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Report

Preserving the positive health effects in innovative pelagic fish products through the value chain (ProHealthPelagic)

Final report

Authors
Hanne Digre
Inger Beate Standal, Vera Kristinova, Grethe Hyldig (DTU), Ingrid Undeland (Chalmers), Turid Rustad (NTNU), Revilija Mozuraityte

SINTEF Fisheries and Aquaculture
Processing technology
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SINTEF Fiskeri og havbruk AS
SINTEF Fisheries and Aquaculture
Address:
Postboks 4762 Sluppen
NO-7465 Trondheim
NORWAY
Telephone:+47 40005350
Telefax:+47 93270701
fish@sintef.no
www.sintef.no/fisk
Enterprise /VAT No:
NO 980 478 270 MVA

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Hanne Digre
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ABSTRACT
This is the final report of the project ProHealthPelagic (2012-2015) funded by the Norwegian Research Council, BIONÆR. The project aim was to investigate potential changes in well documented and tentative health promoting compounds present in herring and mackerel throughout processing operations like salting, marinating and canning. Several experiments have been conducted and the results show: a) The health beneficial lipids are preserved up till 12 months at -27 °C in vacuum packed mackerel fillets. Changes in proteins do take place during frozen storage between 1-7 months of storage. Vitamin D was well preserved both during chilled and frozen storage; b) Storage of mackerel for 9 days at chilled storage led to development of some undesirable compounds (free fatty acids, biogenic amines, TMA, hypoxhantine). Preservation of mackerel by freezing is a better option compared to prolonged chilled storage when it comes to the content of health beneficial compounds; c) Canning of mackerel in tomato sauce did not reduce significantly the amount of healthy compounds; d) Reduction of salt (up to 45%) in marinade did not affect quality of lipids, vitamin D content of pre-marinated and final marinated herring product. The sensory tests showed small differences between the different herring samples and it is mostly the appearance and texture attributes that deviates.

PREPARED BY
Hanne Digre

SIGNATURE


CHECKED BY
Tom Ståle Nordtvedt

SIGNATURE


APPROVED BY
Marit Aursand

SIGNATURE


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Norwegian abstract

Dette er sluttrapporten til prosjektet ProHealthPelagic (2012-2015) finansiert av Norges Forskningsråd, BIONÆR. Prosjektets mål var å undersøke potensielle endringer i veldokumenterte og potensielle helsefremmende forbindelser i rå sild og makrell fra ulike sesonger og gjennom håndtering, prosessering og lagring av råstoffet. Prosjektet har bestått av fire arbeidspakker med fokus på 1) Råstoff, kvalitet og helserelaterte aspekter; 2) Prosessering av sild og makrell til høykvalitets produkter; 3) Marked og konsument; 4) Mekanistiske studier med fokus på grunnleggende mekanismer mht effekten av nye prosesser. Prosjektet har fokusert på to produkter: hermetisk makrell i tomat saus og marinert sild.

Fet fisk som makrell og sild er en viktig del av et sunt kosthold. I tillegg til de velkjente helsefordelaktig langkjedede omega-3 fettsyrer og vitamin D, inneholder disse fiskene også enumettede fettsyrer, proteiner, di-peptider (karnosin, anserine) og frie aminosyrer (taurin). Anbefalinga fra norske myndigheter er at man skal spise fisk 2-3 ganger i uka. Det viser seg derimot at 2/3 av fisken som spises er mager fisk. Et av målene i prosjektet har vært indirekte å øke forbruket av sild og makrell, og på den måten forbedre helsa til det norske folk.

Flere forsøk har blitt gjennomført og resultatene viser følgende:

- De helsemessige gunstige lipidene er bevart opp til 12 måneder ved -27 °C i vakuumpakket makrellfilet. Endringer i proteiner finner sted under fryselagring mellom 1-7 måneders lagring. Vitamin D var godt bevart både under kjøle- og fryselagring.
- Kjølelagring av makrell inntil 9 dager førte til utvikling av noen uønskede forbindelser (frie fettsyrer, biogene aminer, TMA, hypoxhantine). De helsemessige gunstige forbindelsene i lipider og proteiner bevares bedre under frysing sammenlignet med kjølelagring av makrell.
- Hermetiseringsprosessen for produktet "makrell i tomat" reduserte ikke mengden av sunne forbindelser.
- Det er også jobbet med mildere varmebehandling av makrell i tomat (dvs. < 100^{o}C), hvor man studerte ulike flyktige komponenter. Resultatene viser at protein-oksidasjon finner sted under oppvarmingsprosessen, og i større utstrekning ved 80 og 90°C sammenlignet med 50 og 60°C. De sensoriske analyser viser imidlertid at det er visse utfordringer ved å bruke mildere temperaturer i produksjonen av hermetisk makrell i tomat saus.
- Marinering av sild med lavere saltinnhold er studert og tre studier ble gjennomført med råstoff med ulike fettprosent fra ulike sesonger (november 2013, januar 2014 og oktober 2014). Åtte eddik-marinader ble undersøkt hvor NaCl-innholdet varierte fra 14.5% (dagens marinade) til 6.15% (dvs. opp til 58% totalt salt reduksjon), inkludert delvis erstatning av NaCl med KCl. Resultatene viser at kvaliteten på halvfabrikata marinerte sildeprodukter varierer med sesong. Lagringstemperatur hadde ingen effekt på de målte parameterne. Farge, tekstur, lipid oksidasjon (harskning), nivåer av frie fettsyrer og vekt av sildbiter var påvirket av marineringstid, men kun tekstur var påvirket av de ulike saltkonsentrasjonene i marinadene. Sildebitene ble betydelige mykere i marinader med 43% og 58% redusert salt. Oksidasjonen varierte avhengig av fettinnhold i silda (sesongvariasjoner), men var ikke påvirket av saltnivå, temperatur (etter lagring i 35 dg) eller marineringstid. Den mikrobiologiske kvaliteten var god for alle marinadene (Kim tall < 400 etter 10 måneder). Sensorisk vurdering av løksild produsert fra de ulike marinadene viste ingen nevneverdige forskjeller sammenlignet med dagens produkt. Prosjektet viser at det er mulig å produsere marinert sild med lavere NaCl nivå, som har god kvalitet, samt ivaretar krav til mattrygghet. Marinade med 25% totalt salt reduksjon hvor NaCl er redusert med 43% og erstattet med 18% KCl viste de beste resultatene og kan anbefales dersom det er ønskelig å redusere saltinnholdet i marinert sildeprodukter.

Prosjektet har gitt økt kunnskap om samspillet mellom ulike produksjonsprosesser og stabiliteten til de helsefremmende komponentene i pelagisk fisk. Prosjektet har jobbet tett med industrien og bidratt til utvikling av marinerte sildeprodukter med lavere saltinnhold, samt studert prosessen med mildere varmebehandling av hermetisk makrell i tomat saus, samt lagringsstabilitet av makrell.

1 Introduction

The project aim was to investigate potential changes in well documented and tentative health promoting compounds present in raw herring and mackerel throughout processing operations like salting, marinating and canning. With the intention to maximize the healthiness of the end products, the investigations were carried out during novel/modernized versions of these processes where e.g. the amount of salt has been reduced, the thermal regimes of the canning process has been modified and natural antioxidants have been added. Among well documented health promoting compounds in pelagic fish are the omega-3 fatty acids and vitamin D, while monounsaturated fatty acids (MUFAs) (C20:1 and C22.1), di-peptides (carnosine, anserine) and free amino acids (taurine) constitute more tentative candidates, as clinical evidence to date are less established. In order to understand the full impact of the suggested process development the sensory quality of marinated herring was evaluated in relation to reductions in salt.

The ultimate aim of the project was to increase the consumption of herring and mackerel especially for young people, in order to increase healthiness of the Norwegian population. According to the Norwegian dietary guidelines it is recommended to eat fish 2-3 times a week (about. 200 g/week). Median fish consumption for the Norwegian population is relatively high (65g per day for an adult and 6-19 g per day for children (VKM, 2006). However, two third of the consumption consist of lean fish products. The Norwegian Scientific Committee for Food Safety estimated that the average fish consumption insufficient to cover the recommended intake of D vitamin and omega -3 without resorting to dietary supplements or increased fatty fish consumption (VKM, 2006).

The Norwegian and European diet contains too much salt (NaCl), and the Health Directorate in Norway recommends decreasing the daily intake of salt from 10 to 5 gr. Lowering the salt content in marinated herring products has also been focused in this project. The project results may open up for targeted development of new pelagic fish products that could increase market shares, especially for herring products in Norway. The Norwegian Seafood Council has for the last two years been focusing on marketing of pelagic fish products in Norway, resulting in volume increase of 2% from 2009 to 2010. This growth is mainly linked to mackerel. The product "mackerel in tomato" has grown steadily in recent years due to very successful product development (e.g. "single box", and different flavors and package types). However, both the mackerel and herring consumption in Norway has still a potential to grow further, and there is a need to develop new products tailor-made for young people. The results of this project will give the pelagic industry increased competence that can be used for such innovative product development of healthy fish products.

1.1 Health beneficial compounds in herring and mackerel.

Consumption of fatty fish such as herring and mackerel provides numerous important nutrients linked both to their lipids, proteins and water soluble compounds. Herring and mackerel are fatty fish species with lipid contents varying between 8 and 20% and 17 and 35%, respectively (Falch et al., 2006). The lipids of these species are rich in omega-3 fatty acids with documented preventive effects against cardiovascular disease (CDV), positive effects on early neurodevelopment, central nervous system disorders etc. (VKM, 2011). An average recommended intake of EPA and DHA is 250 mg/day (VKM, 2011). The amount of LC omega-3 fatty acids was found to be between 1 and 2.7g/100g in herring and between 3.8 and 6.5g/100g in mackerel (Falch et al., 2007). Therefore, increased consumption of these fish species could help to cover the recommended daily intake of LC omega-3 fatty acids.

