in a conventional smoking facility equipped with an automatic control for temperature, humidity, and density of wood smoke based on a predetermined program used for commercial production of smoked salmon. Relative humidity was kept at 50% and smoke was produced from oak in an external smouldering-type generator. Hot smoking in the kiln was divided into three stages: (1) a preliminary drying and smoking at 30°C for 2 h; (2) a drying and smoking at 40°C for 2 h; (3) cooking at 75°C for 30 min. Within each stage, the kiln was programmed to repeat cycles of drying for 5 min at air circulation of 2800 circles/min, followed by 20 min smoking, at 720 circles/min air circulation before refreshing ("killing the smoke") for 5 min at 720 circles/min air circulation. For cold smoking, the processing time in the kiln was 4 h at 24°C in cycles of drying, smoking and refreshing as explained for the hot smoking process. During the smoking process, the temperature inside the kiln was monitored using thermometers (teste 926 thermometers

AG, Germany) placed at three different location. After smoking, the products were allowed to cool at room temperature and smoking yield calculated as:

$$\text{Smoking yield}\,(\%) = \left(\frac{W_s}{W_r}\right) \times 100$$

where W_s is the weight of smoked fish and W_r is the weight of brined raw material.

Smoked fish was transported to the laboratory and upon arrival 2 days postprocessing, each group was sub-sampled. The rest was further divided equally into two subgroups for air (A) and vacuum (V) packaging. About 20–30 fish were put in a high-barrier film bag (40PA/70LDPE, 250 mm × 400 mm × 0.120 mm, Plastprent, Iceland) prior to packaging using HENKOVAC packaging machine (Heavy duty 2000, Hertogenbosch, The Netherlands). Five packs per subgroup (air/vacuum) were packaged and stored at refrigerated conditions (4 ± 1°C) and samples taken for analysis on day 2, 10, 16, 22, and 28 of storage.

Moisture content (MC), Water activity (a_w) and salt measurements

Moisture content (MC) was determined as the weight reduction of minced fish muscle after drying at 103 ± 1°C for 4 h (ISO 1993). Results were expressed as g water/100 g muscle. Water activity (a_w) was measured at room temperature by carefully placing about 5 g of minced sample in a clean sample cup. The sample cup was placed into a sample chamber and closed to engage the latch. Reading was complete when the instrument beeped (Novasina AW-Center, AWC503 RS-C, Axiar AG, Switzerland). The salt (NaCl) content of the samples was determined based on AOAC (2000) and expressed as g salt/100 g muscle.

Total lipid and phospholipid content

Lipids were extracted from 25 g samples of fish muscle (80 ± 1% water) with methanol/chloroform/0.88% $KCl_{(aq)}$ (at 1/1/0.5, v/v/v) according to the Bligh and Dyer (1959) method. The lipid content was determined gravimetrically and the results were expressed as g lipid per 100 g wet muscle.

Phospholipid content (PL) of the fish muscle was determined using a colorimetric method, based on complex formation of phospholipids and ammonium ferrothiocyanate (Stewart 1980), before reading the resultant solutions absorbance at 488 nm (UV-1800 spectrophotometer, Shimadzu, Kyoto, Japan). The reported PL was based on a standard curve prepared with phosphatidyl-choline in chloroform (5–50 μg/mL) and results expressed as g PL per 100 g lipid.

Free fatty acid (FFA)

Free fatty acid content was determined according to Bernardez et al. (2005) based on complex formation with cupric acetate-pyrimidine, followed by absorbance reading at 710 nm. The FFA concentration was calculated as μmol/L quantities of oleic acid, based on a standard curve spanning a 2–22 μmol range. Results were expressed as g FFA/100 g lipid.

Lipid oxidation

Lipid hydroperoxides (PV) were determined using the ferric thiocyanate method described by Santha and Decker (1994) with modifications according to Karlsdottir et al. (2014), except that 3 ± 0.5 g of sample was used instead of 5 g, and subsequent to extraction and centrifuging at 5100 rpm for 5 min. at 4°C, 200 μL of the chloroform layer was collected and mixed with 800 μL of chloroform: methanol solution. The results were expressed as μmol lipid hydroperoxides per kg wet muscle.

Thiobarbituric acid-reactive substances (TBARS) were measured as described by Lemon (1975) with modifications. A 3 ± 0.5 g muscle sample was homogenized with 10 mL of trichloroacetic acid (TCA) solution (7.5% TCA, 0.1% propyl gallate and 0.1% EDTA mixture prepared in distilled water), using an Ultra-Turrax homogenizer (IkaLabortechnik, T25 basic, Germany) at 8000 rpm for 10 s. The homogenate was then centrifuged at 5100 rpm for 20 min at 4°C (TJ-25 Rotor Centrifuge Beckmann, California, USA). A 100 μL of supernatant was collected and mixed with 900 μL of 0.02 mol/L thiobarbituric acid solution in 1.5 mL Eppendorf and heated in a water bath for 40 min at 95°C. The samples were cooled down on ice after which, 200 μL was placed in duplicate into a 96-well microplate reader (NUNC A/S Thermo Fisher Scientific, Roskilde, Denmark) for absorbance reading at 530 nm (Sunrise Microplate Reader, Tecan GmbH, A-5082 Grödig, Austria). The results were expressed as μmol of malomaldehyde diethylacetal/kg wet muscle and calculated based on a standard curve prepared using tetraethoxypropane.

Fatty acids profile

The fatty acid composition was determined following derivatization of extracted TL to fatty acid methyl esters (FAME), by gas chromatography (Varian 3900 GC, Varian, Inc., Walnut Creek, CA) equipped with a fused silica capillary column (HP-88, 100 m × 0.25 mm × 0.20 μm film), split injector and flame ionization detector (FID) based on AOAC (2000) programme. The result of each fatty acid was expressed as percentages of the total FAME.

Total plate counts (TPC)

To determine microbial quality of smoked fish, aerobic plate counts (TPC) were carried out. Pooled samples were analyzed in duplicate per subgroup, observing strict hygiene to prevent cross-contamination. Twenty five grams of minced muscle were mixed with 225 mL of cooled maximum recovery diluent (MRD, Oxoid) in a stomacher bag to obtain a 10-fold dilution. Blending was done in stomacher for 1 min. Successive 10-fold dilutions were done as required. Aliquots were plated in triplicate on the plate count agar (PCA). In all cases, the pour plate technique was used. Enumeration of TPC was performed after 3 days incubation at 22ºC and results expressed as log CFU/g muscle.

Data analysis

Microsoft Excel 2010 (Microsoft Inc., Redmond, WA) was used for plotting graphs. Analysis of variance (ANOVA) was calculated using the Number Cruncher Statistical System 2000 program (NCSS Statistical Software, Kaysville, UT). The program calculates multiple comparisons, using Duncan's test to find out if sample groups differ ($P < 0.05$).

Results and Discussions

Chemical composition and smoking yield

Salt concentration in fish muscle was not significantly different ($P > 0.05$) between the groups and smoking methods (Table 1). However, a tendency of higher NaCl concentration in hot smoked subgroups (3.48–3.87 g/100 g) compared to cold smoked subgroups (3.18–3.56 g/100 g) was observed mainly due to greater dehydration in hot smoked fish. All subgroups had a NaCl concentration above the critical level of 3% considering the minimum salt content needed to inhibit the growth of food poisoning organisms specifically C. botulinum (Lund and Peck 2000), while providing smoked products with an acceptable salty flavor (Cardinal et al. 2001). Water activity values ranged between 0.944 and 0.953 (Table 1), which are below the critical level of 0.97 for the formation of botulinum toxin (Lund and Peck 2000). Water activity was inversely correlated with NaCl concentration whose preservative effects are ascribed to the decrease in water activity (Marc et al. 1997; Rorvik 2000). During storage, both NaCl and water activity remained apparently stable in all subgroups.

Preservation characteristic of smoked product is associated with the dehydration effects as well as the antimicrobial and antioxidant activity of the smoke constituents (Rorvik 2000; Goulas and Kontominas 2005). In commercial practice, processing firms invest in better technologies to control

Table 1. Raw material water content and smoked capelin and sardine yield, salt content and water activity (A), and phospholipids data as a function of storage time based on Duncan multiple range tests (B). ± = standard deviations (n = 3).

	RM water content and smoked fish yield, NaCl and aw						Phospholipid (g/100 g total lipid) in raw and smoked fish during storage											
						Storage time (days)	2	10		16		22		28				
A	Water (g water/100 g)	Yield (%)	NaCl (g/100 g)	Water activity	B	RM	A	Air	Vac	Air	Vac	Air	Vac	Air	Vac	Air	Vac
C1H	72.7 ± 0.68	76.14 ± 3.29	3.48 ± 0.46	0.949 ± 0.002		5.8 ± 0.5	4.9 ± 0.5	4.7 ± 0.4	5.0 ± 0.1	4.3 ± 0.8	4.4 ± 0.5	4.0 ± 0.9	4.5 ± 0.0[2]	4.4 ± 0.3	3.7 ± 0.2		
C1C	72.7 ± 0.68	81.50 ± 2.83	3.18 ± 0.38	0.952 ± 0.001	***	5.8 ± 0.1[a]	5.2 ± 0.6[ab]	4.1 ± 0.6[b]	4.3 ± 0.7[b]	3.5 ± 0.0[c]	4.2 ± 0.7[bc]	2.3 ± 0.5[d]	2.7 ± 0.7[d]	1.7 ± 0.2[d]	1.9 ± 0.5[d]		
C2H	76.9 ± 0.74	71.23 ± 2.21	3.74 ± 0.52	0.948 ± 0.000[1]	*	10.2 ± 0.2[a]	7.4 ± 0.4[b]	6.3 ± 0.3[c]	7.2 ± 0.2[b]	6.5 ± 1.1[c]	6.7 ± 0.3[c]	5.1 ± 0.1[c]	5.6 ± 0.2[c]	5.5 ± 0.3[c]	5.8 ± 0.7[c]		
C2C	76.9 ± 0.74	77.10 ± 2.07	3.32 ± 0.29	0.953 ± 0.002	***	10.2 ± 0.2[a]	8.2 ± 0.5[b]	6.4 ± 0.4[c]	6.5 ± 0.6[c]	5.2 ± 0.2[d]	5.7 ± 0.8[cd]	2.0 ± 0.6[e]	2.1 ± 0.4[e]	2.0 ± 0.4[e]	2.1 ± 0.2[e]		
SH	75.5 ± 1.05	70.06 ± 2.64	3.87 ± 0.44	0.944 ± 0.000	**	6.7 ± 0.0[a]	4.8 ± 0.1[b]	3.8 ± 0.0[c]	3.0 ± 0.1[c]	3.3 ± 0.7[c]	3.3 ± 0.6[c]	2.3 ± 0.4[d]	3 ± 0.0[c]	2.0 ± 0.1[d]	2.6 ± 0.3[d]		
SC	75.5 ± 1.05	74.16 ± 2.70	3.56 ± 0.42	0.947 ± 0.004	**	6.7 ± 0.0[a]	5.6 ± 0.2[b]	2.9 ± 0.3[cd]	3.0 ± 0.1[c]	2.4 ± 0.4[de]	2.3 ± 0.0[e]	1.1 ± 0.3[f]	1.6 ± 0.2[f]	1.2 ± 0.2[f]	1.7 ± 0.5[f]		

RM, Raw material; A, Analyses before packaging; Air, Air packaged; Vac, Vacuum packaged; C1, high-lipid capelin; C2, low-lipid capelin; S, sardine; H, hot smoked; C, cold smoked.

[1]Values equal to 0.000 are values less than 0.0005.

[2]Values equal to 0.0 are values less than 0.05.

*Significant difference at a level $P < 0.05$; **Significant difference at a level $P < 0.01$; ***Significant difference at a level $P < 0.001$. Different letters (superscript) indicate significantly different values between phospholipids samples within a row.

production parameters so that they maximize their gains. Processing yield is one such factor. In the present study, yield was observed to be significantly different ($P < 0.05$) between the groups and smoking methods, with hot smoked subgroups as well as low-lipid capelin (C2) and sardine (S) recording relatively low yields (Table 1). This may be explained by the fact that during smoking, dehydration occurs due to the evaporation of water on the fish surface and the diffusion of water from the fish muscle to the surface. One of the factors influencing water diffusivity during smoke drying of fish is the chemical composition, particularly, the lipid content (Cardinal et al. 2001). Water diffusion was higher in the low lipid fish, resulting to high dewatering corresponding to lower yield in the groups.

Lipid changes in smoked fish

Lipid content in raw fish was significantly different between groups ($P < 0.05$), with values of 10.3, 7.5 and 3 g lipid/100 g muscle for C1, C2, and S groups in that order (Fig 1). The capelin groups C1 and C2 caught within the same month (February) differed significantly in lipid content. The observation is in agreement with an earlier study by Arason et al. (2014) that lipid content of capelin varies considerably between and within seasons.

On smoking, an increase in lipid content was observed in all sample groups, hot smoked fish having significantly higher lipid levels than their cold-smoked counterparts (Fig. 1). Increased lipid content was also influenced by the initial lipid content as the greatest increase was in fatter capelin C1. Total lipid was based on wet muscle and more water was lost during hot smoking thus increasing dry matter per unit weight. Similarly, based on the groups' initial lipid content and lipid being a constituent of dry matter, its increase was higher when smoking high lipid groups. Upon air and vacuum packaging (Fig. 1), no significant change ($P > 0.05$) in lipid was observed in samples tested over storage period in respective groups. This demonstrates generally but not invariably that the total lipid remained apparently stable over the storage period in all groups. Similar results with nominal variations in total lipid were reported during drying of migaki-nishin (Shah et al. 2009) and in sardine stored in ice (Chaijan 2009).

