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Effect of protein and lipid oxidation in the changes of color in salted and dried herring and klippfish

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Abstract

Color in fish products is sometimes used as quality index. Behind the development of color in fish product are a series of chemical reactions as protein and lipid oxidation as well as Maillard reactions. Transition metals and the composition of the muscle influences can triggered such reactions. The complexity of such reactions is higher when the proteins suffer denaturation due to salting processes. Salt also influences in the degree of oxidation.

In this project, changes in color of two fish products were studied: salted and smoked herring fillets and klippfish. Protein oxidation was measured as carbonyl groups. Lipid oxidation was measured as peroxide value (PV) in salted and dried herring and thiobarbituric acid quantification (TBARS) in fully salted cod. In order to determine discoloration, browning intensity was measured.

In the salted and smoked herring fillets the process to obtain the desired color is very expensive. To achieve a golden brown color is necessary to smoke the fillets for a long period. It was added transition metals to the salting brine resulting in higher oxidation. The use of glucose and xylose as alternative to sucrose was investigated resulting in even higher browning intensity (xylose>glucose>sucrose). This study shows that the use of xylose or glucose as an alternative to the sucrose or the addition of prooxidants as transition metals can optimize the process reducing the time the fillets need to be at the smoking process.

In the klippfish part, fully salted cod cubes were prepared to simulate the salted process. Cod cubes were divided in dark and white muscle. Copper and heme were added to the salting brine. The cod cubes were brined and then kench cured. Protein and lipid oxidation were measured after each salting process. The results show that copper and heme work as prooxidant and may be the responsible of discoloration of klippfish. Copper can be found in salt impurities, thus its importance. The other important factor in the klippfish process is removing all residues of blood or viscera.

Sammendrag

Fargen til fiskeprodukter brukes som en av flere kvalitetsparametre. Fargen i fisk er et resultat av en rekke ulike kjemiske reaksjoner slik som protein- og lipidoksidasjon i tillegg til Maillard reaksjonen. Overgangsmetaller og muskelsammensetningen har innflytelse på disse reaksjonene. Kompleksiteten til reaksjonene øker når proteinene er denaturert på grunn av salteprosesser. Salt har også innflytelse på oksidasjonshastighet.

I dette prosjektet er det sett på fargeendringer til to fiskeprodukter: saltet og røkt sildefilet og klippfisk. I saltet og røkt sild er det et ønske om en gyllenbrun farge mens klippfisk skal være så hvit som mulig. Målet med prosjektet var å undersøke hvilke saltebetingelser som gav høyest brunfarge i sildefilet og lavest bruning i klippfisk. Proteinoksidasjon ble målt som økning mengde karbonylgrupper. Lipidoksidasjon ble målt som peroksidverdi (PV) i saltet og tørket sild og som thiobarbitursyrereaktive forbindelser (TBARS) i fullsaltet torsk. Bruningsintensitet ble brukt til å bestemme fargeendringer.

I saltede og røkte sildefileter er prosessen for å oppnå riktig farge kostbar. For å få en gyllenbrun farge er det nødvendig å røke filetene i lang tid. Overgangsmetaller tilsatt i laken resulterte i økt oksidasjon. Tilsats av glukose og xylose som alternativ til sukrose resulterte i enda høyere bruningsintensitet (xylose>glukose>sukrose). Denne studien viser at bruk av xylose eller glukose som et alternative til sukrose eller tilsats av prooksidanter slik som overgangsmetaller kan forbedre prosessen og redusere nødvendig tid for å få ønsket farge på produktet.

I studiet av klippfisk, ble terninger av torsk fullsaltet for å simulere salteprosessen. Det ble laget terninger fra mørk og lys muskel. Kobber og heme ble tilsatt til saltlaken. Torsketerningene ble lakesaltet og deretter "kench cured". Protein- og lipidoksidasjon ble malt etter begge trinn i salteprosessen. Resultatene viser at kobber og heme er prooksidanter og kan være årsaken til misfarging i klippfisk. Kobber kan finnes som forurensing i salt og er derfor viktig for misfarging. En annen viktig faktor i prosessen er å fjerne alle rester av blod og innvoller.

Contents

1. Introduction

Quality determines the acceptance of a product. The term "quality" can be related to the appearance, freshness and also, to the degree of spoilage of a product. It begins with the raw material and will also be affected during the process. Quality comprises both the satisfaction of the consumer as well as the safety of the product. To determine the quality not only sensory methods are used but also chemical, physical and microbiological methods.