However, the healthy omega-3 fatty acids are highly susceptible to oxidation resulting in rapid quality loss. During lipid oxidation, endogenous antioxidants are consumed and various primary and secondary oxidation compounds are formed, the latter which reduce the sensory quality (undesirable taste and flavour) and may lead to significant losses of protein functionality through cross reactions with proteins (Larsson and Undeland, 2010). Since both antioxidants and omega-3 fatty acids are partly consumed in oxidation

reactions, the nutritional value of the product is also reduced. In this context, it should be stressed that certain lipid oxidation products per se may counteract some of the positive effects obtained from intact omega-3 fatty acids (VKM, 2011). Formation of off-flavor due to oxidation will also have a negative impact on consumer acceptance of seafood products (Børresen, 2008). Therefore, minimizing oxidative deterioration during processing and storage is highly important for several reasons.

Herring and mackerel also contain high amount of long chain monounsaturated fatty acids (MUFAs), such as cetoleic acid C22:1n-11. MUFAs have gained increased interest, and emerging evidence suggests that this class of fatty acids may be cardioprotective (Willet, 2006), increase peroxisomal beta oxidation and limit hyperplasia in obese (Thomassen, 1985). Recently it has been reported that the MUFA cetoleic acid has positive effect of the elongation of short chain omega-3 fatty acids to LC omega-3 PUFAS in human liver cells (http://nofima.no/nyhet/2015/12/silda-er-sunnere-enn-vi-trodde/#relatertboks2). It is therefore important to increase knowledge on the stability of MUFAs as a function of processing parameters. It is expected that they to some extent will be subjected to oxidation, but not to the same degree as the LC omega-3 fatty acids.

Fatty fish is one of the few dietary sources of vitamin D. Vitamin D deficiency cause rickets in infants and children, osteomalacia in adults, and are related to CDV (Undeland et al., 2009). Deficiency and insufficiency of vitamin D are commonly reported in epidemiological studies from Nordic countries (Holick and Chen, 2008). Circulating levels of 25-hydroxyvitamin D over 75nmol/L or 30ng/mL have been suggested to be required to maximize the health beneficial effects from vitamin D. To achieve this, during winter, a daily intake of 25 µg/day of vitamin D may be needed (Holick and Chen, 2008). In 2012, the Nordic Nutrition Recommendations for daily intake of vitamin D were raised from 5µg/day to 10µg/day for young children and adults since it is estimated that people in the Nordic countries may not get enough vitamin D during the dark winter season. For elderly people, the raise was from 10 to 20µg/day (Nordic Nutrition recommendations, 2012).

Fish is also considered as an excellent protein source due to the high protein quality, i.e. content of all essential amino acids and high digestibility (FAO/WHO 1991). It is well known that marine lipids are susceptible to oxidation. However, reaction of lipid radicals with proteins results in changes both in the amino acid side groups and in the backbone. This can result in loss of functional properties – oxidation of proteins in processed meat has been found to result in reduced water holding capacity as well as textural changes. Oxidation of proteins may also result in changed solubility, hydrophobicity, conformation and altered susceptibility towards proteases (Morzel et al., 2006). This could explain the low digestibility and reduced nutritional value of oxidized proteins.

Fish proteins have in the last 10 years gained attention as a source of bioactive peptides. Such peptides can be formed either during the gastrointestinal digestion or in the product itself as a result of endogenous proteolytic enzymes in marinated fish. Carnosine, anserine and ophidine are antioxidative peptides naturally present in skeletal muscle tissues, in amounts that depend on species, age and diet (Chan and Decker, 1994). The free sulfonic acid taurine has been shown to have a positive effect on the cardiovascular system, reduce blood cholesterol and have antioxidative activity (Undeland et al., 2009). During pre-handling and processing of pelagic fish, there are many steps in which both proteins, peptides and amino acids may leach out from the tissue. Critical operations are thought to be the thawing, pre-salting and marinating processes where the fish loses muscle liquid and is in contact with large volumes of liquid. To maximize health properties of pelagic fish, it is therefore highly valuable to know how these compounds are retained during different processing steps.

1.2 Catch and use of herring and mackerel

The NVG (Norwegian spring spawing) herring is caught along the Norwegian coast from around August to February. A few companies in Norway process the herring into deskinned fillets or pieces that are pre salted and marinated in plastic barrels. This semi preservative is sold to herring plants in Europe where the marinated herring is stored at <4°C for 1 to 10 months. The barrels are then drained and the herring is weighted into the primary packing together with sauce, spices and vegetables. The final products, containing 3-5% salt, are then distributed over the whole of Europe. The herring industry primarily uses fresh herring as raw material for these products. However, it is expected that frozen herring and herring fillets will in future account for a larger part of the raw material available on the market (Digre et al., 2014). However, no studies exist focussing on avoiding changes and losses in omega-3 acids, MUFAs, vitamin D, proteins, peptide and free amino acids during salting/marinating of frozen and fresh herring caught during different seasons.
Many traditional marinated fish product have a high salt content, which could counteract the beneficial effects of the fish. Increased salt concentration of the marinating brine decreased marinated herring meat properties (Szymczak, 2012). Therefore, some improvements in current salting/marinating procedures like reducing salt without compromising qualities of the product are needed and have been one of the focuses in this project.

Mackerel (*Scomber scombrus*) is caught in the North Sea by purse seiners or trawlers from September to February, with the main catch period in September/October. The mackerel is landed at bulk-processors, which mainly produce whole frozen, gutted frozen or frozen fillets, and then exported as semi-products to the end-producers mainly located in other countries. A few processors in Norway produce end-products of mackerel; one of these products is mackerel in tomato sauce, which was the targeted product in this project. The product mackerel in tomato sauce consists of different processing steps involving thawing of mackerel (even though some fresh mackerel is used in the season), cutting, filling of mackerel and sauce in the box and finally autoclaving.

Due to the thermal processes, damage of lipids and proteins can occur and influence the total quality of canned fish product. Because of the thermal treatment, lower amounts of omega-3 fatty acid were found in canned fish compared to raw material (Selmi et al., 2008, Medina et al., 2000). Improvements in thermal regimes could help maintain the nutrition value of the fish. Medina et al. (2000) found that heat treatments involving the highest temperatures for the shortest time gave canned tuna product with the highest omega-3 amounts and lowest lipid degradation. Oxidation of fish during canning and storage is also dependent on the type of filling media. The muscle of canned fish in tomato sauce usually contain more omega-3 fatty acids than canned fish in oil, most probably due to antioxidative properties of tomatoes and the lack of extraction by the media (Kolakowska, 2003).

1.3 Consumer preferences

Consumer liking of a food item depends on its sensory quality. Decisions concerning eating quality preference for fish products as well as other food products are made during consumption. The structural and flavour active components from the fish products are perceived by the sensory systems, and this information is combined with the consumer's recollection of previous eating experiences. However it is difficult for the consumer to give a precise and well defined description of the sensory characteristics of a product. By using descriptive sensory analysis carried out with a trained sensory panel it is possible to obtain an objective and quantified sensory description. Consumer liking and the sensory characteristics of a product can be related by use of different multivariate techniques and give the needed knowledge for the fish industry to produce high quality products with a high consumer preference.

2 Organization of the "ProHealthPelagic" project

The project has been organized with a multidisciplinary research team and with an advisory board with representatives from the industry listed below and illustrated in the figure below (Fig. 1).

Multidisciplinary research team:

Personnel	Main fields of competence
PhD Hanne Digre	Fish processing technology, fish quality, project man. of pelagic research projects
PhD Revilija Mozuraityte	Lipid oxidation, food chemistry, fish quality, lipid technology
PhD Inger Beate Standal	NMR, food chemistry and quality, multivariate data analysis, lipid technology
PhD Grethe Hyldig	Sensory and texture of fish and fish products
PhD Henrik H. Nielsen	Post mortem proteolysis in fish muscle tissue, proteolytic enzymes, proteins
Prof. Ingrid Undeland	Lipid and protein oxidation, lipid technology
Prof Turid Rustad	Biochemistry of marine raw materials, processing of food, quality

Advisory board:

Participants	Relevance
SINTEF F&H Sealab	Dr. Marit Aursand is well experienced in research management, food technology, lipid technology
Stabburet	Processors of canned mackerel products
Hopen Fisk	Semi-finished marinating herring products
Athena Seafood	Marketing of pelagic fish products, exporter

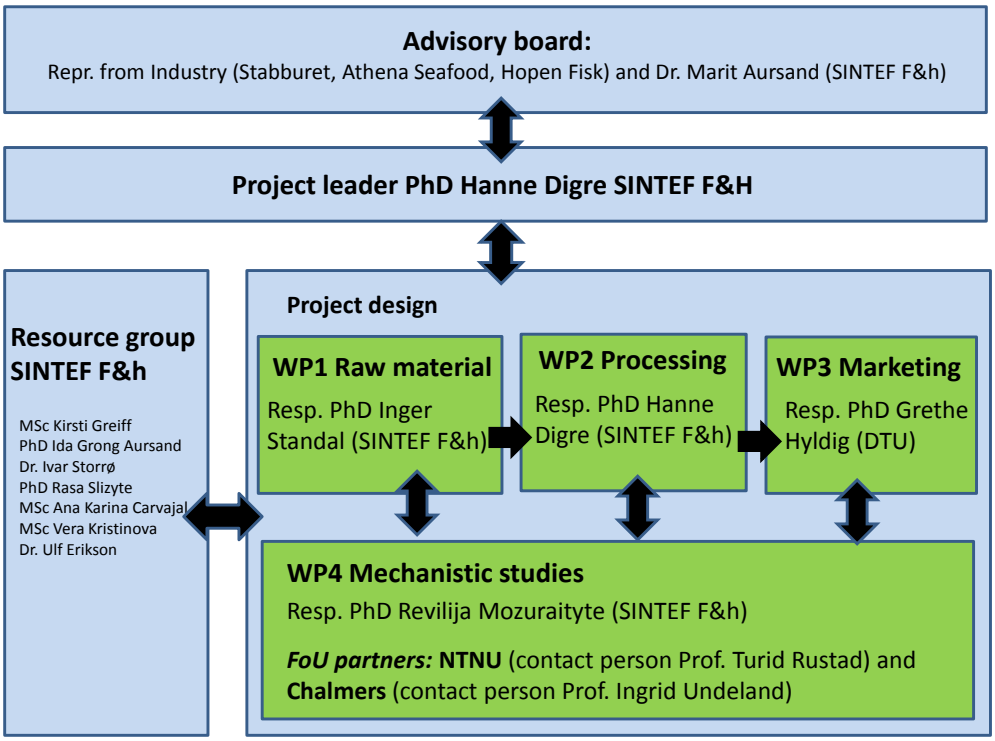

Figure 1. Organization of the project "ProHealthPelagic"

3 The objectives of the project

Main objective of this project has been to preserve health promoting compounds in traditional and novel pelagic fish products through the value chain, in order to increase the consumption of herring and mackerel in Scandinavia and especially Norway.