The main lipid components in fish are triglycerides and phospholipids. Capelin groups (C1 and C2) had different initial PL content (Table 1). In general, all groups had low PL proportion indicating that majority of the lipid in capelin and sardine is triglycerides. Kas'yanov et al. (2002) reported relatively high triglyceride content in capelin, with PL ratio of 11.1%, somewhat similar to the low lipid (C2) capelin (10.2 g/100 g lipid) in the present study. Since PL is a membrane lipid, it is relatively constant with minor seasonal variations (Burri et al. 2012). The low values observed in the high-lipid capelin (C1), may be accounted for by high lipid content (10.32 g/100 g muscle) in the group. On the other hand, PL obtained for raw sardine was comparatively lower than previously reported (Chaijan et al. 2006). This was probably because sardine used in the study was purchased from artisanal fishermen who did not use chilling medium on-board fishing vessels (Odoli et al. 2013). High temperatures may have led to accelerated PL hydrolysis prior to frozen storage and processing as low PL correlated well with high initial FFA content in the group.

Both smoking methods led to PL hydrolysis, particularly in the hot smoked groups (Table 1). Accelerated PL hydrolysis, might be due to the greater activities of phospholipases at high temperatures (Chaijan et al. 2006; Shah et al. 2009). Phospholipid generally declined with storage time, but differently by groups (Table 1). During storage, hot smoked and vacuum packaged fish had low PL hydrolytic activities compared with cold smoked and air packaged groups. Besides, low-lipid capelin (C2) and sardine (S) that had high initial PL proportion had higher PL hydrolytic activities. FFA evolution that is mainly due to lipid hydrolysis by lipolytic enzymes had greater development in C2 and sardine that had a relatively high initial PL proportion and higher PL hydrolytic activities during storage. On day 16 of storage, cold smoked fish

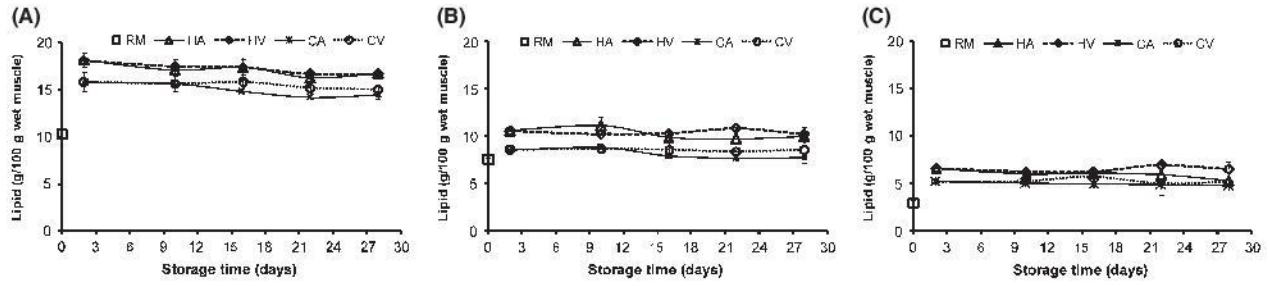

Figure 1. Changes in capelin (A = high lipid (C1) and B = low lipid (C2)) and sardine (C = S) muscle lipid (RM = raw material) upon smoking and chilled storage of hot-smoked (Air = HA and vacuum = HV) and cold-smoked (Air = CA and vacuum = CV) packaged samples (n = 3).

attained low PL proportion comparable to their hot smoked counterparts on day 28, suggesting hot smoking slowed down lipolytic enzymatic activities.

Capelin groups had lower FFA proportion in raw material than Sardines (Fig. 2), indicating that hydrolysis of glycerol-fatty acid esters occurred to some extent during postmortem handling of sardine, mainly due to inappropriate handling methods (nonicing). Hydrolysis of glycerol-fatty acid esters has been reported to be an important change that occurs in fish muscle lipid postmortem with the liberation of free fatty acids (Chaijan et al. 2006). Upon smoking FFA was observed to have evolved more in hot smoked as well as fish groups that had high PL ratio. This suggests that during hot smoking, the temperature of 30–40°C for 4 h in the first two stages prior to cooking stage at 75°C for 30 min may have accelerated lipolytic enzymatic activities, which were mainly phospholipases as evolution was more in high PL groups.

During chilled storage of smoked fish, FFA content was found to evolve particularly with storage time, but progressed differently by fish groups and smoking methods. In capelin, evolution was rapid in cold smoked groups surpassing values of corresponding hot smoked groups between day 10–16 and day 16–22 for C1 and C2 accordingly. More so, the evolution was more rapid in low-lipid capelin (C2) that had higher PL. Similarly in sardines, rapid FFA evolution occurred in cold smoked groups between days 2–10 attaining 26 g/100 g lipid at the end of storage, compared to 17 g/100 g lipid for the hot smoked group. The higher FFA content in cold smoked fish can be explained by the group's high water content and lipolytic enzymatic activities that were almost stopped in the hot smoked fish. In general, FFA evolution was in agreement with the PL hydrolytic trends. Whilst, FFA had a progressive increase during storage, PL proportion decreased. It has been suggested that majority of FFA evolving in fish under refrigeration condition is derived from PL (Lopez-Amaya and Marangoni 2000). The results therefore demonstrate FFA evolution during chilled storage of smoked fish was influenced by the smoking method (more rapid in cold smoked groups) as well as the PL ratio (more rapid with high PL ratio).

Lipid oxidation

Lipid oxidation in smoked capelin and sardine was evaluated by primary (PV) and secondary (TBARS) oxidation products (Fig. 3A–F). The PV and TBARS content in raw materials was significantly different between fish groups; in capelin, high lipid group (C1) had higher values than low lipid group (C2). Unexpectedly, raw sardine obtained the highest PV value (313 μmol/kg) despite its muscle constituting low lipid proportion, whereas TBARS was not statistically different from C1 ($P > 0.05$). It is likely that faster lipid oxidation occurred during postmortem handling of sardines as earlier observed with phospholipids and FFA content. Unlike phospholipids and FFA, PV and TBARS decreased in all groups except for PV in low-lipid capelin (C2) that had the least PV in raw fish. Hot smoking entails higher temperature which is generally known to accelerate lipid oxidation (Marc et al. 1997). Based on the PV and TBARS results, the influence of temperature on lipid oxidation during smoking may have been surpassed by the effects of smoke phenolic compounds with antioxidant properties (Guillen and Errecalde 2002). But temperature differences and product moisture content during hot and cold smoking, contributed to the differences in PV and TBARS obtained in the groups. Hot smoked groups were more dehydrated and thus saturated with lipid than their cold smoked counterparts.

The PV content increased in the early storage time and decreased toward the end of storage in all groups except air packaged hot smoked C1 that had progressive PV increase throughout (Fig. 3A–C). Increase in PV at early storage time was due to the formation of hydroperoxides which are primary lipid oxidation products whose content depends greatly on the ratio between formation and decomposition. Lipid hydroperoxides accumulate rapidly during the initial oxidation process but with extended storage time, the rate of hydroperoxides cleavage and

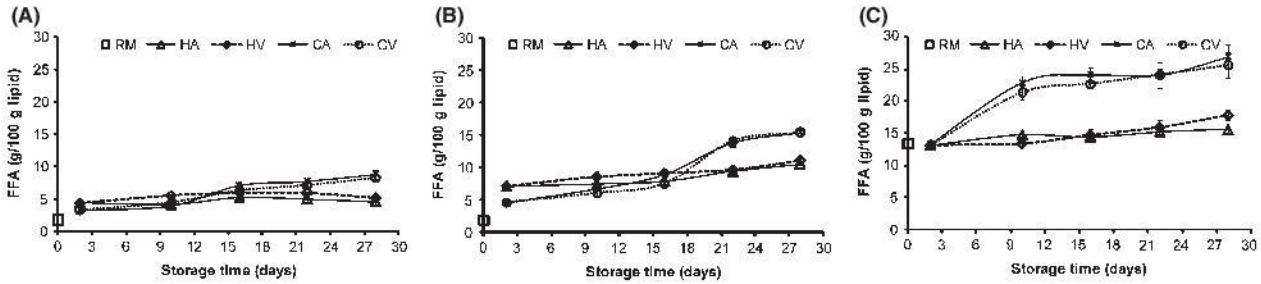

Figure 2. Evolution of free fatty acids in capelin (A = high lipid (C1) and B = low lipid (C2)) and sardine (C = S) muscle lipid (RM = raw material) upon smoking and chilled storage of hot smoked (Air = HA and vacuum = HV) and cold smoked (Air = CA and vacuum = CV) packaged samples (n = 3).

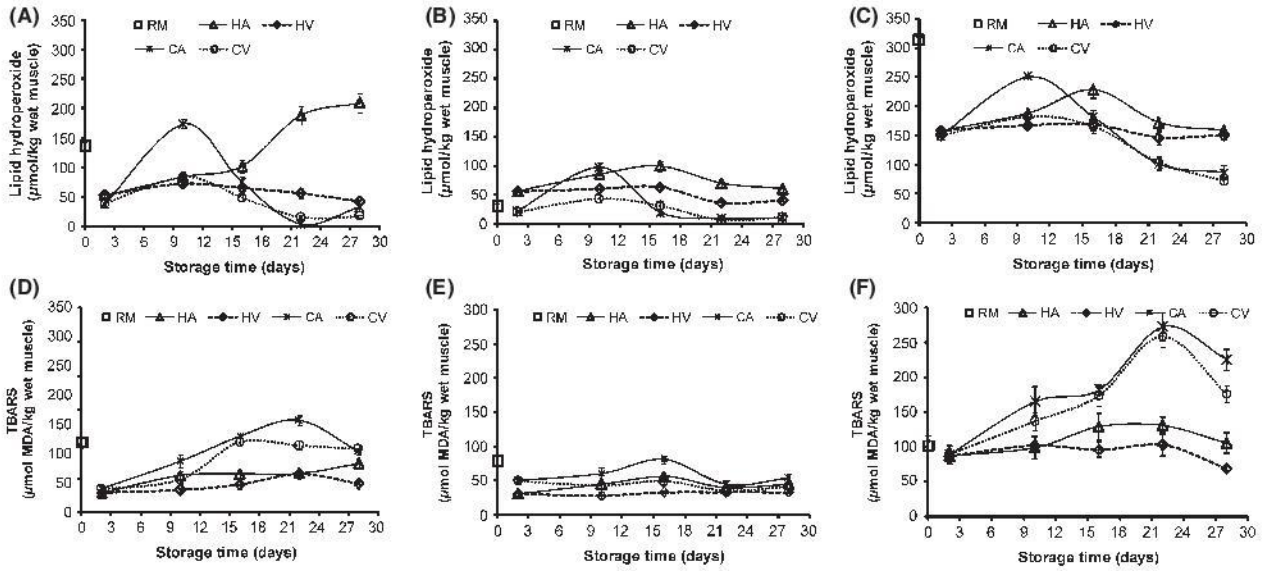

Figure 3. Lipid hydroperoxides formation (A, B, and C) and thiobarbituric acid-reactive substances formation (D, E, F) in capelin (high lipid (C1) = A/D; low lipid (C2) = B/E) and sardine (S = C/D) muscle lipid (RM = raw material) upon smoking and chilled storage of hot smoked (Air = HA and vacuum = HV) and cold smoked (Air = CA and vacuum = CV) packaged samples (n = 3).

reactions exceed the formation (Undeland et al. 1999). In cold smoked groups a significant decrease in PV was obtained ($P < 0.05$) after day 10 and later (after day 16) in hot smoked. This indicates cold smoked fish were more vulnerable to lipid oxidation processes shown by fast accumulation and decomposition of hydroperoxides into secondary oxidation products. The vulnerability to lipid oxidation during chilled storage of cold smoked groups was in harmony with the phospholipids and FFA results. In regard to fish groups, sardine accumulated elevated PV content during early storage probably due to the high content in raw fish (Chaijan et al. 2006). Generally capelin group (C2) that had low lipid content accumulated less PV throughout the storage time, indicating the group's preference for smoking among the studied fish.

TBARS a marker for decomposition of secondary lipid oxidation products increased during the storage with a remarkable decrease at the end (Fig. 3D–F). Smoked fish obtained higher TBARS content, when the groups corresponding PV content was lower (Fig. 3). This as earlier explained was due to the breakdown of hydroperoxides in the later stages of lipid oxidation to secondary oxidation products. TBARS content in low-lipid capelin (C1) did not decline toward the end of storage implying delayed formation that correlated well with the group's progressive hydroperoxides accumulation observed. The decrease in TABRS after extended storage time may be explained by the malomaldehyde capability of cross-linking amino acids to form amidine linkages and/or its interactions with other components of fish which are the end products of lipid oxidation (Undeland et al. 1999). On packaging, lipid oxidation was more intense with air packaging, implying the group's susceptibility to oxidation compared to vacuum packaged. This is due to the unsaturated fatty acids reacting with molecular oxygen, usually via a free radical mechanism, to form hydroperoxides which are the primary oxidation products (Chotimarkorn et al. 2010).