Our senses can perceive the smell, flavor, texture and appearance of food. Such sensory parameters are crucial in the acceptance of a product. Sensory analysis is also used in quality control in the food industry. Trained and qualified sensory panel test the product to determine if the product fulfills the quality standards. The quality parameters of raw fish, includes the appearance of the skin, eyes, belly and gills, the smell of the fish and the stiffness (Huss, 1995).

Even if the inspection has shown the product to have a high quality, customers have the last decision on buying or not buying a product. In most of the cases, the consumer is only allowed to use the appearance of the product to judge if it has the expected quality. Appearance is what people perceive with the eyes, for instance, the color.

In salted and smoked herring fillets a golden brown color is expected, while in fully or heavily salted and dried cod (klippfish), the whiter the fish, the better. During processing of fish, the molecular structure of heme proteins (myoglobin and hemoglobin) may change. In addition chemical reactions such as lipid and protein oxidation, non-enzymatic browning and enzymatic activity are also responsible for color changes (Sanchez-Zapata et al., 2008)

The aim of this project is to study how lipid and protein oxidation influence the development of color in salted and dried Atlantic herring (*Clupea harengus*) and in heavily salted Atlantic cod (*Gadus morhua*).

In the first part of the project factors that influenced the development of golden brown color in salted and dried herring were studied. In this case the discoloration is desired and is believed to be affected not only by lipid and protein oxidation but also by browning reactions. The influence of the presence of transition metal ions was studied as well as the influence of different types of sugars.

In the second part, factors to understand yellow discoloration in klippfish were studied. Discoloration of the flesh surface has been a major problem in heavily salted fish. Changes in color have been correlated to the presence of transition metals and hemoglobin. The presence of metal ions and hemoglobin as prooxidant agents in lipid and protein oxidation reactions was studied.

1.1. Structure and composition of fish

Water is the main component in fish; and the water content varies between 69 and 82g/ 100g. There is a correlation between the content of water and content of fat in the fish. Figure 1 shows that it is possible to estimate the fat content based on its water content (Stroud, 2001).

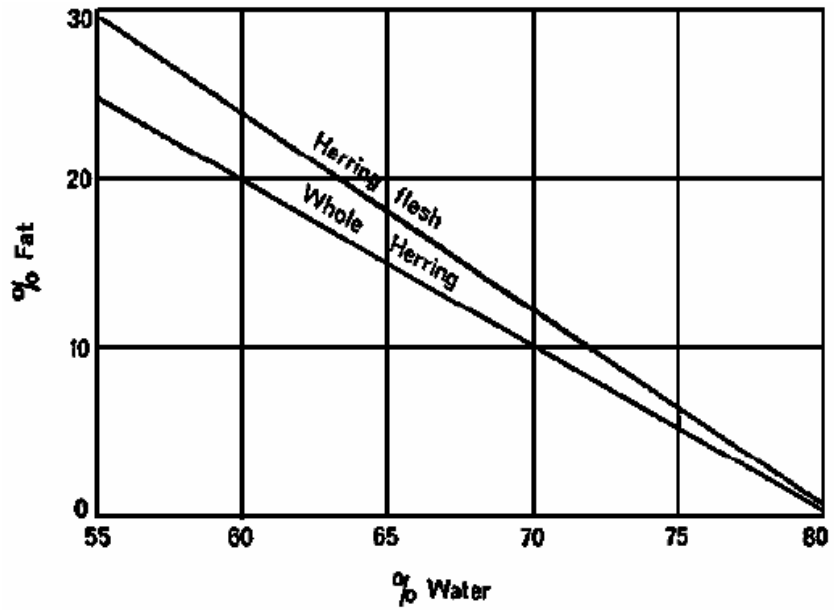

Figure 1. Fat content in herring (Stroud, 2001)

Proteins usually make up around 20% in fish composition. Structure of the proteins in the muscle is linked to nutritional and sensory characteristics of fish. The proteins in fish muscles can be divided in three groups.

- Myofibrillar proteins include actin, myosin and regulatory proteins as tropomyosin, troponin and actinin. Myofibrillar proteins constitute the largest group of total proteins in fish, 70-80%. They are responsible of the muscle movement and form myofibrils that constitute the contractile structure. Myofibrillar proteins are soluble in high ionic strength salt solution, thus they are also referred to as salt soluble proteins (SSP) (Gallart-Jornet, 2006, Ruiter, 1995).
- Sarcoplasmic proteins include albumins, globulins and enzymes. They are referred to as water-soluble proteins (WSP), due to their solubility in water or in solutions with low ionic strength. Sarcoplasmic proteins are located in the

sarcoplasma. Their content in fish is variable and characteristic of each species (Gallart-Jornet, 2006).