The main goal is to be achieved through the fulfilment of several sub goals:

1. To identify which handling and process steps during the production of salted/marinated and canned products of herring and mackerel that are the most challenging for the stability of omega-3 fatty acids, MUFA, vitamin D, proteins, di-peptides and selected free amino acids
2. To determine the effect of novel processes comprising e.g. use of frozen raw material, salt reduction, modified thermal treatment and additions of natural antioxidants on the stability of omega-3 fatty acids, vitamin D, proteins, di-peptides and free amino acids
3. To understand the biochemical changes taking place during novel processing methods and how these are related to sensory quality
4. To identify how health benefits of pelagic fish products and their individual health promoting components are effectively communicated to the consumers in order to increase the demand for and, consequently, the consumption of these products.

4 Impact for the research, fish processing industry and the society in general

- The results of the project show that vacuum packed mackerel fillets keep a good quality during frozen storage at -27 °C up to 12 months. The health beneficial lipids are preserved up to 12 months at -27 °C. Also the content of low molecular weight metabolites such as free amino acids, IMP and inosine, relevant for taste and food safety, are to a large extent stable during the 12 month frozen storage , however, the quality at the time of freezing the fish is critical. The time prior to freezing/canning of fresh fish, and the handling of the fish will influence the quality and food safety of the raw material, and thereby the final product e.g. mackerel in tomatoes sauce. Changes in proteins do take place during frozen storage between 1-7 months storage, but there is a lack in knowledge relating these changes to the quality parameters of fish. Vitamin D was well preserved both during chilled and frozen storage in this project.
- Storage of mackerel for 9 days at chilled storage led however to development of some undesirable compounds (free fatty acids, biogenic amines, TMA, hypoxhantine). The development of such compounds can reduce the healthiness of the final product. Therefore, preservation of mackerel by freezing is a better option compared to prolonged chilled storage when it comes to the content of health beneficial compounds.
- Traditional canning processes involve temperatures higher than 100^{o}C. The project results showed that the LC omega-3 fatty acid amount was only slightly lower in the canned mackerel muscle compared to the raw material before canning. However, the reduction of the LC omega-3 fatty acid amount is most probably because of the diffusion of lipids from muscle to the sauce. Vitamin D level was not significantly changed during canning. Therefore, the results show that canning of mackerel in tomato sauce did not reduce significantly the amount of healthy compounds.
- Reduction of salt (up to 45%) in marinade did not affect quality of lipids, vitamin D content of pre-marinated and final marinated herring product. Therefore, reduction of salt could improve healthiness of product (reduce daily intake of salt) and reduce the processing cost.

5 The project research areas and results

5.1 WP 1 – Raw material, quality and health-related aspects

Background:

The quality of fish raw material varies according to a wide range of factors including; biological conditions, catching methods, on board handling and storage. The same factors also affect the content of health-beneficial compounds such as LC- PUFAs, MUFA, vitamin D and proteins in addition to the content of amino acids and other low molecular weight metabolites relevant for food quality and safety.

In addition to varying initial qualities, the quality of fish changes during storage, and both lipids, proteins, peptides may be affected. However, also several labile low molecular weight metabolites are broken down during storage, this includes nucleotides, amino acids, and osmolytes such as TMAO.

The main objective of this WP was to screen seasonal variation in herring and mackerel and to find the relationship between pre-freezing, storage and thawing on the fish quality and content of the selected health promoting compounds.

Comprehensive analyses (all the compounds mentioned above) of mackerel fillets submitted to different storage conditions has been performed. The seasonal variation in herring and the effect of further processing is reported in WP2, while the results on the storage study of mackerel is reported below.

Figure 2. Catching and processing mackerel and herring (Photo: SINTEF).

Research tasks:

Task 1.1: *To analyse the condition of the raw material as affected by seasonal variation*

Task 1.2: *To evaluate the effect of different chilling, thawing and storage methods on the raw material, and the impact on the end product quality.*

Deliverables:

D1.1: Input to the peer reviewed scientific publications in WP2-4

Activity conducted:

Atlantic mackerel has been collected for two seasons, February 2013 and September 2014, and has been submitted to quality analyses during chilled/frozen storage, and after a canning into mackerel in tomatoes sauce. Additional, a study performed in a student project focused on different methods for thawing mackerel. The quality is evaluated by gross chemical composition, quality of lipids and proteins, vitamin D and content of low molecular weight metabolites relevant for food quality and safety.

Results:

Stability analyses during storage of mackerel

Stability of mackerel fillets during chilled storage at +4 °C (4 and 9 days after catch) and frozen storage at –27 °C (1, 7, 12 months after catch) has been studied for the February 2013 catch, also for different parts of the fillet. When analyzing the frozen fish, the vacuum packed fillets were thawed over night at 4°C.

The gross chemical composition was stable during the storage conditions; fat content was 21 ± 0.5 % of wet weight, water content was 62.4 ±0.7 %. A slightly higher amount of fat was obtained in dark muscle (20%) compared to light muscle (16%) in the mackerel fillet. Lipid oxidation was not observed at the storage conditions neither in the fillet nor in the light and dark muscle separately. The content of DHA and EPA was stable, 10 and 7% (w/w) of the total fatty acid composition, respectively (corresponding to 2.1 and 1.5 g/ 100g fish respectively). Also the content of MUFAs was stable, C22:1n-11 was the most abundant MUFAs at a level of 15% of total fatty acids (3.2 g/100 g fish). However, hydrolysis of lipids (determined by free fatty acid analyses) was observed, and this was most pronounced during chilled storage at +4°C (*Figure 3*). Vitamin D analysis (WP4) shows that the vitamin D level in mackerel was stable during chilled and frozen storage (0,18 µg/g dry weight, corresponding to 6 µg/100 g fish).

The properties of the proteins were influenced by frozen storage. Oxidation of proteins was determined as increase in carbonyl groups and decrease in thiol groups. For the myofibrillar proteins, a significant increase in carbonyl groups was observed from month 1 to month 7. A reduction of thiol-groups was observed from month 1 to month 7 for both myofibrillar and sarcoplasmic proteins. Freezing reduced the solubility of both myofibrillar and sarcoplasmic proteins. Water holding capacity of all muscle types was significantly lower after frozen storage for 7 and 12 months compared to after 1 month frozen storage and chilled storage.

The lower water holding capacity of 7 and 12 months frozen stored samples compared to chilled samples could be due to formation of ice crystals and increasing concentration of solutes and enzymes in unfrozen solutions leading to protein denaturation and decreased water holding capacity.

The observed loss in water holding capacity after 7 and 12 months frozen storage could also be due to protein oxidation. There was a significant correlation between the formation of carbonyls in myofibrillar proteins and loss of WHC during storage of Atlantic mackerel. In addition, protein oxidation has been reported to trigger protein denaturation and insolubilization due to the chemical and structural changes which have negative effects on water holding capacity.

Also, oxidative modifications lead to polymerization and aggregation of the proteins. However, more knowledge is needed on the relationship between protein oxidation during frozen storage and the quality parameters of muscle foods, especially in fish.

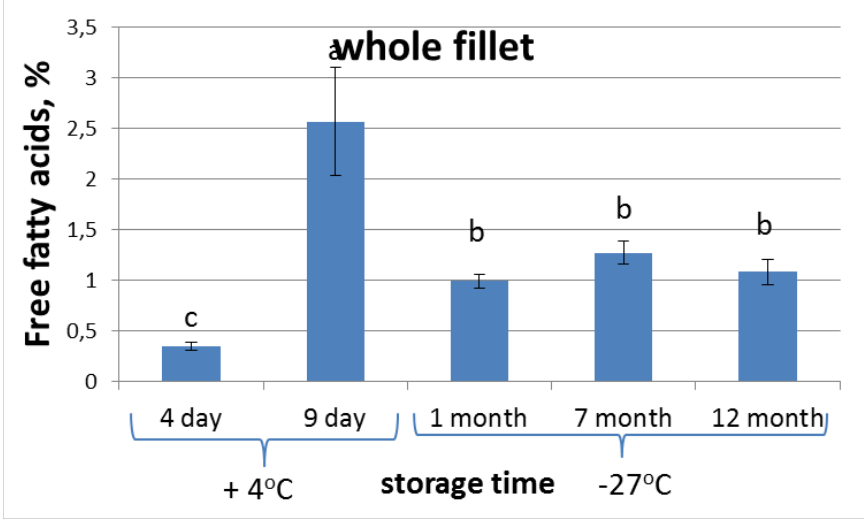

Figure 3. Content of free fatty acids in mackerel fillets stored at +4 °C and -27 °C, respectively.