Changes in fatty acid composition

In capelin (C1 & C2) muscle lipid, the most abundant group of fatty acids was represented by monoenic acids (MUFAs) accounting for 53–55%, polyunsaturated fatty acids (PUFAs) 19–20% and saturated acids (SFAs) 19% of total fatty acids in raw fish (Table 2). This is in agreement with a study by Bragadóttir et al. (2002) who reported fatty acid profile of capelin to have extraordinary high concentration of MUFAs (46–57%). In contrast sardine muscle lipid constituted SFAs as the main group, accounting for 41%, while PUFAs had a ratio of 27% and MUFAs constituted the least ratio (17%) of total FAs. Sardine muscle lipid has been reported to comprise 45.9% SFAs, 35.7% PUFAs and 16.7% MUFAs (Chaijan et al., 2006), that depicts similar compositional trends to our finding but different percentage values. The difference in proportion can probably be explained by the heterogeneous nature and composition of fish fats in view that fish used in present study was obtained from a geographical different area to the allusion. Factors such as size, age, reproductive status, environmental conditions mainly water temperature can affect the fatty acid composition of fish (Saito et al. 1999).

Table 2. The Fatty acid composition present in the highest concentrations in the lipid extracted from the muscle of fresh and smoked capelin and sardine stored at refrigeration for 28 days. ± = standard deviations (n = 3).

Fish group		Storage day	Fatty Peak area of the respective FA in % of total peak area														
			C14:0	C16:0	C16:1n7	C18:0	C18:1n9	C18:1n7	C20:1n9	C18:4n3	C22:1	C20:4n6	C20:5n3	C22:6n3	∑SFA	∑MUFA	∑PUFA
C1	RM	0	6.37^{a***}	11.56*	8.72***	1.03	8.47	2.75	13.51^{a**}	2.16^{a***}	16.17^{a**}	1.83	7.23^{ac***}	6.22^{a***}	19.55	53.1^{a**}	20.8^{ac***}
	H	2	5.29^{b}	13.13^{a}	8.78	1.21	8.68	2.98	10.19^{b}	1.93^{b}	11.81^{b}	1.44	9.66^{b}	11.1^{b}	20.3	45.6^{b}	27.7^{b}
		28A[1]	5.84^{ab}	12.25	8.83	1.11	8.67	2.87	11.91	2.19^{a}	13.03^{c}	1.78	8.42^{abc}	8.33^{ac}	19.75	49.65^{a}	24 ^{cd}
		28V	5.57^{ab}	12.63^{ac}	8.44	1.19	8.38	2.85	11.08^{b}	2.18^{a}	12.97^{c}	1.59	9.02^{bc}	9.69^{bc}	20	46.9^{b}	25.95^{bd}
	C	2	6.32^{a}	11.43^{bc}	7.94^{a}	1.05	8.7	2.63	13.72^{a}	2.25^{a}	16.08^{a}	1.87	6.96^{a}	7.39^{ac}	19.4	52.5^{a}	21.9^{ac}
		28A	6.26^{ab}	11.76	9.75^{b}	1.09	9.08	3.03	12.66	2.02^{b}	15.18^{d}	1.82	7.7^{ac}	6.08^{a}	19.7	53.05^{a}	21^{ac}
		28V	6.11^{ab}	11.69	8.91	1.1	9.1	2.88	13.38^{a}	1.95^{b}	15.67^{a}	1.89	7.46^{ac}	6.52^{a}	19.5	53.3^{a}	21.05^{ac}
C2	RM	0	6.41	11.6*	7.98	1.14	9.79*	3.03	14.03^{ac***}	1.54	16.74^{a***}	1.92^{a*}	6.68a**	6.09^{a}	19.7	55	19.3^{a***}
	H	2	6.14	12.31	8.06	1.17	8.88^{a}	2.97	12.1^{b}	1.37	13.9^{b}	1.57	8.43^{a}	9.53^{b}	20.2	49.2	24.1^{b}
		28A	6.05	12.21	7.36	1.27	8.74^{a}	2.86	12.1^{b}	1.5	14.32^{ab}	1.65	8.21^{a}	9.88^{b}	20.15	48.7	24.5^{b}
		28V	5.98	11.9	7.82	1.16	8.63^{a}	2.81	12.59^{b}	1.55	14.96^{ab}	1.81	8.07^{a}	9.21^{bd}	19.6	50.15	23.75^{bc}
	C	2	6.64	10.9b	8.14	1.07	10.11^{b}	3.03	14.85^{a}	1.53	14.16	1.21^{b}	8.25^{b}	9.47^{c}	19.1	51.9	23.5^{c}
		28A	6.21	11.81	8.29	1.17	9.26	2.98	13.01^{bc}	1.49	15.24^{a}	1.81	7.88^{a}	7.65^{ad}	19.75	52.2	21.95^{a}
		28V	6.11	11.89	8.37	1.18	9.39	3.07	12.69^{b}	1.59	15.14^{a}	1.79	7.89^{a}	7.67^{ad}	19.75	51.95	22.1^{ac}
S	RM	0	3.92	24.8^{ac**}	2.57^{a**}	8.59**	5.85^{a***}	2.26	0.56^{a**}	0.47^{ac**}	3.81^{a**}	0.14^{a*}	4.14	16.39^{ab***}	40.9^{ab***}	16.5^{a**}	26.5**
	H	2	4.36	23.9^{a}	3.44^{bd}	7.45^{a}	6.8^{b}	2.45	2.19^{b}	0.7^{b}	5.1	0.31	4.81	16.27^{ab}	38.9^{bc}	21.7^{b}	27.6^{a}
		28A	4.29	23.01^{b}	3.7^{b}	7.64^{b}	6.67^{bc}	2.5	2.79^{c}	0.58^{c}	5.95^{b}	0.43^{b}	4.62	15.44^{bc}	38.1^{c}	23.55^{c}	26.6
		28V	4.24	24.16^{ab}	3.27^{cd}	7.85^{b}	6.29^{ac}	2.32	2.03^{b}	0.61^{cd}	5.51^{bc}	0.33	4.6	16.14^{ab}	39.4^{bc}	21.15^{b}	27.05^{a}
	C	2	4.28	24.07^{ab}	3.3^{cd}	7.57^{b}	6.64^{bc}	2.4	1.94^{be}	0.66^{d}	5.15^{bc}	0.3	4.69	16.16^{ab}	39.4^{bc}	21^{bd}	27.9^{a}
		28A	4.25	25.08^{ac}	3.02^{ac}	8.9^{bc}	6.47^{bc}	2.4	1.56^{e}	0.55^{a}	4.73^{ac}	0.26^{a}	4.78	14.91^{c}	41.7^{ad}	20^{bd}	26.55
		28V	4.26	26.04^{c}	2.69^{a}	9.57^{c}	6.5^{bc}	2.34	0.99^{a}	0.45^{a}	4.04^{a}	0.23^{a}	4.3	15.1^{c}	43.6^{d}	18.35^{d}	25.2^{b}

[1]Packaging (A, Air packaged; V, Vacuum packaged); RM, Raw material; C1, high-lipid capelin; C2, low lipid capelin; H, hot smoked; C, cold smoked.

*Significant difference at a level P < 0.05; **Significant difference at a level P < 0.01; ***significant difference at a level P < 0.001. Different letters (superscript) indicate significantly different values between samples (same group) within a column.

Among the MUFAs, erucic acid C22:1 (16% FAs) and eicosenoic acid C20:1n9 (14% FAs) were most abundant in capelin groups, whereas in sardine oleic acid C18:1n9 (6% FAs) was the major MUFAs. The SFAs were significantly higher in sardine muscle lipid and constituted palmitic acid C16:0 as predominant, with a proportion of 25% and 12% of FAs in sardine and capelin groups, respectively. There is consensus that long chain PUFAs, especially EPA (C20:5n-3) and DHA (C22:6n-3) have beneficial health outcome to consumers (Stołyhwo et al. 2006; Karlsdottir et al. 2014). Our results indicate both EPA and DHA to be present in studied fish but in varied amounts depending on the species. In capelin EPA and DHA amount were comparable with ratio of about 7% and 6%, respectively while sardine had majorly DHA constituting 16% against 4% for EPA of total FAs. Chaijan et al. (2006) found out DHA to be more abundant than EPA, with a value of 3.21 times greater. In considering health benefits of the two essential PUFAs (DHA + EPA), sardine appear to be the most valuable source of EPA + DHA. However, since these small fish are consumed as muscles not extracts and considering the lipid content of the two species in question, capelin groups (7–10 g/100 g muscle) beside sardine (3 g/100 g muscle), it is deduced that both species may be equally valuable as source of essential PUFAs.

The effects of processing and packaging technologies on fatty acid compositions of fish muscle lipid have widely been studied. Nonetheless, to our knowledge, there are no published studies on the fatty acid composition of packaged smoked capelin and sardine. Upon smoking and during storage (Table 2), significant changes ($P < 0.05$) in MUFAs were observed in both capelin groups and sardine muscle lipid. SFAs remained stable in capelin groups ($P > 0.05$) but significant changes were obtained in sardine. On the contrary, MUFAs content declined in capelin groups with values corresponding to the rise in PUFAs content given that SFA appeared to be stable. In sardine MUFAs had a tendency of increasing even as PUFAs increased and SFAs content reduced. During storage, a decline on PUFAs was recorded with rapid decline in air packaged than vacuum packaged groups that related well with the formation and decomposition lipid oxidation index suggesting the losses might be due to oxidation.

Microbial changes

The aerobic plate count in raw fish was log 2 CFU/g, 2.7 CFU/g, and 4 CFU/g for C1, C2, and S in that order (Fig. 4). The low bacterial count, especially in capelin groups indicate that the requirement of high initial quality of the raw material for use in fish smoking was fulfilled. After smoking, a decline in total plate count was observed in all groups but statistically significant ($P < 0.05$) with hot smoking. Total counts in capelin and sardine groups are reduced to the limit of detection (log 1 CFU/g) after hot smoking and log 2.2, 2.4, and 3.4 CFU/g in C1, C2, and S, respectively after cold smoking (Fig. 4). This occurrence could be attributed to the effects of dehydration and antimicrobial activity of the smoke constituents (Rorvik 2000) besides the high temperature under hot smoking. Dehydration during smoking resulted in an increase in salt content that additionally reduced products water activity. On the other hand, high temperature (75°C for 30 min) during hot smoking may have denatured the psychotrophs that probably constituted the main microflora of defrosted fish.

During storage of packaged smoked fish, a lag phase of bacteria was observed in all groups (Fig. 4), probably due to cold shock on the microbes and also the occurrence of antimicrobial smoke constituents. From day 10 of storage, high microbial growth was evident, especially in air packages. Microbial growth may be because of the diminishing intensity of antimicrobial smoke constituents, presence of oxygen, and re-establishment, or succession by cold-loving bacteria. Total counts reached log 7 CFU/g and log 4 CFU/g in cold- and hot-smoked air packaged capelin groups and log 8 CFU/g and log 5 CFU/g in cold and hot smoked sardine, respectively, on day 28. Aerobic counts of log 5 CFU/g has been used in smoked fish as limit for consumption (Hansen et al. 1995; Leroi et al. 2000). This indicates that air packaged cold smoked fish in all groups had surpassed the limit for consumption on day 28. Specifically cold smoked air packaged capelin groups reached

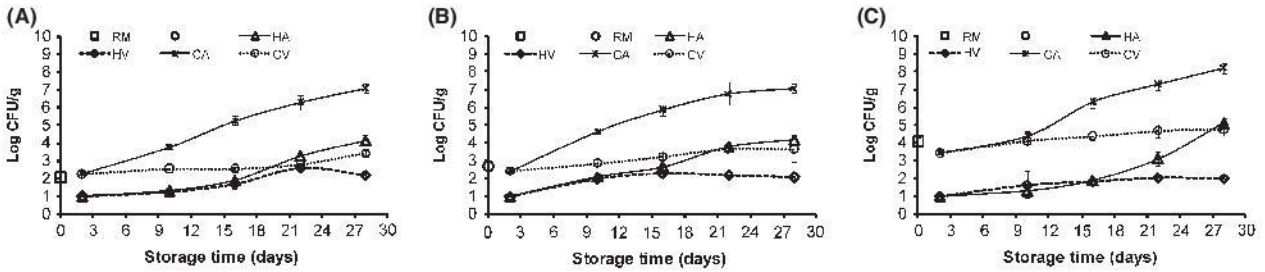

Figure 4. The total plate count in capelin (A = high lipid (C1) and B = low lipid (C2)) and sardine (C = S) fish (RM = raw material) upon smoking and chilled storage of hot smoked (Air = HA and vacuum = HV) and cold smoked (Air = CA and vacuum = CV) packaged samples (n = 3).

the limit on day 16, while sardines consumed on that day surpassed by 1 log cycle. On the other hand, aerobic counts in air-packaged hot-smoked capelin groups did not reach log 5 CFU/g consumption limits, while corresponding sardine group had attained the limit on day 28.

Vacuum packaged groups showed delayed growth ($P < 0.05$) obtaining lower aerobic counts throughout the storage, indicating counts were not observed to reach log 5 CFU/g consumption limit. This could be due to the presence of low O_2 level, retention of antimicrobial smoke constituents and to a lesser extent the low storage temperature used in the study that may have inhibited aerobic microflora development.

Conclusions

The present study indicates accelerated lipid degradation occurred during hot smoking, but the products became more stable on chilled storage than counterpart cold smoked. Smoked capelin muscle lipid hydrolysis was more pronounced in low lipid than in high lipids, whereas accelerated lipid oxidation occurred in the high lipid group. Besides, smoked capelin muscle lipid was more stable than that of sardine. Both smoked capelin and sardine were considered a valuable source of essential PUFAs. Vacuum packaging ensured products microbial quality as well PUFAs stability and its use is recommended for smoked fish considering the right level of salt and water activity to prevent type E C. Botulinum poisoning. Based on oxidative stability and the fact that low-lipid capelin had higher PL proportion at the end of storage time despite accelerated hydrolytic activities, it is the preferred group for introduction as the smoked product. However, a consumer study needs to be done to ascertain products acceptability.

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Conflict of Interest

None declared.

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PAPER V

V

Influence of lipid content and packaging methods on the quality of dried capelin (*Mallotus villosus*) during storage

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Under review

Influence of lipid content and packaging methods on the quality of dried capelin (*Mallotus villosus*) during storage

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ABSTRACT Open-sun dried small fish is an important source of protein for low income people in many developing countries. Capelin annual catch exceeds half a million tons in Iceland, with only a small quantity (<20%) of female with roe used for human food. There is a potential to use dried male capelin as a new product for human consumption. Three batches of dried male capelin (*Mallotus villosus*) differing in lipid content packed in open and sealed polyethylene and vacuum bags were studied during 5 months of storage at 22 °C to establish the effects of lipid content and packaging methods on dried capelin stability. Lipid composition, lipid hydrolysis and oxidation, sensory attributes and microbial activity were evaluated. Batches differed in composition and stability, with low lipid capelin constituting higher proportion of polyunsaturated fatty acids than high lipid capelin. Lipid oxidation was influenced by lipid content and packaging mode, as accelerated oxidation occurred in high lipid and open packed capelin. Lipid hydrolysis was less influenced by packaging and was greater in low lipid capelin. High lipid capelin in open bags scored highest for rancid odor. All batches were micro-biologically stable with colony-forming unit counts increasing less than log 1 during storage. The influence of lipid content on hydrolysis and oxidation as well as sensory properties during storage at room temperature was demonstrated. A stable and safe product at room temperature could be produced by the raw materials and drying method used, if products are vacuum packed.

Key words: Dried capelin, lipid content, packaging, stability

1 Introduction

Capelin (*Mallotus villosus*) is a small pelagic schooling fish that is economically and ecologically important in the northern hemisphere (ICES 2009). Large populations occur in the Atlantic and Pacific Oceans but important commercial fisheries have developed in the Atlantic Ocean (Carscadden et al. 2013). In Iceland, it is caught during spawning migration in winter, from December to March. Lipid content in capelin depends on maturity and season, and varies from about 4% to 20% body weight (Bragadóttir et al. 2002). There are even variations in lipid content of capelin caught within the same month of the year (Cyprian et al. 2015a).

Capelin catch in Iceland exceeds half a million tons in recent years (Statistics Iceland 2015). A small quantity of it specifically female capelin with roe is used for human food, with about 80% used for fishmeal and oil production (Statistics Iceland 2015). So there is a potential to increase the use of capelin for human consumption. In Eastern Africa particularly Kenya, Tanzania and Uganda the consumption of dried small pelagic species is widespread (IOC 2012; Oduor-odote et al. 2010). The increased consumption of small dried pelagic species has been ascribed to the increase in human population, decline in catches of table fish and improved processing (IOC 2012). Consumer preferences for the products are not only because of the taste and flavor, but also because of low price and good stability during storage (IOC 2012). This provides a possible avenue for utilizing capelin for human consumption by marketing dried capelin in East Africa and elsewhere. Blue whiting (*Micromesistius poutassou*) is already dried for markets in Nigeria.

Storage is an important part in the distribution and marketing of processed food. Storage life is determined by processing method, product composition, packaging and storage conditions. Air drying is advantageous over other preservation methods because it is affordable and efficient (Jain 2006; Bellagha et al. 2002). Quality deteriorations such as lipid hydrolysis that influence the formation of oxidation products occur during storage of dried fish (Doe 2002). Air dried products are also easily contaminated by microbes mainly air borne molds as they are often processed and sold unpackaged in an open-air environment (Park et al. 2014). Earlier studies have concentrated on the influence of drying conditions on quality than the influence of storage conditions because dried fish are considered safe during storage (Cyprian et al., 2015b; Hwang et al. 2012; Dewi et al. 2011; Oduor-odote et al. 2010; Kilic 2009; Wu and Mao 2008).

Packaging is an important part of the food industry. Food packaging has a preservation function and delivers safe, wholesome and attractive foods to the market (Kilcast and Subramaniam 2000). The methods of packaging can influence the preservation of quality of dried fish. Open air packaging is generally used for dried small pelagic species (mostly lean fish) in the intended markets and can expose dried capelin saturated with oil to oxygen. Vacuum packaging eliminates the air and is known to improve the shelf life and overall quality of muscle foods (Etemadian et al. 2012). The present study was aimed at assessing lipid deterioration and microbial quality during storage of dried capelin. The effects of lipid variation and packaging methods on lipid hydrolysis and oxidation, and microbial quality were analyzed. Samples were stored at 22±2 °C in order to simulate warm climate as expected in the distribution and marketing of this kind of product.

2 Materials and methods

2.1 Raw material, processing and sampling

Three batches of frozen male capelin (*Mallotus* v*illosus*) caught on 1st (C1), 13th (C2) and 28th (C3) February, 2013 in the North Atlantic were obtained from HB Grandi fishing company, Reykjavik, Iceland. The fish had been stored in chilled seawater (0 °C) for two days post-catch before freezing at -25 °C in blocks weighing 25 kg each and kept frozen for 3 months until the study time. Batches differed significantly ($p < 0.05$) in lipid content with values of 10% (10.32±0.65), 9% (8.83±0.46%) and 7.5% (7.54±0.48%) for C1, C2 and C3, respectively. Before drying, the frozen fish was thawed overnight at 18-20 °C. Ten fish from each group were tagged for weighing during drying to determine when the samples attained hygroscopic equilibrium moisture. Fish were arranged separately by groups in a single layer on plastic meshed trays and dried in a tunnel drier under controlled temperature, relative humidity and air speed (Cyprian, et al., 2015b).

After drying the fish (DC1, DC2 and DC3) was taken to Matis laboratory and each group divided into three equal portions which were further divided into 30 equal size samples, of these 10 were put into open polyethylene bags, 10 in sealed polyethylene bags and 10 were vacuum packaged in 10 high-barrier film bags (40PA/70LDPE, 250 mm × 400 mm × 0.120 mm, Plastprent Iceland). Packed fish were stored at room temperature (22±2 °C) and sampled every 30 days for

five months when two bags were picked randomly for analyses from each packaging treatment for each batch, 18 bags in all.

2.2 Chemicals

Chemicals used in this study were of analytical grade purchased from Sigma-Aldrich (Steinheim, Germany), Sigma-Aldrich (St. Louis, MO, USA) and Fluka (Buchs, Switzerland).

2.3 Water content and water activity

Water content of raw and dried capelin was determined as the difference in weight of minced fish before and after drying at 103±1 °C for 4 h (ISO, 1993a). Results were expressed as g water/100 g mince. Water activity (a_w) was measured using an Aqua Lab instrument (Novasina AW-Center, AWC503 RS-C, Axiar AG, Switzerland) as described by Cyprian and others (2015b).

2.4 Total lipid

Lipid was extracted from 25 g of minced fish (with 80±1% water content) with methanol/chloroform/0.88% $KCl_{(aq)}$ (at 1/1/0.5, v/v/v) according to the Bligh and Dyer (1959) method. The lipid content was determined gravimetrically and the results were expressed as g lipid per 100 g mince.

2.5 Fatty acid composition

The fatty acid composition of the total lipid extracts was determined by gas chromatography of fatty acid methyl esters (FAME). Methylation of fatty acids was carried out according to AOAC (2000). The lipid extract was vaporized to constant weight at 55 °C under nitrogen. About 70 mg of the pure lipid extract was dissolved in 1.5 mL 0.5N NaOH (in methanol) and incubated at 100 °C for 7 minutes. After cooling, 2 mL BCl3, 12% in methanol were added and incubated again at 100 °C for 30 minutes. The sample was cooled and 1 mL of standard solution (1 mg/mL 23:0 methyl ester in isooctane) and 5 mL saturated NaCl solution were added and mixed. After phase separation, the isooctane layer was transferred into a test tube containing 1mm bed of anhydrous Na_2SO_4. This was repeated with 1 mL of clean isooctane. The combined isooctane layers, containing the FAMEs were then transferred to GC vials (2x750 µL). The FEMEs were

separated by GC (Varian 3900 GC, Varian, Inc., Walnut Creek, CA, USA) equipped with a fused silica capillary column (HP-88, 100 m x 0.25 mm x 0.20 μm film), split injector and flame ionization detector fitted with Galaxie Chromatography Data System (Version 1.9.3.2 software). The result of each fatty acid was expressed as g fatty acid per 100 g lipid.

2.6 Phospholipid and free fatty acid

Phospholipid (PL) content was determined on the lipid extract by a colorimetric method, based on a complex formation of phospholipid and ammonium ferrothiocyanate (Stewart 1980), before reading the resultant solutions absorbance at 488 nm (UV-1800 spectrophotometer, Shimadzu, Kyoto, Japan). The reported PL was based on a standard curve prepared with phosphatidyl-choline in chloroform (5-50μg/ml) and results expressed as g phosphatidyl-choline equivalent/100 g lipid. Free fatty acid (FFA) content was determined on 3 mL of the lipid extract based on a complex formation with cupric acetate-pyrimidine (Bernardez et al. 2005), followed by absorbance reading of the upper layer at 710 nm. The FFA concentration was calculated as μM quantities of oleic acid, based on a standard curve spanning a 2-22 μmol range. Results were expressed as g FFA/100 g lipid.

2.7 Lipid oxidation

Lipid hydroperoxides (PV) were determined using the ferric thiocyanate method described by Santha and Decker (1994) with modifications according to Karlsdottir and others (2014) and Cyprian and others (2015b). The results were expressed as μmol lipid hydroperoxides per kg mince. Thiobarbituric acid-reactive substances (TBARS) were measured as described by Lemon (1975) with modifications according to Cyprian and others (2015a) and Cyprian and others (2015b). The results were expressed as μmol of malomaldehyde diethylacetal/kg mince.

Fluorescent compounds were determined with a Perkin-Elmer LS 50B fluorescent spectrometer (Perkin–Elmer, Massachusetts, USA). The absorbance of the organic phase resulting from the lipid extraction described earlier (Bligh and Dyer 1959) was measured at 393/463 and 327/415 nm excitation/emission maxima according to previous studies (Nguyen et al. 2012; Karlsdottir et al. 2014). The fluorescence shift (OFR) was calculated as the ratio between the two relative

fluorescence intensity (RF) values at excitation/emission maxima 393/463 nm and 327/415 nm, i.e. OFR = $RF_{393/463nm}$/ $RF_{327/415nm}$.

2.8 Microbial analyses

To determine microbial quality of dried capelin, aerobic plate count (TPC), molds and yeast counts were carried out according to general microbiological principles. Samples were analyzed in duplicate per package. Twenty five grams of minced fish were mixed with 225 ml of cooled Maximum Recovery Diluent (MRD, Oxoid) in a stomacher bag to obtain a 10-fold dilution. Blending was done in stomacher for one minute. Successive 10-fold dilutions were done as required. TPC were determined by plating aliquots on plate count agar (PCA) using pour plate technique. Enumeration of TPC was performed after 3 days incubation under aerobic conditions at 30.0 ± 1.0 °C. Molds and yeast were determined from the aliquots by spread plating onto Dichloran Rose-Bengal Chloramphenicol agar (DRCB-Agar) in Petri dishes. Molds and yeast were counted separately after 5 days incubation at 22± 1 °C. The results were expressed as log CFU/g minced sample.

2.9 Sensory evaluation with Generic Descriptive Analysis (GDA)

A sensory panel of 10 members participated in the GDA of the dried capelin. All participants were trained according to international standards (ISO, 1993b) including detection and recognition of tastes and odors, and in the use of scales. The members of the panel were familiar with the GDA method and experienced in sensory evaluation. The panel used an unstructured scale from 0 to 100 (Stone and Sidel 1985) to evaluate rancid odor and stock-fish odor. About 5 cm portions were cut from individual capelin after removing the head and tail. The samples were placed in aluminum boxes coded with 3-digit numbers that did not indicate sample group nor storage time or packaging method. Each panelist evaluated duplicates of samples in a random order in two sessions. A computerized system (FIZZ, Version 2.0, 1994–2000, Biosystemes, France) was used for data recording.

2.10 Data analysis

Data analyses were done using Microsoft Excel 2010 (Microsoft Inc. Redmond, Wash., U.S.A.). One way analysis of variance (ANOVA), Duncan's test (Post-hoc) and Pearson correlation

analysis were performed on means of the variable values in the statistical program NCSS 2000 (NCSS, Utah, USA). Multivariate comparison of different variables and samples was performed using Principal Component Analysis (PCA) on mean level corrected variable values using full cross-validation in the Unscrambler ® statistical program (Version 8.0 CAMO, Trondheim, Norway).