Regarding low molecular weight metabolites, approximately 40 compounds was identified and quantified (by nuclear magnetic resonance (NMR)) such as free amino acids, nucleotides, and organic acids. The amino acid histidine dominated as the most abundant compound, followed by taurine, lysine, alanine, leucine, valine and tyrosine. The dipeptides anserine and carnosine (reported in other fish such as cod and haddock, Standal et al., 2007, Martinez et al., 2005) was not detected, in accordance with previous results reporting low content of these dipeptides in mackerel (Konosu and Yamaguchi (1982). The results show that bioactive compounds, such as taurine, and compounds relevant for taste of fresh fish, such as inosine (Ino) and inosine-monophosphate (IMP) are well preserved during frozen storage of 1- 12 months at -27 °C. The results show a slight increase in dimethylamine (DMA) both during chilled and the frozen storage, the endogenous enzyme (TMAOase) present in fish metabolizes TMAO to DMA and formaldehyde, the latter of which are proposed to cause toughening of cod muscle during frozen storage (Careche, 1998).

During chilled storage (+4°C) changes in composition occur that most likely will impact the quality of the final product. At day 9 after catch, the content of the biogenic amine histamine was 110 ±20 ppm, which is higher than the acceptable limit for seafood set by EU (100 ppm) and by the FDA (50 ppm). The amino acid lysine is completely degraded to cadaverine. The osmolyte Trimethyl-amine N-oxide (TMAO) is almost completely degraded into trimethylamine (TMA) which is associated with unpleasant "fishy" odor and taste.

The degree of freshness if often expressed in terms of a value K, described by the percentage of Ino and hypoxhantine (Hyp) of the total sum (ATP/ADP/AMP/IMP/Inos/Hyp). K values differ for different fish species- but in general the limits for consumption is ca 80 (% of hypoxhantine and inosine of total sum). In this study, the frozen fish had a K value similar to the fresh fish at day 4, while the chilled stored fish had a K value of 93% at day 9 (*Figure 4*).

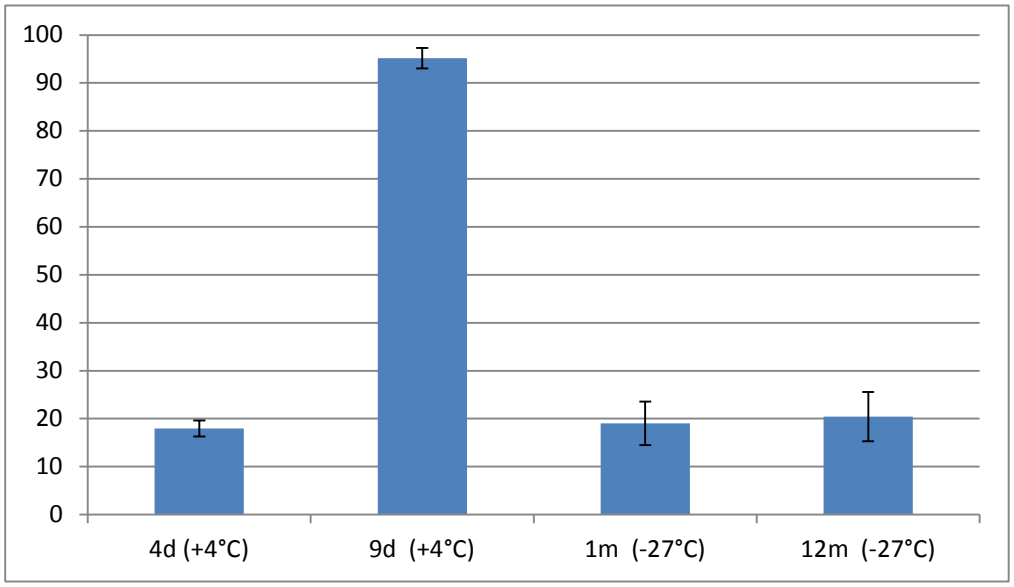

Figure 4. K values calculated for mackerel fillets stored at chilled (+4°C) and frozen storage (-27°C). The K value is described as the percentage of inosine (Ino) and hypoxhanthine (Hyp) of the total sum ATP/ADP/AMP/IMP/Inos/Hyp.

To sum up, the results show that freezing at -27 °C preserves the healthy lipids, vitamin D and low molecular weight metabolites such as free amino acids, IMP and Inosine. Changes in proteins take place during frozen storage between 1-7 month storage; however, more knowledge is needed on the relationship between protein oxidation during frozen storage and the quality parameters of muscle foods, especially in fish. During chilled storage (+4°C), from day 4 to day 9 after catch, low molecular weight compounds relevant for food quality degrades into non-favorable compounds.

Also mackerel caught in September 2014 have been sampled, and analyzed fresh (6d) and frozen (6 months and 12 months). The fat content was 27 ± 2 %, and the water content was 54.6 ±1.3 %. Due to the limited budget, the focus on the September mackerel were on the effect of processing, rather than on a thorough mapping of seasonal variation compared to February mackerel. Experiments investigating alternative production processes were included (namely antioxidant addition to sauce and different temperature regimes) (WP2 and WP4).

Thawing of mackerel:
Of the thawing methods that were studied, thawing in a steam oven at 30°C and 100% humidity resulted in the highest average water content and water holding capacity. To retain a high water holding capacity the fish has to be cooled down after thawing to reabsorb some of the drip loss. Thawing in a temperature controlled water bath gave the highest content of water soluble proteins, and the highest values were found for the fillets thawed at low temperatures (20°C). This work has been published in a student thesis, see chapter 6 for the reference's.

Publication I (in preparation):

Authors: Inger Beate Standal, Revilija Mozuraityte, Turid Rustad, Leili Alinasabhematabadi, Annette Almgren, Ingrid Undeland

Title: Quality changes in Atlantic mackerel (*Scomber scombrus*) during chilled and frozen storage: focus on health beneficial compounds

Abstract:
The stability of health beneficial compounds (omega-3 fatty acids, vitamin D, proteins) in mackerel during chilled (+4 °C) and frozen storage (-27°C) has been studied. The faith of compounds relevant for safety and taste of the fish has also been studied (i.e. low molecular weight metabolites). Results show that the mackerel is stable in composition (water, lipid content and fatty acid profile) during the storage conditions. No significant lipid oxidation was observed during fresh or frozen storage of mackerel fillets (-27°C). Vitamin D was preserved both during chilled and frozen storage. The results show however, that changes in proteins take place during frozen storage between 1-7 month storage, however, more knowledge is needed on the relationship between protein oxidation during frozen storage and the quality parameters of muscle foods, especially in fish. Frozen storage shortly after catch preserves the beneficial compounds studied. At chilled storage (+4°C) low molecular weight compounds relevant for food quality degrades into non-favorable compounds. Therefore, the current results shows that preservation of mackerel by freezing is a better option compared to prolonged chilled storage when it comes to preserving compounds relevant for food safety and quality.

5.2 WP 2 – Processing of herring and mackerel into high quality end products - traditional and modernized (novel) approaches

Background:
Salting, marinating and canning involve processing steps that may reduce the nutritional value of fish. The herring industry uses both fresh and thawed herring as raw material, and the choice will probably influence the nutritional value of the end product. Addition of salt to the muscle increases lipid oxidation. Therefore, the hypothesis that oxidation of omega-3 fatty acids and proteins in marinated herring may be reduced by reducing the salt content was raised. In order to maintain the desired sensory quality, salt replacers may be employed. Temperature is important for biochemical and physical changes during canning and optimisation of temperature regimes could be a way to preserve health promoting compounds. The right antioxidants could also help to slow the oxidation of health beneficial compounds in both marinated and canned products. In this work package, we have focused on reducing the salt content in marinated herring products. The work done with the canning process for mackerel in tomato sauce is reported in WP 4.

The main objective of this WP was to evaluate how possible modification of traditional salting, marinating and canning technologies affect health promoting compounds, sensory quality and the shelf life of salted, marinated herring and canned mackerel products.

Figure 5. Processing of marinated herring (Photo: SINTEF).

Research tasks:
To optimize the traditional marinating and canning processes the following subtasks are needed:

Task 2.1: *Pre-salting of marinated herring*
Task 2.2: *Development of marinated herring products with high consistent quality*
Task 2.3: *Canned preservation of mackerel (reported in WP 4)*

Deliverables:
D2.1: 1-2 peer reviewed scientific publications;
D2.2: Optimized recipe for salted and marinated herring in order to keep the nutritional value of the product;
D2.3: Protocol for optimized recipe for canned mackerel in tomato in order to keep the nutritional value of the product.

Results:
Marinated herring with reduced salt content in the brines:
Three studies were conducted with raw herring with different fat content obtained in different seasons (November 2013, January 2014 and October 2014, see *Figure 6*). Eight vinegar-marinades were investigated where NaCl content varied from today's marinade up to 58% total salt reduction, including partial replacement of NaCl with KCl. The results show that the quality of semi-marinated herring varies with season. Storage temperature had no effect on all the measured parameters. Colour, texture, lipid oxidation (rancidity), free fatty acids and weight of the herring pieces was affected by the marination period, but only texture was affected by the different salt concentrations. The herring pieces were significantly softer in marinades with 43% and 58% salt reduction. Lipid oxidation varied in dependence on the fat content in the herring (seasonal variation), but was not affected by salt concentration, temperature or marination period. The microbial quality was excellent for all the marinades (total viable count < 400 after 10 months). In the marinades with the lowest salt content, the temperature was an important factor where lower temperature (under 0°C) increased the mortality. A study with the final product (Løksild) manufactured from the semi-marinated herring with different salt content in the pre-marinades was also conducted. In the final product, the NaCl content was reduced up to 21% in relation to today's product. Sensorial test of the final product showed no major differences compared to today's final product (see WP 3).

This project shows that it is possible to produce marinated herring with lower NaCl content which have good quality and at the same time fulfils food safety requirements. Marinade with 25% total salt reduction where NaCl was reduced by 43% and replaced with 18% KCl showed the most promising results and can be recommended if it is desirable to reduce salt content in marinated herring products.

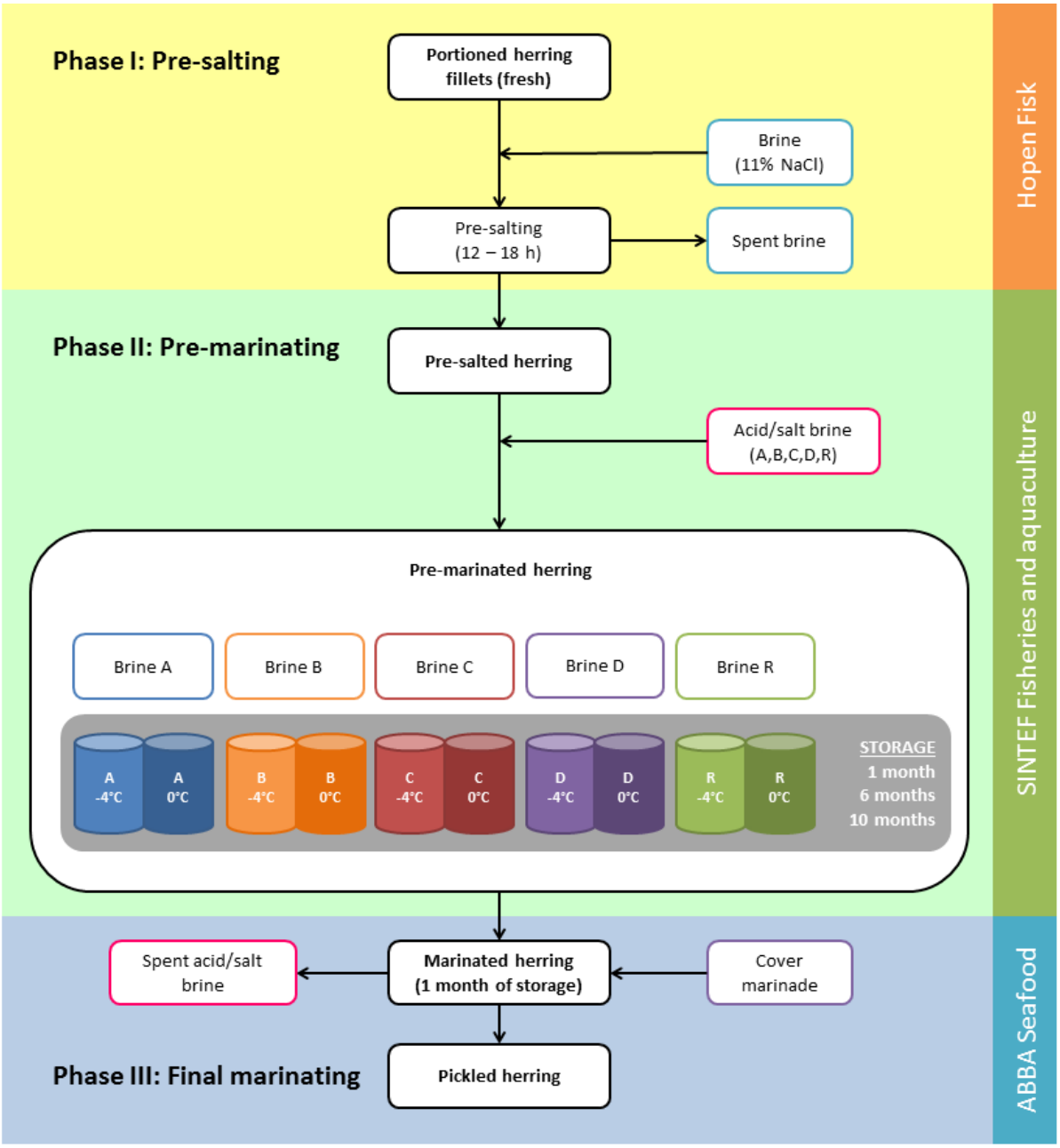

Figure 6. Industrial marinating process for pickled herring, depicting three production phases: I) Pre-salting, II) Pre-marinating, and III) Final marinating. Phase I and the production part of phase II was carried out at Hopen Fisk, the storage part in phase II was carried out at SINTEF Fisheries and aquaculture, and phase III was carried out at ABBA Seafood.

Publication II (in preparation):

Authors: Vera Kristinova, Hanne Digre, Revilija Mozuraityte, Kirsti Greiff

Title: The effect of reduced salt concentration and replacement of NaCl with KCl in brines on the quality attributes of herring during marinating – part I: Total salt reduction 12 – 25%

Publication III (in preparation):

Authors: Vera Kristinova, Revilija Mozuraityte, Kirsti Greiff, Ida Aursand, Hanne Digre

Title: The effect of reduced salt concentration and replacement of NaCl with KCl in brines on the quality attributes of herring during marinating – part II: Total salt reduction 25 – 58%

5.3 WP 3 – Market and consumer

Background:

The marinated herring is a well-known product for many consumers and when changes are made to the product the consumer will respond to this. One of the goals was to reduce the salt contend to get a healthier product, but without changing the sensory quality and there by the consumers preferences. The structural and flavour active components from the fish products are perceived by the sensory systems, and this information is combined with the consumer's recollection of previous eating experiences. However it is difficult for the consumer to give a precise and well defined description of the sensory characteristics of a product. By using descriptive sensory analysis carried out with a trained sensory panel it is possible to obtain an objective and quantified sensory description. By relating the consumer liking and the sensory characteristics of the product, by use of different multivariate techniques, the results gives needed knowledge for the fish industry to produce high quality products with a high consumer preference.

In the consumer survey there were questions for setting up effective strategies of communication to the consumer. The communication strategies of "health related" benefits of salt reduced fish products are important in order to increase consumer demand and, consequently, their consumption.

Figure 7. Sensory tests by trained panel (Photo: DTU)

Research tasks:
Task 3.1: *Evaluation of the acceptability/preference of the products*
Task 3.2: *Analysis of the sensory quality by descriptive objective sensory profile*
Task 3.3: *Multivariate regression and the Preference Mapping technique*
Task 3.4: *Identification of effective communication strategies*

Deliverables:
D3.1: Report on results from consumer test;
D3.2: Report on results from Sensory analysis;
D3.3: Report on correlation between results from task 3.1 and 3.2;
D3.4: One peer reviewed scientific publications

Results:
Qualitative consumer test:
The consumer test was conducted with 60 consumers and they were recruited among fish eaters in such a way that there were equally distribution between female and male and as close to equally distributed in age. The distributions are shown in *Table 1*.

Table 1. The distribution of the consumers.

	20-39	40-59	60-79	Total
Female	8	14	8	30
Male	14	8	8	30
Total	22	22	16	60

The marinated herring samples used in the consumer test was chosen based on the sensory profiling to get the largest variation between the products. The samples chosen were: B0, C0, D0 and R0.
The consumer test was conducted as a central location test. The consumers were asked to score if they liked the sample or not on a 9-point category scale, with a neutral point at 5. The samples were served one at a time and the consumer had water and crackers to clean their mouth between the samples. After they have tasted all four samples they got a questioner including questions on their motives and barriers for eating fish products with focus on marinated herring and canned mackerel products.

Conclusion:
All four marinated herring product were liked. B0 got the lowest liking score, it is the sample with a salt reduction. The most liked was C0, this is the sample with both a salt reduction and a substitution of Na with K.

Descriptive objective sensory profile:
In the batch of marinated herring arrived at DTU December 2013 there was a headspace in all jar. This head space resulted in oxidation and the sensory profile for these 10 samples was decided not to be used in the further work.

The next batch was pre-marinated at SINTEF and sent to Abba where the samples were packed with the final marinade in PET jars. Each PET jar contained 1.5 kg drained herring.

Sensory profiling:
The herring samples were analysed by sensory profiling performed by a trained sensory panel at the Technical University of Denmark. The panel consisted of 10 assessors selected, tested, and specifically trained in descriptive analysis of herring according to the ISO 11035 standard (1994). The evaluations were performed in separated booths under normal daylight and at ambient temperature according to the ISO 8589 standard (1988). The assessors used water and flat bread to clean the palate between samples. Data were collected using a computer system (FIZZ Network Version 2.0, Biosystems, France).

A vocabulary was developed and three sessions were used for training the assessors to evaluate the sensory attributes on an unstructured 15 cm line scale with anchor points 1.5 cm from each end. The sensory attributes were: appearance: colour, cohesiveness; odour: sourish, spicy, sweet, herring; flavour: sourish, salty, sweet, fat/oily, bitter; texture, firm, grainy, juicy.

Sample preparation:
A new PET jar with marinated herring was opened and samples were taken randomly. 10 bites were placed in a sieve for draining and afterwards minced. For each assessor the samples was prepared in a petri dish with one whole bit was placed with the skin side down and a spoon full of minced sample. The samples were kept at 2°C for 30 minute before serving. The assessors used the whole sample for measuring appearance and texture and the minced sample for measuring odour and taste. The samples were assessed in duplicates.