3 Results and discussion

3.1 Lipid, water content and water activity

Lipid content of raw fish decreased from early to late February and was significantly ($p < 0.05$) different between the batches with values of 10%, 9.0% and 7.5% in C1, C2 and C3, respectively. The corresponding water content increased and was 73%, 75% and 77%. This is in agreement with the variation in lipid content of capelin that is highest in late fall and gradually decreases during migration in early winter to spawning in March/April (Bragadóttir et al. 2002) and with the general inverse relationship between water and lipid content in fish (Cyprian et al. 2015a; Henderson et al. 1984).

The difference in lipid content of dried batches was also significant ($p < 0.05$) with values of 31%, 28% and 25%, and corresponding water content of 23%, 19% and 18% in DC1, DC2 and DC3, respectively and water activity 0.758, 0.683 and 0.676. High lipid content reduced drying rate with the less fatty capelin ending drier than the fattest capelin. The lipid is thought to play a role in limiting the drying process (Cyprian et al. 2015b) by replacing the aqueous phase that serves as vector for transfers or acting as a physical barrier to heat transfer. Dried capelin batches had a moisture content < 25% and water activity < 0.80 that is enough to inhibit the growth of harmful bacteria and assure product safety (Kilic 2009). During storage, lipid and water content in dried packed capelin did not change significantly ($p > 0.05$) in any of the groups (data not shown).

3.2 Fatty acids (FA) composition

Monoenic (MUFAs) fatty acids constituted 57-60% of the total lipid in dried capelin, followed by saturated (SFAs) 20-21% and polyunsaturated (PUFAs) 19-22% of the total lipid (Table 1).

Raw and smoked capelin muscle lipid has been reported to have a high concentration of MUFAs (46-57%) (Cyprian et al. 2015a) and Bragadóttir et al. (2002). The predominant acids among the MUFAs were long-chain C22:1 (n-11 and n-9) and eicosenoic acid (C20:1n9). Proportions of PUFAs were significantly ($p < 0.05$) higher in DC3 and DC2 compared to DC1. This may be explained by relatively high phospholipid content in DC3 and DC2 (Cyprian et al. 2015a; Parmentier et al. 2007). Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) constituted about 13% of the total lipid.

Packaging methods influenced changes in the proportion of fatty acid groups in dried capelin during storage (Figure 1). Proportion of PUFAs became lower in all batches during storage with a corresponding increase in both SFAs and MUFAs. This is because PUFAs are highly susceptible to oxidation (Karlsdottir et al. 2014; Nguyen et al. 2012). Oxidation of PUFAs appeared to be reduced more by vacuum packaging of fattest capelin (DC1) than in less fat capelin (DC3 and DC2). This as discussed in section 3.3 could be explained by differences in phospholipids proportion (Shah et al. 2009).

3.3 Lipid hydrolysis

Proportion of phospholipids (PL) expressed as g phosphatidyl-choline equivalent/100 g lipid was significantly ($p < 0.05$) lower with increased lipid content of dried capelin being 25%, 15% and 8% of total lipid in DC3, DC2 and DC1, respectively (Figure 2 A, B, C). The main lipid components in fish are triacylglycerols and PL. Diet of the fish affects the proportion of PL by either increasing or decreasing triacylglycerols (Standal et al. 2010; Burri et al. 2012), leading to increase or decrease in total lipids (Mørkøre et al. 2007). Phospholipids are membrane lipids with relatively constant absolute content, explaining why the proportion of PL is higher in less fatty than fatty capelin.

PL hydrolysis in dried capelin was rapid in all batches during early storage and most rapid in DC3 with the highest proportion of PL (Figure 2 A, B, C). The relatively higher PL content at the beginning of storage declining as storage progressed may explain the reduced rate of hydrolysis with time. Rapid PL hydrolysis in DC3 may be attributed to the occurrence of substrate specific enzymes such as phospholipases that catalyze cleavage of hydrophilic phospho-diester bonds but not of lipophillic triacylglycerols. Also, a higher proportion of the

highly susceptible PUFAs especially n-3 PUFAs in PL than triacylglycerols (Parmentier et al. 2007) can explain the difference in rate of hydrolysis between batches of dried capelin, why rapid hydrolytic activities occurred with high PL content (DC3 > DC2 > DC1). Packaging methods (open, sealed and vacuum) did not have significant effects on PL hydrolysis (Figure 2 A, B, C), supporting the explanation that PL hydrolytic processes were largely driven by enzymatic activities (Chaijan et al. 2006; Shah et al. 2009).

Free fatty acids (FFA) content was the highest in dried capelin with the lowest fat content and the highest proportion of PL (Figure 2D, E, F). FFA increased in all groups and most rapidly during early storage and with significantly ($p < 0.05$) higher values for DC3 than the other groups. FFA content increased while PL content declined (significant correlation $r = -0.54$) indicating that FFA evolution was mainly due to PL degradation. Hydrolysis of glycerol-fatty acid esters occurs in fish muscle lipid with the liberation of free fatty acids (Chaijan et al. 2006; Lopez-Amaya and Marangoni, 2000). The development trends of FFA in different groups can be explained by the corresponding PL hydrolysis. Packaging methods did not affect the FFA formation which is in agreement with the PL hydrolytic results (Figure 2 D, E, F). Generally, the results indicate that lipid hydrolysis was mainly influenced by PL content with DC3 demonstrating a greater hydrolysis and suggesting that DC1 was more stable than less fatty capelin (i.e. DC2 and DC3 group).

3.4 Lipid oxidation

Lipid content and packaging methods significantly ($p < 0.05$) affected the hydroperoxides formation, where PV developed fastest in fattier capelin and open bags (Figure 3 A, B, C). The difference between groups and packaging method depends on fat content and access to oxygen necessary for lipid oxidation. Peroxide value increased during storage in open and sealed bags except towards the end of storage when it decreased in the fattier DC1 and DC2 open bags (Figure 3 A, B). The increase was due to the formation of hydroperoxides which are primary lipid oxidation products. Hydroperoxides accumulate during the initial oxidation process but decrease later, as the rate of cleavage and reactions exceed their formation (Cyprian et al. 2015a; Underland et al. 1999). Therefore, the progressive increase in PV during storage in open and sealed bags and a decline observed in DC1 and DC2 open bags towards the end of storage

indicate the vulnerability of dried capelin to lipid oxidation, but more so in DC1 and DC2 open bags. Similar results were obtained with color changes (L* and b* values on CIELAB color scale) (data not shown), associated with lipid oxidation products (Cyprian et al. 2015b). Peroxide value of vacuum packed capelin was stable during storage (Figure 3 C). It is known that vacuum packaging can delay lipid oxidation by limiting access to oxygen thereby preserving the quality of muscle foods (Etemadian et al. 2012).

Thiobarbituric acid-reactive substances (TBARS) were the highest in groups with higher lipid content, and showed a tendency of decreasing with storage time except in DC1 and DC2 open bags increasing again towards the end of storage (Figure 3 D, E, F). The decrease in TBARS during storage may be explained by the low decomposition of hydroperoxides into secondary products of lipid oxidation (TBARS) as PV progressively increased. The results indicate a negative correlation between PV and TBARS ($r = -0.27$). The interactions of protein components (peptides and acid amines) with malonaldehyde to give tertiary lipid oxidation products might have contributed to decrease in TBARS (Underland et al. 1999). Increase in TBARS in DC1 and DC2 open bags towards the end of storage may be attributed to faster decomposition of existing hydroperoxides into TBARS since the corresponding PV were decreasing. Packaging methods had some impacts on TBARS decomposition with vacuum packed capelin being more stable than open and sealed bags. The results are in conformity with PV data in explaining the importance of limiting access to oxygen to minimize lipid oxidation.

At the beginning of storage fluorescence shift ratio (OFR) were not significantly ($p > 0.05$) different between the batches, with OFR values of 1.05, 1.29 and 1.16 in DC1, DC2 and DC3, respectively. Increase in OFR values during storage were dependent on the lipid content and packaging methods (data not shown). The increase was higher in fattier dried capelin (DC1 and DC2) in both open (9.9 and 5.0) and sealed (5.3 and 3.6) bags until towards the end of storage when the fattiest fish (DC1) scored much higher (9.9) than in the other groups. Increases in OFR values occurred in parallel to decreases in TBARS (even though no significant (($p > 0.05$)) inverse correlation $r = -0.10$ was obtained). OFR, tertiary products of lipid oxidation are derived from complex interactions of protein components with secondary lipid oxidation products such as malonaldehyde (Underland et al. 1999). Contrarily, when OFR in open bags DC1 increased towards the end of storage, the corresponding TBARS content increased whereas PV decreased.

This may be due to interactions of hydroperoxides with protein components that has also been reported to form fluorescence compounds (Nguyen et al. 2012). Packaging methods had a considerable influence on OFR development with samples in open bags obtaining higher values than sealed and vacuum packed.

3.5 Sensory evaluation with generic descriptive analysis (GDA)

The generic descriptive analysis (GDA) attributes used in this study to describe dried fish degradation were rancid odor and stock-fish odor (a characteristic of dried fish). The rancid odor increased whereas stock-fish odor decreased during storage (Table 2 A, B). Both lipid content and packaging methods affected rancid odor (Table 2 A). A stronger rancid odor ($p < 0.05$) was obtained in high fat DC3 as well as open packed samples. These results are in agreement with lipid oxidation indicators discussed earlier, showing positive correlations ($r = 0.67, 0.05, 0.13$ with PV, TBARS & OFR, respectively). Rancid odor was below 20 on the scale of 0-100, a limit that has been used to indicate the samples are becoming rancid (Magnusson et al. 2006). The stock-fish odor was slightly reduced as storage time progressed, with higher reduction in fattier and open packed capelin (Table 2 B). The changes were however, not significantly different ($p > 0.05$) by groups during storage. This can be explained by slow accumulation of TBARS with storage since TBARS have a profound impact on food sensory properties (Stapelfeldt et al. 1997). Stock-fish odor was inversely correlated to PV and TBARS ($r = -0.22$ and -0.21, respectively).

3.6 Microbial quality

At the beginning of storage, total plate count (TPC) was not significantly ($p > 0.05$) different between the groups with values of log 5.48, 5.24, 5.38 colony-forming units in DC1, DC2 and DC3, respectively. Slow microbial growth was obtained during storage of dried capelin with TPC increments of less than log 1 colony-forming units by the end of storage. This may be explained by the low moisture content (< 25%) and water activity (< 0.80) in dried capelin batches, slowing down the growth of spoilage bacteria (Kilic 2009). Lipid content appeared to have no effect on TPC development. The lowest TPC was found in vacuum packed capelin a fact attributed to the air elimination retarding aerobic microorganisms' growth. Yeast and molds were generally not detected in the dried capelin except after extended storage in DC1 and DC2 open

bags. This may in addition to the low moisture content be explained by the drying conditions and packaging methods used. Indoor drying, sealing and vacuum packaging minimized contamination by microbes mainly air borne yeast and molds (Park et al. 2014). The results indicate that dried capelin was microbiologically stable during storage.

3.7 Multivariate data analysis

Principal component analysis (PCA) was carried out to gain an overview of the similarities and differences between the variables (Figure 4). Not all variables were analyzed at all sampling points, therefore microbial and fatty acid composition data analyzed at the beginning, middle and the end of storage were not included in the model. The samples varied mainly in phospholipid, total lipid, water content and rancidity along the first principal component (PC-1), explaining 49% of the variations in the samples, mainly due to the difference in sample groups (i.e. lipid content). Low lipid (DC3) samples were located to the left of the scores plot along PC-1 described by phospholipid and stock-fish odor variables whereas high lipid (DC1) samples were located on the right side described mainly by the total lipid, water content and rancid odor. The variables describing DC3 were negatively correlated to those that described DC1 capelin. DC2 capelin that had moderate lipid content were centrally located implying they were marginally described by the variables that described both DC3 and DC1.

Samples also varied with regard FFA, PV and TBARS along the second principal component (PC-2) accounting for 26% of the total variation, mainly due to the difference in packaging methods storage time. As seen in Figure 4, samples are located on the lower side of PC-2 during early storage described mainly by TBARS but also total lipid and on the upper side later during storage described mainly by FFA, PV, and fluoresce. TBARS were negatively correlated with the variables that described samples during later storage. The influence of packaging methods was not clearly portrayed in the model.

4 Conclusions

The study proved that a stable and safe product at room temperature with moisture content less than 25% and water activity of less than 0.80 could be produced by the raw material and drying method used. But the capelin used must be chosen carefully due to its seasonal variation in lipid

content. The influence of lipid content on hydrolysis and oxidation as well as sensory properties during storage at room temperature could be demonstrated even though all the batches in the study were of medium lipid content (7.5- 10%). Lipid hydrolysis analyzed by proportion of PL and FFA was fastest in the lowest lipid capelin which can be explained by higher proportion of PUFAs notably the long chain fatty acids EPA and DHA.

The state of lipid oxidation changed during storage. Primary hydroperoxides (PV) increased first and then decreased during later storage. Secondary oxidation products (TBARs) were higher with increased lipid content and tended to decrease during storage except in higher lipid groups with access to oxygen towards the end of the 5 months at 22 °C. OFR values measuring tertiary oxidation products were highest at the end of storage and highest in the fattiest product in open bags. These conclusions were confirmed by the principal component analysis where the variation in the data (49%) was explained by low lipid with high phospholipid content, and stockfish odor, and highest lipid capelin with total lipids, water content and rancid odor. Oxidation products explained 26% of the variation with early storage, TBARS and total lipids on one side and PV, FFA, OFR and long term storage on the other side. The dried high lipid capelin was more stable during storage if oxygen in the atmosphere was excluded by vacuum packaging with less PV, odor and amount of microbes. The spoilage of lipids was both due to oxidation (PUFAs and oxygen) and hydrolysis. It is recommended to test consumer acceptability of indoor dried and vacuum packed high lipid capelin for markets accustomed to dried small fish.