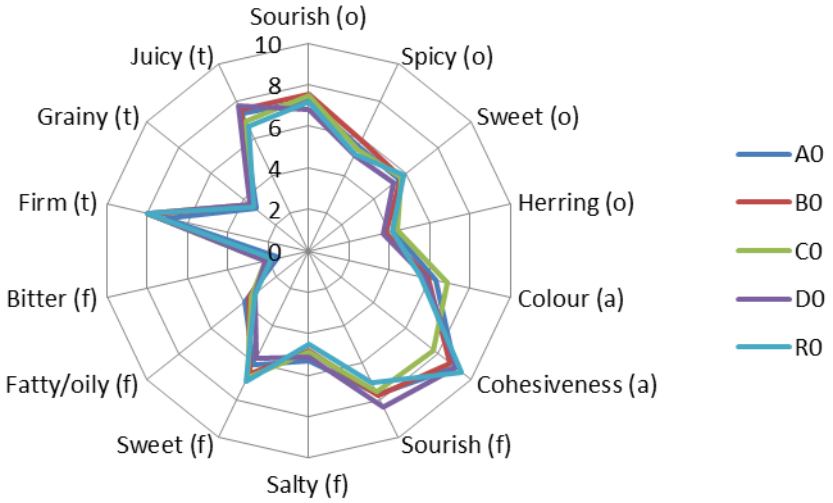

Figure 8. Sensory profile of the five marinated herring samples storage at 0°C. o: odour, a: appearance, f: flavour and t: texture.

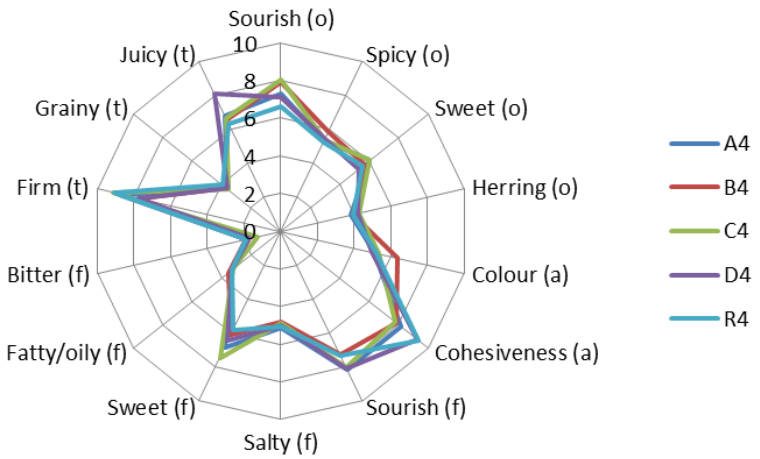

Figure 9. Sensory profile of the five marinated herring samples storage at 4°C. o: odour, a: appearance, f: flavour and t: texture.

Conclusion:
It is small differences between the different samples and it is mostly the appearance and texture attributes that deviates. The deviations are largest in the samples storage at 4°C.

Correlation between sensory profile and consumer liking:
Figure 10 shows the sensory profile of the four samples for the consumer test and the liking score for the four samples can be seen in *Table 2*.

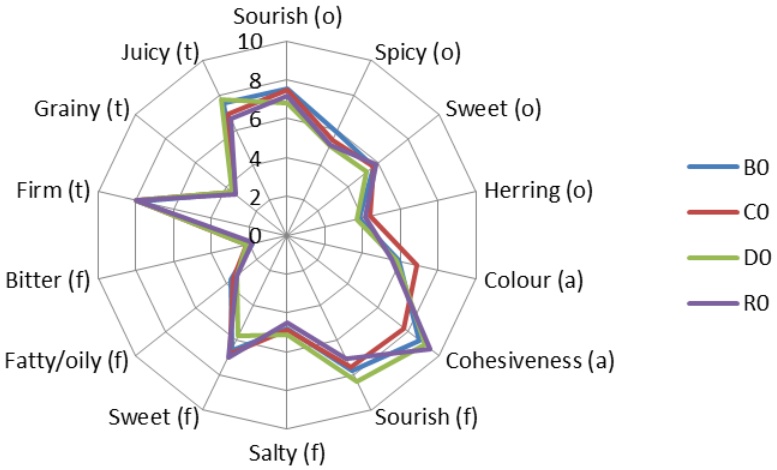

Figure 10. Sensory profile of the four marinated herring samples used in the consumer test. The samples have been storage at 0°C. o: odour, a: appearance, f: flavour and t: texture.

Table 2. Liking scores for the different samples.

	Mean liking score
B0	6.0
C0	6.7
D0	6.4
R0	6.5

These results were used in the preference mapping and the results are shown in *Figure 11*.

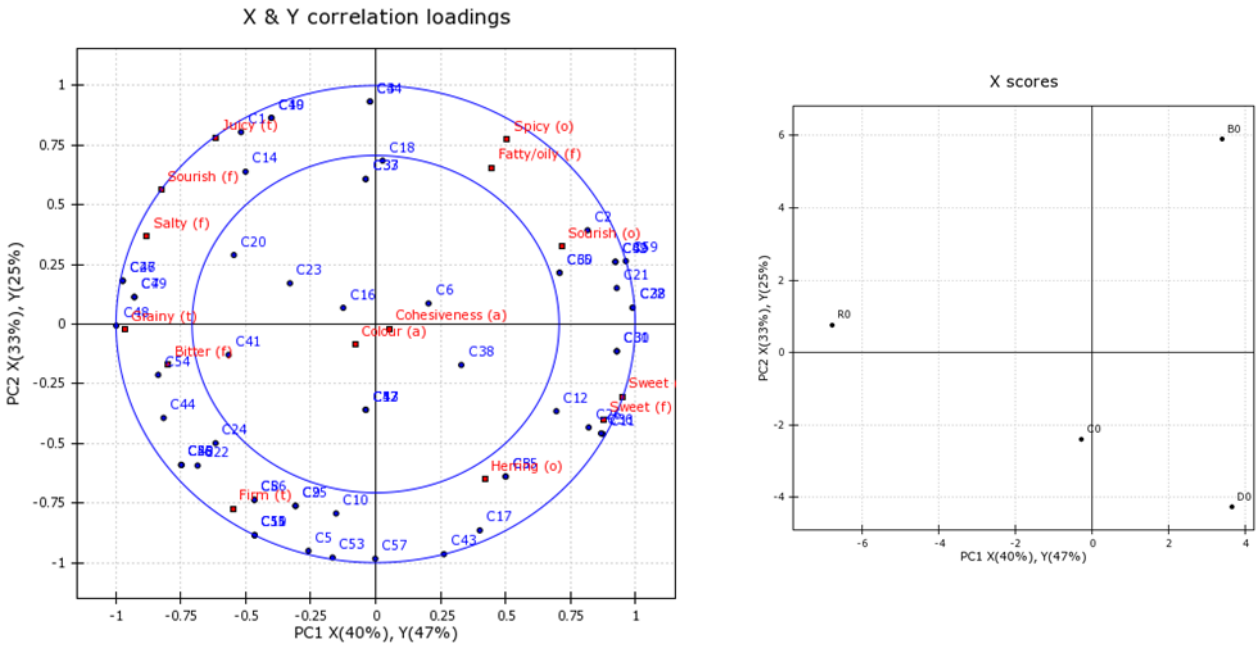

Figure 11. Preference map of B0, C0, D0 and R0.

Conclusion:
There is no clear clustering of the consumers. There are a tendency towards C0 and R0. Based on the results it can be concluded that C0 can be an alternative to R0 (the reference sample).

Communication strategies:

The consumers were familiar with marinated herring products as it can be seen in *Figure 12*.

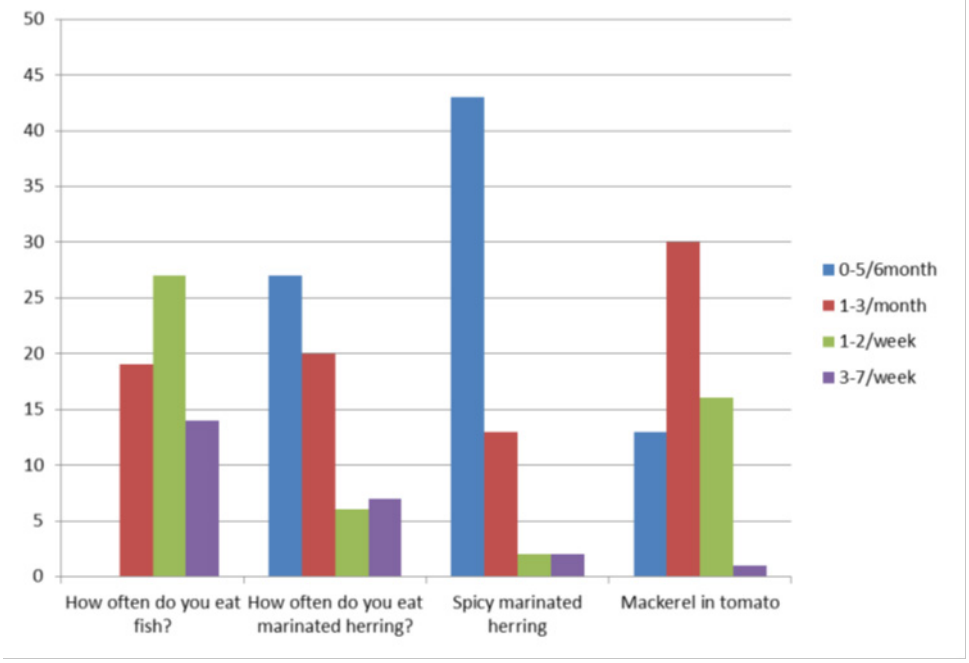

Figure 12. Consumers eating pattern for fish and fish products.

In the questionnaire, the consumers scored 6.6 in average on the question "It is healthy to eat fish" which mean that the consumer knows that fish is good for their health. On the question "To eat fish is without any health risk" the mean score was 4.6 which means that a part of the consumers think that there are a health risk connected to eat fish.

The consumers knows already that fish is healthy therefore it is recommended that the communication strategy for the marinate herring with reduced salt content include the information on the benefit of lowering the health risk by lowering the salt content in the marinated herring product.

Publication IV (in preparation):

Authors: Grethe Hyldig, Vera Kristinova and Hanne Digre

Title: Sensory Properties of Marinated Herring (*Clupea harengus*) products with reduced salt content.

Abstract:
Marinated herring products with reduced salt content and/or substitution for the salt was characterize by sensory profile. Based on the results for the sensory profiling four products were chosen for a consumer test on liking.

5.4 WP 4 – Mechanistic studies: Effect of new processes on protein and lipids in food

Background:
During processing, high temperatures, exposure to air and light, prooxidative components like heme in haemoglobin and myoglobin, may lead to loss or destruction of the valuable healthy components i.e. oxidation of LC omega-3 fatty acids and proteins, loss of vitamin D. Lipid oxidation leads to formation of rancid off-flavours and taste that usually limits the consumer choice of the product. Moreover, oxidation may lead to loss of the nutritionally important fatty acids such as EPA and DHA and antioxidants. Lipid oxidation rate increases with increasing processing temperature. Usually freezing is used to prolong the shelf-life of the fish. However, freezing may affect the functional properties of the proteins e.g. reduce the solubility of proteins (Saeed and Howell, 2002). Oxidation of proteins may also lead to changes in texture, colour and protein digestibility. Therefore, optimal storage temperature conditions are needed in order to maintain the healthy components in the fatty fish like mackerel.

Information on the stability of vitamin D in foods is quite limited. In general a high vitamin D stability is assumed (Kilcast, 1994), but this depends on the processing technique used. Despite the fact that fish is an important dietary source of vitamin D, the stability of vitamin D in fish has been little studied. Aminullah Bhuiyan et al. (1993) found that cholecalciferol is quite stable during smoking of Atlantic mackerel. For perch, Baltic herring, trout and Vendace, Mattila et al. (1999) found cooking at 172 or 200°C for 20 min to cause **<10% loss of D_3**. One study is available on pickled herring, which reports similar values before after the pickling, and also before and after frozen storage of herring (Aro et al., 2005).

Several studies, (Augbourg and Ugliano, 2002; Guizani et al., 2014) reported that increasing the salt content in fish accelerates the rate of oxidative rancidity. However, effect of salt may depend also on the processing and the purity of salt. Theoretically, high salt concentrations should reduce the dissolved oxygen concentration in the marinade and reduce available oxygen for lipid oxidation. If the marinating is performed in a closed unit with no oxygen (oxidation substrate) diffusion, only a low formation of lipid peroxide can occur. Thus, the development of secondary oxidation products will depend on the raw material quality (pre-existing peroxide amount). In Norway fresh herring fillets are used for marinating, therefore the level of oxidation is very low. Therefore, marinating of herring in closed system should not be affected by salt concentration in the marinade. However, no publications have been found reporting on the stability of lipids, proteins and vitamin D during different steps of marinating herring and also not on salt concentration effects.

Summing up the knowledge gaps, WP4 focused on:

- quality of lipids, low molecular weight metabolites and vitamin D in the model product of canned mackerel in tomato sauce, effect of:
  - canning temperature and time
  - addition of vitamin E in tomato sauce
- marinating of herring and changes in protein and lipid quality and vitamin D amount in the pre-salted herring product, pre-marinated herring product and the final marinated herring product, effect of:
  - pre-marinating temperature and time
  - composition of herring (seasonal variation)
  - reduction of salt in the marinade
  - addition of antioxidants (EDTA and caffeic acids) in the marinade

Figure 13. Producing canned mackerel in tomato sauce (Photo: SINTEF).

Research tasks

Task 4.1: Mechanism underlying changes in the amount of lipids, lipid soluble compounds and oxidative stability of lipids.

Task 4.2: Water soluble components and effect of different processing parameter.

Task 4.3: Mechanism underlying the changes in protein properties during the marinating and canning processes.

Deliverables:

D4.1 Data on the stability of herring lipids as affected by marinating conditions;
D4.2 Data on the stability of mackerel lipids as affected by canning conditions;
D4.4 Data on the retention of water soluble peptides and taurine during processing;
D4.4 Data on protein oxidation/functionality changes during processing;
D4.5 1-2 peer reviewed scientific publication

Results:

Effect of canning on lipid quality, low molecular weight metabolites and vitamin D in the model product of canned mackerel in tomato sauce:

The study of canning temperature and -time effect on the quality of canned mackerel in tomato sauce was performed in WP2. Two canning temperatures (112±5°C and 130.5±3°C) and two canning times (15 and 25 min) were applied on the model product. Moreover, 1000ppm of vitamin E was added in the tomato sauce to see of this could reduce oxidation of lipids during canning. Slightly lower amounts of water were obtained in the samples canned at higher temperature compared to lower temperature canning. This can be explained by higher water leakage from the fish to the sauce at higher temperature canning. The sum of EPA and DHA (of total fatty acids) were slightly lower (approx 14%) in the canned mackerel compare to the levels obtained in the raw material (approx 17%). The lower amount of DHA and EPA in the canned sample can be explained by lipid diffusion from the muscle to the sauce. When analyzing TBARS values in the muscle lipids, slightly lower amount of TBARS was obtained in samples that contained vit. E in the sauce. This indicates that antioxidants may improve the oxidative quality of lipids during canning, even though in general the oxidation product amount was relatively low in the freshly canned products.
Regarding the low molecular weight metabolites, the analyses of fish muscle from canned mackerel in tomato sauce shows that the profile of compounds is largely influenced by the tomato sauce, which is soaked into the fish muscle. This can e.g. be seen by the large number of signals from carbohydrates, amino acids, and other compounds such as AMP. The mackerel after canning contained lower amount of IMP and the

inosine content was higher than for the frozen stored and fresh fish. TMA/DMA content was also higher in the canned fish than in the fresh day 4/frozen stored fish. No significant content of cadaverine or histamine was detected. The fish was canned at day 5 after catch, so it is difficult to conclude whether it was the somewhat longer time prior to canning or the canning process that is the reason for the somewhat higher levels of TMA/DMA/Inosine and lower content of IMP compared to in the fresh fish at day 4/frozen stored fish.

Traditional canning process in the production of mackerel in tomatoes is typically performed at above 100°C. The effect of mild heating (below 100 °C) was performed in order to understand the temperature effect on the development of volatile compounds and compared with traditional mackerel in tomato sauce product. In the mild temperature canned fish, aldehyde amount was lower after 9 days of storage (after canning) at +4^{o}C compared to after 2 days of storage. However, a clear conclusion on the temperature effect on volatile compound formation in the mackerel in tomato sauce product was difficult to draw because of the very complicated volatile compound spectra. Amount of furfural and 2-furancarboxaldehyde, 5- methyl- was higher in samples treated at lower temperature. Samples treated with lower temperatures contained more of compounds such as acetic acid, formic acid, 3-methyl butanol, dimethyl disulfide that resulted probably from higher microbial/enzymatic activity. Aldehydes such as hexanal, propanal and 2,4 heptadienal tend to disappear during sample storage and this could be due to interaction between aldehydes and proteins.

Vit D level in the mackerel muscle was relatively stable and canning had no effect on the amount of vit D in muscle. 100g product without sauce would give 5 µg vit D_3; which is half of the daily recommended intake.

Also changes in proteins during milder heating regimes in production of mackerel in tomatoes sauce were studied. Experiments were performed on the fish muscle alone, and with both fish muscle and tomatoes sauce, and temperatures ranging from 50 – 90 °C was used. To sum up, the results show that protein-oxidation takes place during the heating process, and at greater extent at 80 and 90 °C compared to 50 and 60°C. Sensory analyses performed show however that there are certain challenges by using milder temperatures in the production of canned mackerel in tomatoes sauce when it comes to the sensory quality.

Marinating of herring - protein and lipid quality and vitamin D amount in the pre-marinated herring product and the final marinated herring product:

No significant influence of pre-marinating temperature (-4^{o}C or 0^{o}C) on the oxidative quality of the pre-marinated herring was observed. However, slightly lower amount of free fatty acids was obtained in the samples pre-marinated at -4^{o}C and this might be due to lower enzymatic activity at lower storage temperature. The amount of free fatty acids increased with the pre-marinating time (*Figure 14*) as the result of lipolytic enzymes activity as -4^{o}C or 0^{o}C temperatures are not able to inactivate enzymes. The Final marinated herring was made from the pre-marinated herring for 35 days. The amount of free fatty acids was even higher compared to the free fatty acids obtained in the pre-marinated herring for 10 month. The final marinating was performed at higher temperatures (+4^{o}C) and therefore resulted in faster formation of free fatty acids.

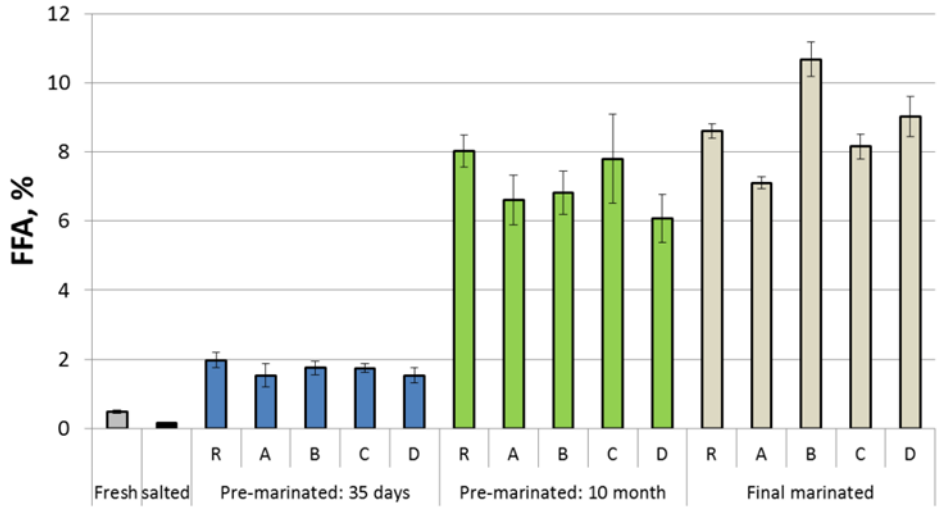

Figure 14. Amount of free fatty acids after different production steps: raw material -fresh, after salting for 18 hours, pre-marinated for 35days and 10 month and final products that were made from pre-marinated herring for 35 days.