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Table 1: Fatty acid composition (g fatty acids/100 g lipid) present in lipid extracted from dried capelin mince (n=4)

Fatty acid	**DC1**[†]	**DC2**	**DC3**
C14:0	6.2±0.15	6.4±0.26	6.1±0.18
C16:0	11.7±0.31	11.7±0.3	11.8±0.22
C18:0	1.2±0.11[a*]	1.2±0.15[a]	0.6±0.31[b]
Other SFA	2.2±0.12[a]	1.5±0.06[b]	2.5±0.33[a]
Total SFA	***21.2±0.56[a]***	**20.6±0.34[b]**	**21.1±0.11**
C16:1n7	8.5±0.30	8.2±0.33	8.5±0.51
C18:1n7	2.9±0.26	2.9±0.30	3.0±0.31
C18:1n9	9.4±0.39	9.4±0.25	9.5±0.44
C20:1n9	15.2±0.00[a]	14.6±0.25[b]	14.6±0.37[b]
C22:1(n-11 & n-9)	20.2±0.11[a]	19.3±0.28[b]	19.4±0.36[b]
Other MUFA	3.8±055[a]	3.3±0.41[a]	2.2±0.25[b]
Total MUFA	**60.1±1.22[a]**	**57.7±0.92[b]**	**57.4±1.06[b]**
C18:2n6	1.2±0.00	1.2±0.08	1.2±0.13
C18:4n3	1.8±0.14	2.1±0.09	2.2±0.16
C20:5n3 (EPA)	6.7±0.00[a]	6.9±0.26	7.2±0.33[b]
C22:6n3 (DHA)	6.1±0.06[a]	7.2±0.61[b]	6.8±0.33[b]
Other PUFA	2.7±0.43[a]	3.9±0.61[b]	4.1±0.29[b]
Total PUFA	**18.7±1.02[a]**	**21.5±1.11[b]**	**21.6±0.97[b]**

[†] Abbreviations: DC1= dried capelin of high lipid; DC2= dried capelin of moderate lipid; DC3= dried capelin of low lipid; SFA= saturated fatty acids; MUFA= monounsaturated fatty acids; PUFA= polyunsaturated fatty acids.

[*] Different letters (superscript [a-b]) within a row indicate significantly different values between samples ($p < 0.05$).

Table 2:Mean sensory scores (scale 0-100) of rancid odor (A) and stock-fish odor (B) of dried capelin (n=2)

Group	Packaging (bags)	A	Storage time (months)						B	Storage time (months)					
			0	1	2	3	4	5		0	1	2	3	4	5
DC1[†]	Open	***	4.5[a]	8.4[b]	10.9[b]	15.3[c]	14.8[c]	16.5[c]		45.7	44.6	47.5	48	46	45
	Sealed	***	4.5[a]	6.6[a]	7.5[a]	7.4[a]	10.3[b]	13.6[c]		45.7	45.2	47.4	41.9	40.5	42.7
	Vacuum	**	4.5[a]	4.9[ab]	4.6[a]	6[c]	5.7[bc]	7.3[c]	*	45.7[a]	45.2[a]	43.2	36.7[b]	38.1	37.1
DC2	Open	***	3.6[a]	5.8[b]	3.4[a]	5.4[b]	9.1[c]	10.8[c]		50	48.7	45.7	45.9	45.9	48.1
	Sealed	**	3.6[a]	4.7[a]	4.5[a]	4.6[a]	7.2[b]	7.3[b]	*	50[a]	48.9[a]	47.7	49.7[a]	42.3[b]	47.5
	Vacuum	**	3.6[a]	3.8[a]	2.8[a]	3[a]	3.6[a]	6.5[b]	**	50[a]	42.2[b]	51.3[a]	48.2[ac]	44.9[bc]	38.8[b]
DC3	Open	**	3.2[a]	4.1[a]	6.2[b]	5.5[b]	6.8[b]	6.3[b]	**	57.8[a]	46.6	45.6	49.7[a]	45.8[b]	46.6
	Sealed		3.2	4	5.1	4.4	4.4	4.3		57.8	49.1	48.2	46.6	48.6	46.9
	Vacuum	**	3.2[a]	3.2[b]	3.5[b]	3.3[b]	2.8[b]	5.9[c]	*	57.8[a]	53.3	49.5[b]	51.1	52.6	51.6

[†]Abbreviations: DC1= dried capelin of high lipid; DC2= dried capelin of moderate lipid; DC3= dried capelin of low lipid.

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ (significant difference in a sensory attribute with storage time, different letters (superscript [a-c]) within a row under the same sensory attribute indicate significant different values)

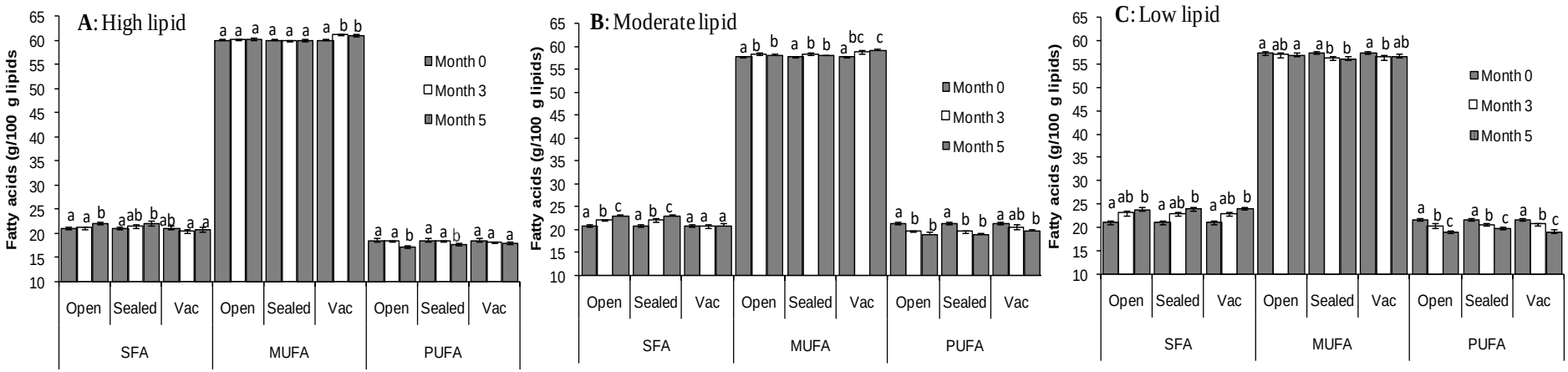

Figure 1: Changes in major fatty acid classes (g fatty acid/100 g total lipid) in packed dried capelin (A, B & C) during 5 months of storage at ambient temperature (n=4).

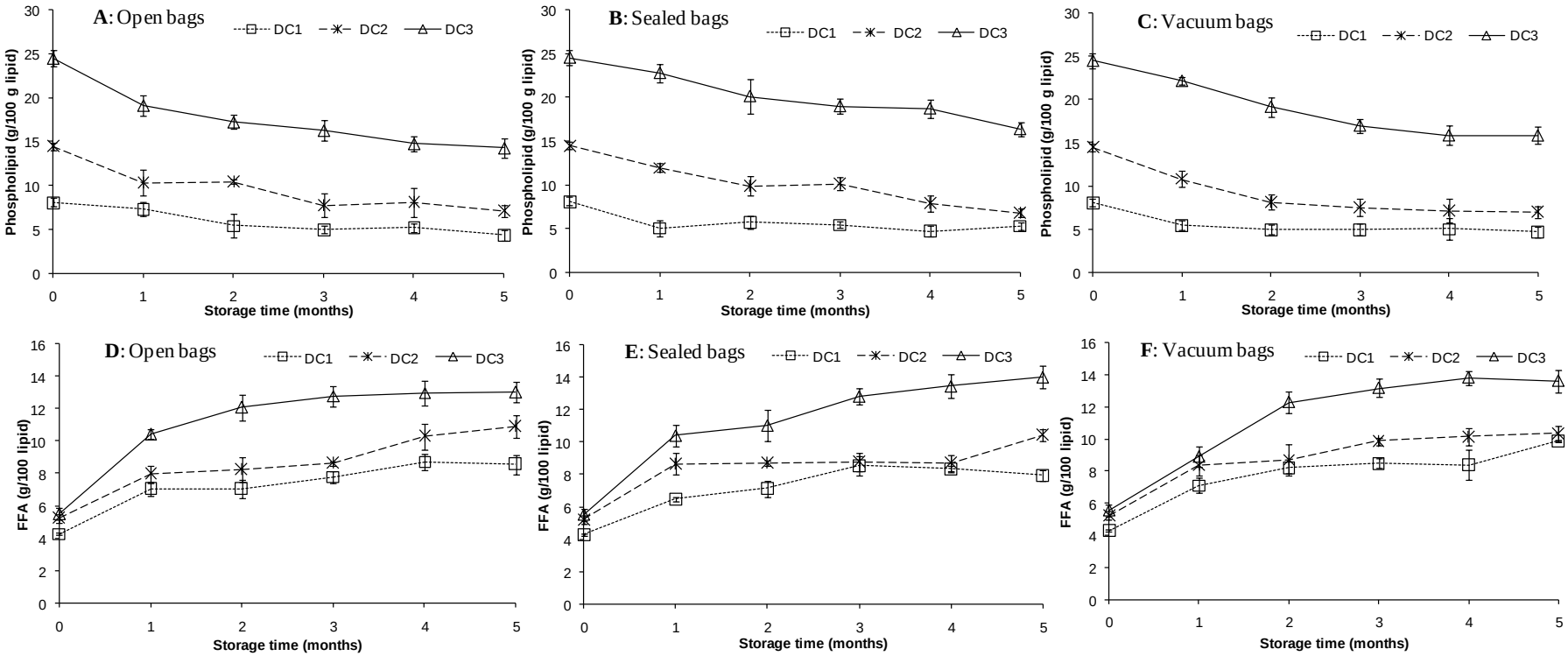

Figure 2: Changes in phospholipid (A, B & C) and free fatty acid (D, E & F) in packed dried capelin during 5 months of storage at ambient temperature (n=4). DC1= dried capelin of high lipid; DC2 = dried capelin of moderate lipid; DC3 = dried capelin of low lipid.

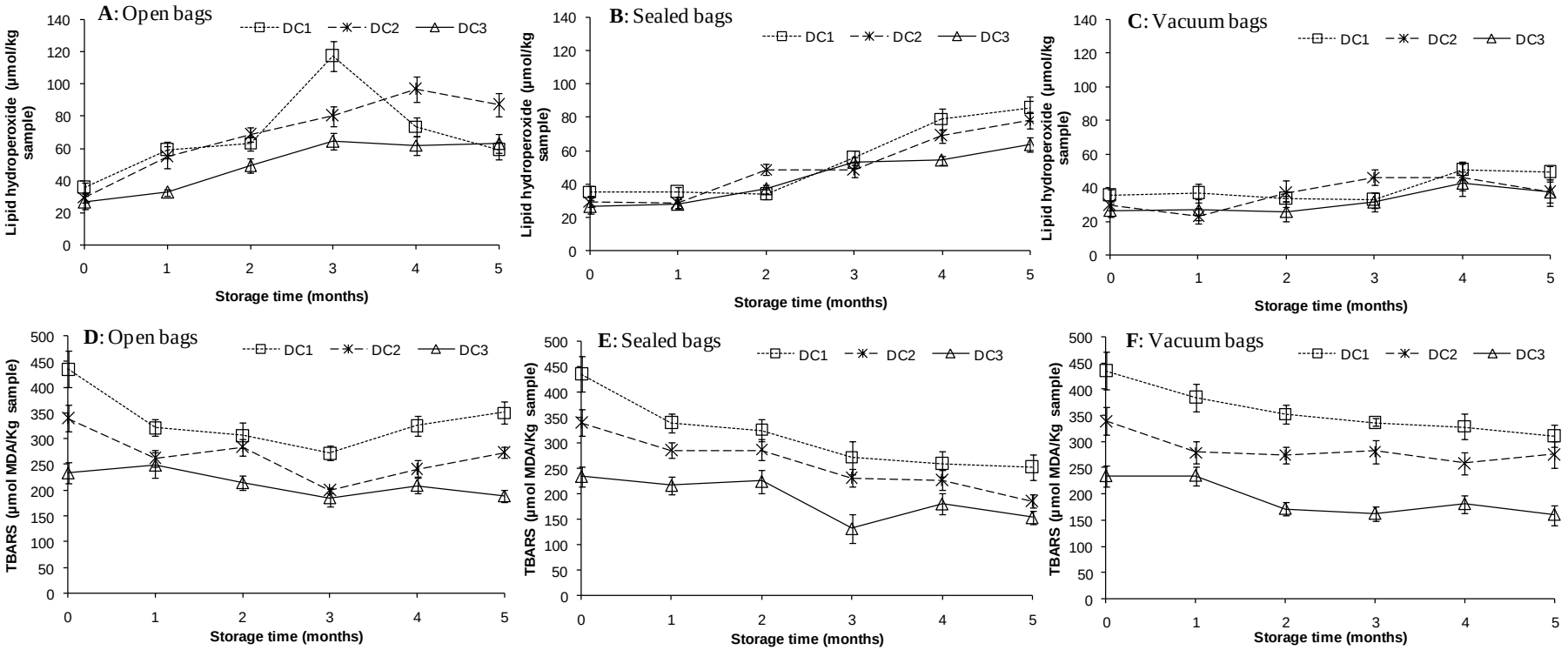

Figure 3: Lipid hydroperoxides formation (A, B & C) and thiobarbituric acid reactive substances formation (D, E & F) in packed dried capelin during 5 of months storage at ambient temperature (n=4). DC1= dried capelin of high lipid; DC2 = dried capelin of moderate lipid; DC3 = dried capelin of low lipid.

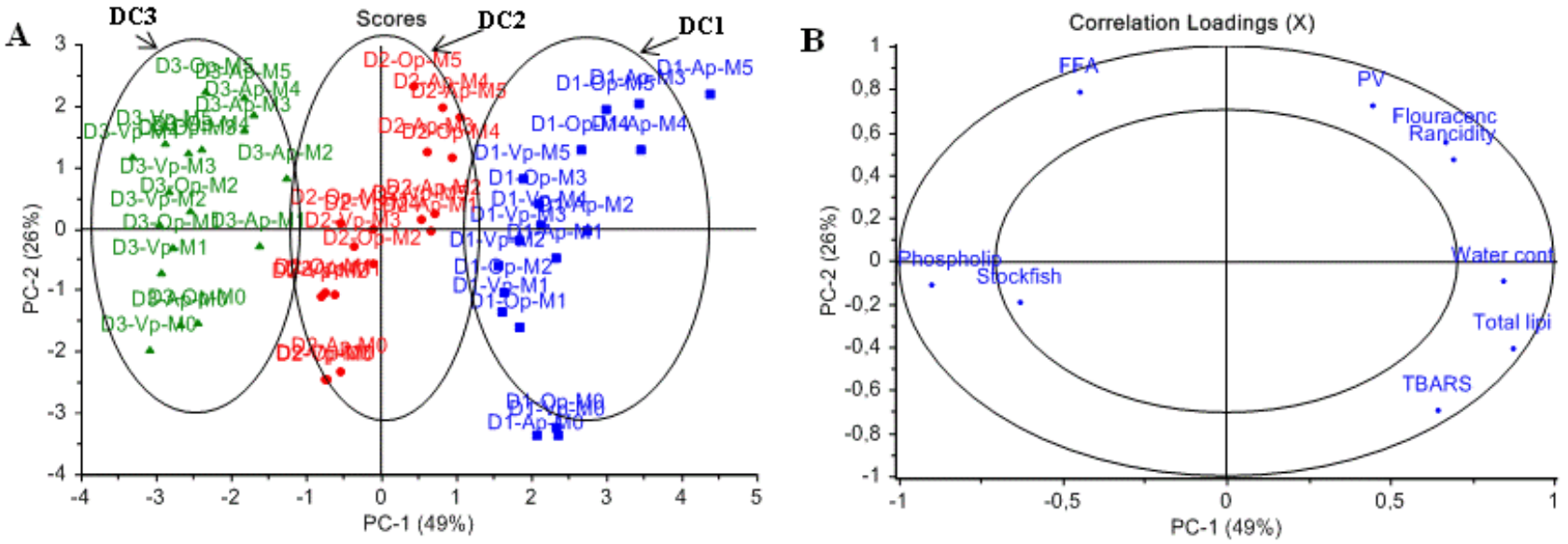

Figure 4: Principal component analysis (A) scores and (B) correlation loadings describing packed dried capelin based on variables evaluated at all sampling points. PC1 49% vs. PC2 26%. DC1 = dried capelin of high lipid; DC2 = dried capelin of moderate lipid; DC3 = dried capelin of low lipid; Op = open bags; Ap = sealed bags; Vp = vacuum bags; M = month of storage.

Marketing potential of improved dried sardine (*Sardinella gibossa*) and capelin (*Mallotus villosus*) in markets accustomed to traditional dried fish

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Under review

Marketing potential of improved dried sardine (*Sardinella gibossa*) and capelin (*Mallotus villosus*) in markets accustomed to traditional dried fish

Running title: Marketing potential of improved dried fish

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ABSTARCT

Traditional dried small fish are an important source of protein for low income people in many developing countries. The aim of this study was to determine the marketing potential of improved dried sardine and capelin as new products in markets accustomed to traditional dried fish. One hundred and twenty participants were recruited among shoppers at supermarkets and open-air markets in Kenya. Each participant received 500 g of each product to be prepared and consumed at home, before evaluating acceptability and willingness to buy. The products obtained high acceptability ratings. Middle income classes were willing to pay up to KSH 600 for 500 g of capelin but KSH 400 for sardine, while low income classes were willing to pay up to KSH 400 the reference price for both products. There is market potential for new dried small fish products that are of improved quality among consumers accustomed to traditionally dried fish.

PRACTICAL APPLICATIONS

Small pelagic fish species are among the important fisheries in the world with sustained catch stocks; nonetheless, small quantities are used fresh, dried or salted for human food. In this paper the marketing potential of improved dried small fish species such as capelin, as new products in the markets accustomed to traditional dried fish is assessed. The study shows that there is market potential for new dried small fish products that are of improved quality among consumers accustomed to traditional dried fish, and that the middle income classes are willing to pay more for improved quality. Consumption of improved dried small species may contribute to reduction of malnutrition that is prevailing in many developing countries, and as well increase the utilization of less valued species such as capelin for human food.

Key words: Marketing, capelin, sardine, acceptability, willingness to buy

1 INTRODUCTION

In Eastern Africa, particularly Kenya, Uganda and Tanzania, there is a high demand for dried fish, mainly small pelagic species (IOC, 2012). Originally the main market for small pelagic fish was the animal feeds industry. However, due to an increase in human population,

reduced availability of high value fish species in local markets and improved fish drying methods, over 80% of small pelagic fish now goes to human consumption (IOC, 2012). Consumer preference for dried small pelagic fish is not only because of the flavor, but also the reasonable price, availability and stability during storage (Peter Oduor-Odote et al., 2010). Dried small pelagic fish are traditionally sold in open-air markets in small portions to meet the needs of the low income class (FAO 2007; Oduor-Odote et al. 2010).

The growth of the middle class in East Africa (African Development Bank, 2011) and reduced availability of high value species (IOC, 2012) has resulted in a demand for premium dried small pelagic fish. Small pelagic fish dried on improved traditional racks that are raised ventilated platforms in open-air, are widely available in open-air markets (Peter Oduor-Odote et al., 2010). But their quality varies extremely due to dependence on weather conditions and often does not meet the local food regulation standards (Kenya Bureau of Standards 2015; Langat and Rey 1999). Quality uncertainty notwithstanding, small quantities of packed fresh water dagaa (*Rastrineobola argentea*) from Lake Victoria dried on racks have gained access to national retail stores, largely supermarkets, although a much higher proportion is still sold unpackaged in open-air markets.

There is awareness of the nutritional and health benefits associated with fish consumption, especially the omega-3 polyunsaturated fatty acids (Bilgin et al. 2008; Stołyhwo et al. 2006). Dried small fish are highly nutritious (Jain & Pathare, 2007) and improving the quality, and marketing in national retail stores is likely to increase consumption among the middle class. This will raise the value of the fishery thereby creating more employment and higher income along the value chain, especially among women who play an important role in the processing and marketing of this fishery (Schuurhuizen, Van-Tilburg, & Kambewa, 2006).

Fresh water dagaa (*Rastrineobola argentea*) landed from the Kenyan part of Lake Victoria was 92,000 MT in 2013 comprising about 54% of total catch of fish in Kenya (Lake Victoria Basin Commission, 2015; Munguti et al., 2014). Sardine landings was reported at 881 tons in 2013 corresponding to about 10% of the 9,000 tons of artisanal marine fish landed in Kenya (Warui, 2014). This is probably an underestimate. Low valued fish are often underreported in Kenya (Malleret-King et al., 2009; Gitonga, 2005) and sardine is caught using

light attraction at night and landed early in the morning before enumerators start work. The total small fish landings in Kenya cannot meet the existing demand (IOC, 2012). Most developing countries import low value fish while they export more valuable species landed from their waters. In 2012 Kenya exported a total of 10,165 metric tons of fish and fish products valued at KES 4,000 million (approximately 40 million USD) (State department of Fisheries Kenya 2012). Over the same period 2,622 metric tons of fish mainly frozen mackerels (62.3%) and sardines (13.7%) were imported largely from Japan, Pakistan and Korea.

Capelin landing in Iceland has exceeded half a million tons in recent years (Statistics Iceland, 2015). A small portion of it particularly female is used for human food, with about 80% reduced to fishmeal and oil (Statistics Iceland, 2015). Female capelin is exported to Japan and Europe as whole fresh/frozen product or roe while male capelin and females without roe is reduced to fish meal and oil (Statistics Iceland 2015; Shahidi et al. 1995). Therefore, there is a potential to introduce dried male capelin as a new product into markets accustomed to small dried fish especially in developing countries.

The choice and acceptability of a food product is mainly based on sensory properties. If a product has low sensory acceptability, no brand or nutritional and/or health benefit promise will manage to get it accepted by consumers (Sosa et al. 2008; Hough et al. 2006). But if a product has high sensory acceptability, there are additional issues that have to be resolved to ensure overall acceptability for instance packaging, price, convenience and cultural habits. In the East African markets, fish price is a major determinant of consumer purchasing decisions (IOC, 2012). Before deciding on the introduction of a new product into the market it is necessary to obtain information about sensory acceptability and possible pricing of the product (Grunert et al. 2009; Sosa et al. 2008). Such information can also aid in deciding on the launching strategy.

Home use and standardized situation tests are universally used in consumer research (Boutrolle et al. 2007; Sosa et al. 2008). Home use tests are more reliable as they are conducted in a setting where the product being tested is normally consumed (Boutrolle et al. 2007; Boutrolle et al. 2005). Larger amounts of products tend to be consumed in home use test and consumers are free to choose when to prepare and consume the product (Boutrolle and others

2007, 2005). Since dried small fish are mainly cooked and consumed in the homes as part of the main course of a meal, home use test was found to be ideal for the current study.

In Kenya, the majority of low income consumers shop for food in open-air markets whereas middle income groups especially in towns and cities do most of their shopping in supermarkets. Therefore Mombasa city and Kwale county in Kenya were selected for the study. Mombasa is cosmopolitan in nature and hosts the main supermarkets along the Kenyan coast, with the majority of its population belonging to middle income class (Ipsos-Synovate 2013; Kenya National Bureau of Statistics 2015). The main sardine landing beaches along the Kenyan coast are in Kwale county where consumption of dried sardine is widespread. Majority of the population in Kwale belong to the low income class (Ipsos-Synovate 2013; Kenya National Bureau of Statistics 2015).

The objective of this study was to determine the marketing potential of dried sardine of improved quality and indoor dried capelin among low and middle income consumers presumed to be represented by respondents shopping in open-air markets and supermarkets respectively. The information is necessary to evaluate the feasibility of improving drying methods and packaging of sardine, and the introduction of new dried fish products such as capelin to the markets accustomed to traditionally dried small fish.

2 MATERIALS AND METHODS

2.1 Samples

Sardine were dried on raised rack drier in Mombasa, Kenya and packed in sealed polyethylene bags weighing 500 g each before the study (water content 24%, fat 9%). Capelin were dried under controlled drying conditions (O. Cyprian, Nguyen, Sveinsdottir, Jonsson, Thorkelsson, et al., 2015) in Iceland and transported by air freight to Mombasa Kenya. Capelin (water content 19.5%, fat 27%) was packaged in the same way as sardine in polyethylene bags weighing 500 g. Processing and packaging complied with local food regulations (Kenya Bureau of Standards 2015; Langat and Rey 1999).

2.2 Subjects/respondents

The study was carried out in Mombasa city and Kwale county that are located in the southern part of the Kenyan coast.

Participants were recruited among shoppers in three supermarkets in Mombasa and three open-air markets in Kwale county over a four week period. Participants were adults willing to take part in the study. Participants contact details were obtained. In the open-air markets a local person from each area was hired during the period of study to collect completed questionnaires. Participants in supermarkets returned completed questionnaire at the supermarkets and those who returned completed questionnaires could win a prize which was announced on the radio. A total of 120 consumers participated; 60 supermarket shoppers and 60 open-air market shoppers.

A home use test was used in this survey. Participants were given a pack of the first product (500 g) with the instructions that they were free to prepare the product as they habitually do when it suited them best together with family and/or friends and complete the questionnaire within one week. Completed questionnaires were returned by participants from supermarkets on a specified date at the supermarket, and were collected during the first visit a week later for participants from open-air markets. Once the completed questionnaires were collected, the respondents received the second product (500 g) to be consumed within one week's time. Product presentation order was balanced while issuing out products with a half the number of participants receiving the sardine first and the other half got the capelin first.