Lipid oxidation did not develop during pre-marinating, as process was performed almost anoxic (no diffusion of oxygen from the atmosphere). However, when preparing the final product, slight increase in the primary (conjugated dienes (CD) and secondary (TBARS) oxidation products was obtained. When preparing the final product, herring pieces have to be taken out from the pre-marinating barrels, drained and added to new barrels with final marinade. During these processing steps, herring pieces come in to contact with oxygen, and therefore some oxidative changes occurs. Moreover, the final marinade contains less amount of salt, and therefore more dissolved oxygen can be presented and this also may lead to some peroxide formation during final marinating. However, the studied salt composition (NaCl and KCl) and concentrations in the pre-marinade had no effect on the oxidative stability of the pre-marinated and final marinated herring. However, after 1 month of pre-marinating, the higher CD, PV and TBARS values were obtained in the November and October herring compare to January herring. It might be that higher lipid content in January herring also contained higher omega-3 fatty acids amount and therefore the lipids were more prone to oxidation.
Pre-marinating of herring using marinades that contained EDTA (200ppm) or caffeic acid (500ppm) was performed. Addition of caffeic acid (antioxidant) in the marinade, slightly increased the PV and CD values in the pre-marinated herring and this shows that iron catalysed oxidation is involved. This shows that antioxidants might help to reduce oxidation during pre-marinating of herring. However, antioxidants did not affect the development of free fatty acids.

During marinating the vitamin D_3 level in the herring did not change significantly and it remained at approx. 0,25 µg vit D/g WW of herring. The level found in herring was about four times higher than that found in mackerel, and it would be enough to eat 40g marinated herring to cover the daily recommended dose of vitamin D_3.

Publication V (in preparation):

Authors: Revilija Mozuraityte, Vera Kristinova, Turid Rustad, Nils-Gunnar Carlsson, Ingrid Undeland, Hanne Digre

Title: Changes of proteins and lipids during marinating of herring

Abstract:
Marinated herring is a healthy and popular dish in Scandinavia. Consumption of fatty fish, such as herring, provides numerous important nutrients, such as lipids (rich in LC omega-3 polyunsaturated fatty acids), proteins and water soluble compounds. However, the healthy marinated herring also contains about 6-8% salt depending on the recipe. This work studies the changes in proteins and lipids during marinating of herring and the effect of reduced sodium (Na+) content. The results show enzymatic changes such as formation of free fatty acids was not effected of neither salt amount or headspace presence and increase during marinating time. Herring lipids were oxidative stable during pre-marinating of herring. Reduction of sodium and replacement by potassium had no effect on the oxidation stability of lipids. During marinating the vit D level in the herring did not changes significantly and it stayed appox. 0,25 µg vit D/g WW of herring.

6 Deliverables

Reports and Student thesis:

Authors	Title	Information
William Topper Breien	**Hurtig innsalting av sildefilet**	SINTEF Report A24694 –Open Student project, Aug 2013
Guro Møen Tveit	**Marinert sild med mindre salt**	SINTEF Report– Restricted Student project, Aug 2013
Katrine Horne Iversen	**Oxidative and hydrolytic changes in lipids of pelagic fish during different processing steps**	Master thesis – Open May 2014
Kristine Taraldsvik	**Reduction of salt during marinating of herring and the effect on stability of lipids**	Master thesis – Open June 2015
Christina Grønstad	**Varmebehandling av makrell Effekt på proteinstabilitet**	Master thesis-Restricted June 2015
Christina Grønstad	**Tining av makrell – effekt av ulike tinemetoder**	TBT450-Bioteknologi Student Project, 2014
Charlotte Staurem	**Sommerprosjekt 2014 - Makrell i tomat**	SINTEF Report nr A26265-restricted. Aug 2015
Leili Alinasabhematabadi	**Stabilitet til proteiner i makrell ved kjøle- og fryselagring**	Master Thesis, open

Presentations at Conferences:

Information	Authors and title
Oral presentation	Standal, IB.; Mozuraityte, R.; Undeland, I.; Rustad, T. **Content of health beneficial compounds in Atlantic Mackerel during cold- and frozen storage, 45th WEFTA meeting** 12-15 October, 2015, Nantes, France,
Oral presentation	Digre, H.; Mozuraityte, R.; Aursand, I.G.; Greiff, K.; Hyldig, G.; Nielsen, H.H. **Reduction of salt in marinated herring (*Clupea harengus* L.) products** 1st International PLEASURE Conference, 18-19 June 2014, La Rochelle, France
Oral presentation	Mozuraityte, R.; Digre, H.; Aursand, I.G.; Greiff, K.; Rustad, T.; Hyldig, G.; Nielsen, H.H.; Undeland, I. **Reduction of salt in marinated herring (*Clupea harengus* L.) products** 44th WEFTA meeting 2014, 9-11 June 2014, Bilbao, Spain
Poster	Mozuraityte, R.; Kristinova, V., Greiff, K; Rustad, T., Undeland, I., Digre, H. **Stability of marine lipids during marinating of herring: Effect of headspace, time and salt concentration in the marinade** 28th Nordic Lipidforum Symposium, 3-6 June 2015, Reykjavik, Iceland
Oral presentation + poster	Digre, Hanne; Mozuraityte, Revilija; Standal, Inger Beate; Aursand, Ida Grong; Greiff, Kirsti; Rustad, Turid; Undeland, Ingrid; Hyldig, Grethe. **Preserving the positive health effects in innovative pelagic fish products through the value chain.** 43rd WEFTA Meeting, 2013-10-09 - 2013-10-11

Publications in preparation:

Paper No	Authors	Title
I	Inger Beate Standal, Revilija Mozuraityte, Turid Rustad, Leili Alinasabhematabadi, Annette Almgren, Ingrid Undeland	Quality changes in Atlantic mackerel (*Scomber scombrus*) during fresh and frozen storage: focus on health beneficial compounds
II	Vera Kristinova, Hanne Digre, Revilija Mozuraityte, Kirsti Greiff	The effect of reduced salt concentration and replacement of NaCl with KCl in brines on the quality attributes of herring during marinating – part I: Total salt reduction 12 – 25%
III	Vera Kristinova, Revilija Mozuraityte, Kirsti Greiff, Ida Aursand, Hanne Digre	The effect of reduced salt concentration and replacement of NaCl with KCl in brines on the quality attributes of herring during marinating – part II: Total salt reduction 25 – 58%
IV	Grethe Hyldig, Vera Kristinova and Hanne Digre	Sensory Properties of Marinated Herring (*Clupea harengus*) products with reduced salt content
V	Revilija Mozuraityte, Vera Kristinova, Turid Rustad, Nils-Gunnar Carlsson, Ingrid Undeland, Hanne Digre	Changes of proteins and lipids during marinating of herring

7 Conclusions

WP 1 – Raw material, quality and health-related aspects:

- The results show that freezing at -27 °C preserves the healthy marine lipids, vitamin D and low molecular weight metabolites such as free amino acids, IMP and Inosine in mackerel.
- Changes in proteins take place during frozen storage between 1-7 month storage, however, more knowledge is needed on the relationship between protein oxidation during frozen storage and the quality parameters of muscle foods, especially in fish.
- At chilled storage (+4°C) low molecular weight compounds relevant for food quality degrades into non-favorable compounds after 9 days. Therefore, the current results shows that preservation of mackerel by freezing is a better option compared to prolonged chilled storage when it comes to preserving health beneficial compounds.

WP 2 – Processing of herring and mackerel into high quality end products -traditional and modernized (novel) approaches:

- This project shows that it is possible to produce marinated herring with lower NaCl content which have good quality and at the same time fulfils food safety requirements. Marinade with 25% total salt reduction where NaCl was reduced by 43% and replaced with 18% KCl showed the most promising results and can be recommended if it is desirable to reduce salt content in marinated herring products.
- Studies on milder heat treatment of mackerel in tomato sauce (ie, <100 ° C) shows that protein oxidation occurs during the heating process, and to a greater extent at 80 and 90 ° C compared to 50 and 60 ° C. The sensory analysis shows that there are some challenges using milder temperatures in the production of canned mackerel in tomato sauce.

WP 3 – Market and consumer:

- All four marinated herring product were liked. B0 got the lowest liking score, it is the sample with a salt reduction. The most liked was C0, this is the sample with both a salt reduction and a substitution of Na with K.
- It is small differences between the different samples and it is mostly the appearance and texture attributes that deviates. The deviations are largest in the samples storage at 4°C.
- There is no clear clustering of the consumers. There are a tendency towards C0 and R0. Based on the results it can be concluded that C0 can be an alternative to R0 (the reference sample).
- The consumers knows already that fish is healthy therefore it is recommended that the communication strategy for the marinate herring with reduced salt content include the information on the benefit of lowering the health risk by lowering the salt content in the marinated herring product.

WP 4 – Mechanistic studies: Effect of new processes on protein and lipids in food:

- The lipids in mackerel showed to be oxidatively stable during canning of mackerel. Addition of vit E could even more reduce the amount of secondary oxidation in the final canned products. The amount of vit D was also stable during the processing of mackerel into canned product. 100g of canned mackerel product without sauce would give 5 µg vit.
- Herring lipids were relatively oxidative stable during pre-marinating of herring. Reduction of sodium and replacement by potassium had no effect on the oxidation stability of lipids. Therefore, healthier (less sodium) marinated herring products could be produced without significant effect on the quality. The formation of free fatty acids developed during processing time and was not affected by studied amounts of salt in marinades. During marinating the vit D level in the herring stayed approx. 0,25 µg vit D/g WW of herring.

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