2.3 Questionnaire

Respondents rated their liking of the product appearance, flavour and texture using a 9-point hedonic scale ranging from 1 (dislike extremely) to 9 (like extremely). Respondents were also asked about their general willingness to buy and willingness to buy 500 g of the products at Kenya shillings (KES) 200, 400, 600 and 800 on a 9-point scale from 1 (very unlikely) and 9 (very likely). The participants were informed that the price of dried sardine in the market was around KES 400 (reference price). Socio-economic demographic questions (gender, religion, education level, occupation, household size and who consumes dried fish in the household) was also included in the questionnaire since they have been found to relate to the preferred type of

food and reason for purchase(Green et al. 2003; Obiero et al. 2014). The questionnaires were translated into Swahili and briefly explained to participants when they received the first sample. The whole process, across the study areas lasted four weeks.

2.4 Data analysis

Data was analyzed using the Statistical Package for the Social Sciences (IBM - SPSS Inc. version 20.0). Descriptive analyses were done by use of means, standard error, percentages and frequency distribution of responses. The influence of product presentation order, product liking rating and willingness to buy based on the product type and shopping location were tested with ANOVA (score = product x subject/consumers) followed Duncan's means separation test (Post-hoc) for differences between groups. P values of < 0.05 were considered significant.

3 RESULTS AND DISCUSSION

3.1 Socio-economic profile of fish consumers

A hundred and twenty completed questionnaires were collected (60 supermarket shoppers and 60 open-air market shoppers) which is considered to be an adequate number for a consumer test (Hough et al. 2006). The socio-demographic characteristics of the two groups differed (supermarkets and open-air markets) except for gender that was relatively similar in both groups (Table 1). Although more than half of the participants were women, the proportion was less than expected as females are the primary shoppers of households in most parts of Africa (Obiero et al. 2014; Schuurhuizen et al. 2006). Women were less willing than men to participate in the study, possibly because of a high rate of illiteracy among the women. Majority of respondents shopping in open-air markets were Muslims unlike the supermarkets which were dominated by Christians. This was expected given that the population of Kwale county is largely Muslims while a higher proportion of the population in Mombasa is Christians. Religion is thought to have an influence on respondents' habits and could affect product acceptability. A majority of Muslims believe in observing a certain ritual of slaughtering procedures for animals, negatively influencing some believers from accepting dried fish produced by non Muslims.

Majority of the respondents shopping in open-air markets (72%) had only elementary education or high school education level (28%) (Table 1). Majority of respondents shopping in

supermarkets had completed high school (63%), followed by those with a university degree (30%) and elementary education (7%). Most of the respondents shopping in open-air markets were working as fishermen (27%), businessmen (27%) and farmers (25%) with 47% of these shoppers having a household size of 7-9 people. The respondents shopping in supermarkets were mainly working for private companies (48%) and government (30%) with 46% of them having a household size of 4-6 people.

Respondents shopping in open-air markets consumed dried fish more frequently (33%, more than or equal to four times a week) than those shopping in supermarkets (5%, four times a week or more often) (Table 2). This indicates that shoppers in open-air markets consume dried fish on a regular basis. Consumption of dried fish was negatively influenced by education, with high consumption frequency among less educated consumers (Table 2). Educated consumers are generally more aware of the health and other benefits associated with fish consumption. In a study on preferences for fish and seafood using an 'evoked set' analysis education was found to positively influence preference for fish (Kinnucan et al., 1993). Health benefits are however questionable when it comes to low quality products that may be contaminated. Kenyan consumers consider dried small pelagic fish to be an inferior quality product sold mainly in rural areas (Peter Oduor-Odote et al., 2010), where a majority of the population are poor with limited education. A majority of respondents shopping in the supermarkets are most likely middle class as they were mainly working for private companies or government and had relatively small households. Therefore, they are able to purchase more expensive protein sources than dried fish whose quality is uncertain and not often available in the supermarkets.

Dried fish is largely sold in open-air markets, with 95% and 58% of respondents shopping in the open-air markets and supermarkets respectively purchasing dried fish in open-air markets (Table 1). Forty two percent of supermarket shoppers and 5% open-air markets shoppers purchased dried fish from supermarkets. The low proportion of consumers purchasing dried fish from supermarkets is mainly attributed to restricted sales of dried fish to low price open-air markets and people with low income in Kenya (Peter Oduor-Odote et al., 2010) with only small quantities reaching national retail stores/supermarkets.

3.2 Product acceptability

The products were generally well received with acceptability rating scores ranging between seven and eight (Table 3). Although the overall acceptability values were relative for both products, respondents shopping in open-air markets rated both products higher than those shopping in supermarkets. Open-air shoppers rated the products higher as are familiar and regular consumers of dried fish. The result is in agreement with a study by Boutrolle et al. (2005) who reported that greater familiarization with a product resulted in higher acceptability ratings. Dried capelin had significantly ($p<0.05$) higher appearance rating irrespective of the respondents' shopping location. On the other hand, sardine obtained the higher flavor rating. Liking of texture was not significantly different ($p>0.05$) between the two locations, with a higher rating for capelin at both locations. Capelin appears to be attractive to consumers but the flavor was moderately ranked and needs to be improved. Both dried capelin and improved processed sardine were acceptable in the Kenyan markets. This implies that new dried fish products of superior quality can in general be accepted in Kenya and East African markets accustomed to dried small fish.

The socio-demographic did not appear to influence product acceptability except for level of education (Table 4). Education primarily determines the consumers occupation and in this case the income (Obiero et al., 2014; Green et al., 2003; Kinnucan et al., 1993). Most respondents shopping in the supermarkets commented that poor quality dried products (contaminated with soil) and unavailability in national retail stores limited dried fish consumption among the group. Even though the majority of respondents shopping in supermarkets were irregular dried fish consumers, they rated products highly implying that they might accept new dried fish products such as capelin if quality could be improved. Gender, religion and household size had no significant influence on product acceptability.

3.3 Respondents' willingness to buy

The results shows consumers were willing to buy the products irrespective of the shopping location (Table 5). The average values of willingness to buy capelin and sardine were relatively high and very close, with a significant difference ($p<0.05$) only obtained based on respondents shopping location. Those shopping in open-air markets were more willing ($p<0.05$)

to purchase the products than those shopping in supermarkets. This may be because consumers shopping in open-air markets were more familiar with dried fish.

On the specific amount of money the respondents were willing to pay for 500 g of the product, open-air markets and supermarkets shoppers had high rating for both products at KSH 200, but significantly higher rating was obtained for capelin than sardine (Table 5). Consumers were not willing to pay more than the reference price of KSH 400 for improved dried sardine, but supermarket shoppers were willing to pay up to KSH 600 for dried capelin (Table 5). The unwillingness of consumers to pay more than the reference price for sardine could be that they were not able to see the difference of improved dried sardine from traditionally dried. Sosa and others (2008) reported food product choice and acceptability to be based on the sensory properties. Sardine was dried in enclosed drier that depended on weather conditions and may have only reduced contaminations from the environment (such as soil) but not lipid oxidation that affects color development during drying resulting in unattractive products. Also the reference price used in the study was based on the supermarket price of a product similar to the improved sardine used in this study and therefore more expensive than traditional dried sardine sold at about KSH 300 in open-air markets.

These results indicate that a high market potential exists for dried small fish among the low income class but can be enhanced among the middle income consumers if quality is improved, especially appearance, as willingness to buy capelin at a higher price than sardine appeared to be based on the appearance (Table 3).

4 CONCLUSIONS

Dried capelin and improved dried sardine received relatively high acceptability ratings. However the products differed in definite attributes with capelin obtaining significantly higher ratings for appearance, while sardine obtained significantly higher flavor ratings. Consumers shopping in supermarkets considered to represent middle income groups were willing to pay up to KSH 600 for 500 g of capelin. Consumers shopping in open-air markets who consume dried fish on regular basis were willing to buy 500 g of dried capelin and sardine at up to KSH 400. The consumers of traditional dried small fish as well as new consumers especially in the middle income class might accept new dried fish products if overall quality could be guaranteed. A

follow-up study covering a large geographical area is recommended to assess business feasibility.

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Table 1 Socio-economic profile of respondents shopping in supermarkets (Mombasa) and open-air markets (Kwale) in Kenya

Variables	Response	Village markets % (n=60)	Supermarkets % (n=60)	Average % (n=120)
Gender	Male	43.3	41.7	42.5
	Female	56.7	58.3	57.5
Religion	Islam	80.0	33.3	56.7
	Christian	20.0	66.7	43.3
Education level	Elementary	71.7	6.7	39.2
	High school level	28.3	63.3	45.8
	University level	0.0	30.0	15.0
Occupation / working with	Government	5.0	30.0	17.5
	Private company	19.0	48.3	29.2
	Farmer	25.0	0.0	12.5
	Fisherman	26.7	3.3	15.0
	Businessman	26.7	6.7	16.7
	Unemployed	6.7	11.7	9.2
Household size	1 - 3	3.3	8.3	5.8
	4 – 6	28.3	58.3	43.3
	7 - 9	46.7	30.0	38.3
	> 10	21.7	3.3	12.5
Purchase location	Open market	95.0	58.3	76.7
	Supermarket	5.0	41.7	23.3
Consumer in family	Child/children	5.0	18.3	11.7
	Adults	1.7	13.3	7.5
	All members	93.3	68.3	80.8

Table 2.

Dried fish consumption pattern among respondents at the coast of Kenya divided by shopping location and education level

	% respondents consumption frequency					
Education level/shopping location	Less than once a month	Once a month	2-3 times a month	Once a week	2-3 times a week	More often
Elementary education	4.3	6.4	14.9	10.6	29.8	34
Secondary education	22.2	5.6	38.4	11.1	11.6	11.1
University degree	25.6	20.9	18	18.2	8.2	9.1
Village markets	1.7	8.3	10	16.7	30	33.3
Supermarkets	30	8.3	33	11.7	11.7	5

Table 3.

Overall acceptability of improved dried sardine and indoor dried capelin. Average values based on a 9-point hedonic scale ranging from 1 (dislike extremely) to 9 (like extremely) (n=120)

Sensory attribute	Capelin		Sardine	
	Open market	Supermarket	Open market	Supermarket
Overall acceptability*	8.0 ± 0.3^{a}	7.1 ± 0.3^{b}	7.7 ± 0.2^{a}	7.0 ± 0.4^{b}
Appearance***	8.4 ± 0.3^{a}	7.8 ± 0.5^{b}	6.8 ± 0.4^{c}	6.8 ± 0.3^{c}
Flavour***	6.7 ± 0.2^{a}	6.3 ± 0.5^{a}	7.6 ± 0.2^{b}	7.4 ± 0.1^{b}
Texture	7.2±0.5	7.4±0.3	7.0± 0.4	7.1±0.2

[a] Different letters (superscript) indicate significantly different values between samples within a row. *$p < 0.05$, ***$p < 0.001$

Table 4.

Overall acceptability (1 = "dislike extremely" to 9 = "like extremely") and willingness to buy (1 = "very unlikely" to 9 = "very likely") of indoor dried capelin and improved dried sardine by demographic variables. Responses from open market and supermarket compiled

Variables	Character/response	Overall Acceptability		Purchase intent	
		Capelin	Sardine	Capelin	Sardine
Gender	Male	7.4±0.4	7.3±0.6	7.8±0.3	7.5±(0.2
	Female	7.6±0.2	7.4±0.3	7.9±0.1	7.6±0.5
	P-value	0.517	0.623	0.769	0.645
Religion	Islam	7.8±0.2	7.5±0.0	8.0±0.2	7.7±0.2
	Christian	7.2±0.3	7.2±0.3	7.6±0.4	7.5±0.1
	P-value	0.83	0.223	0.207	0.45
Education level	Elementary	7.9±0.3[a]	7.6±0.2[a]	8.2±0.3[a]	7.9±0.2[a]
	High school	7.5±0.2[a]	7.7±0.3[a]	7.8±0.2[ab]	7.6±0.3[a]
	University	6.6±0.5[b]	6.4±0.2[b]	7.1±0.5[b]	6.9±0.2[b]
	P-value	**0.028**	**0.045**	**0.046**	**0.039**
Household size	1 to 3	8.3±0.5	7.4±0.7	8.1± 0.5	8.3± 0.3
	4 to 6	7.4±0.3	7.3±0.2	7.6±0.2	7.3±0.2
	7 to 9	7.4±0.3	7.4±0.2	7.8±0.3	7.8±0.2
	Equal to/more than 10	8.3±0.4	7.5±0.3	8.4±0.3	7.5±0.3
	P-value	0.203	0.929	0.402	0.091

[a] Different letters (superscript) indicate significantly different values between samples within a column for a specific variable ($p < 0.05$)

Table 5.

Willingness to buy (1 = "very unlikely" to 9 = "very likely") indoor dried capelin and improved dried sardine at specified amount (90 KSH = I USD)

Willingness to buy	Capelin		Sardine	
	Open market	Supermarket	Open market	Supermarket
Unlikely/likely to buy	8.3±0.2[a]	7.3±0.2[b]	8.0±0.1[a]	7.2±0.2[b]
At KSH 200	8.6±0.2[a]	8.8±0.1[a]	7.9±0.1[b]	7.8±0.2[b]
At KSH 400	6.7±0.3[a]	7.5±0.2[b]	6.1±0.2[a]	5.2±0.3[c]
At KSH 600	4.0±0.2[a]	6.4±0.2[b]	3.2±0.2[c]	2.1±0.2[d]
At KSH 800	1.4±0.1	3.8±0.3[a]	1.2±0.1	1.2±0.1

[a] Different letters (superscript) indicate significantly different values between samples within a row. $p < 0.001$