- Connective tissue proteins constitute about 1 to 12% of the total of protein in fish.

Fish muscle can be divided in two types: white and dark muscle. White muscle has anaerobic metabolism while dark muscle has aerobic metabolism. Dark muscle is placed as a central line running along the lateral side of fish. Figure 2 shows that the proportion of dark muscle in fatty fish is higher than in lean fish, and also contains higher concentrations of fat and vitamins (Gallart-Jornet, 2006, Murray and Burt, 2001).

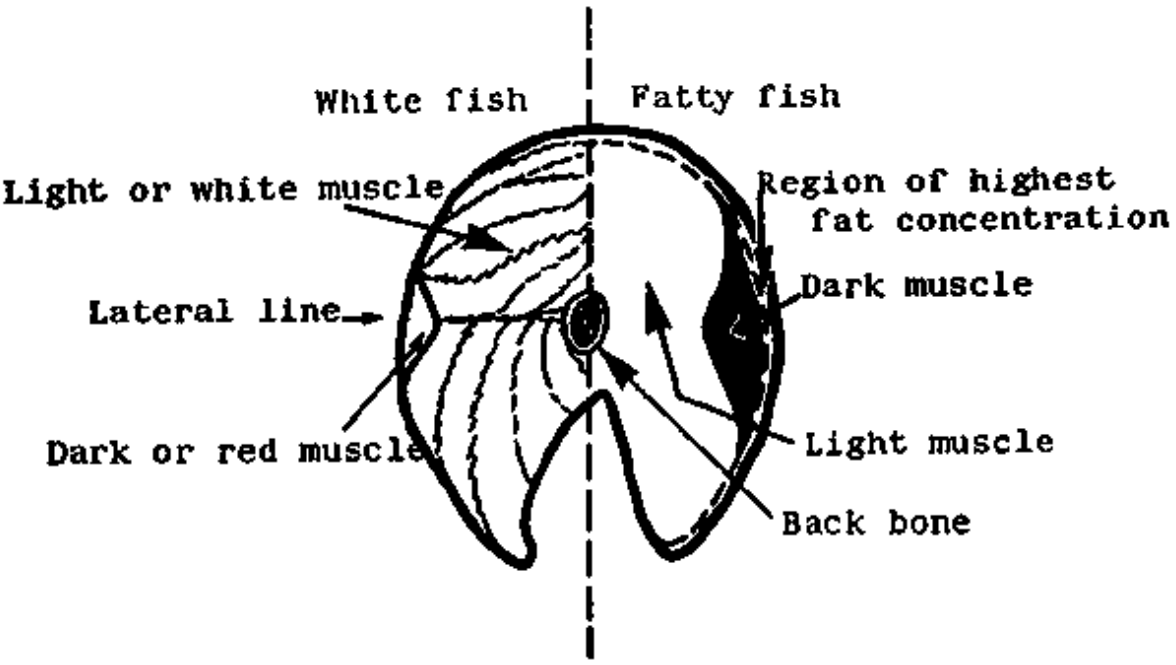

Figure 2 Dark and white muscle in fatty and lean fish (Murray and Burt, 2001) .

Blocks of muscle that are held together by connective tissue make up the flesh. Therefore the surface of cut fillet seems to be smooth and continuous (figure 3) Fat in lean fish is concentrated mainly in the liver (Murray and Burt, 2001). While in fatty fish the fat is found in the myocommata, under the skin, in the belly cavity and in the muscle.

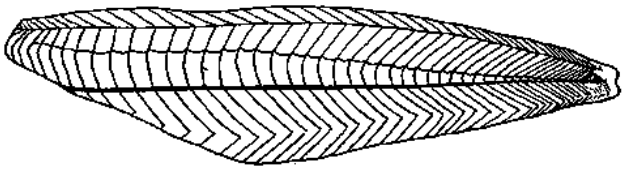

Figure 3. Diagram of a cod fillet (Murray and Burt, 2001).

The content of lipids is variable; lean fish such as cod present lipid content less than 1%, while fat fish lipid content are superior to 10%. Lipid content also varies depending on the stage of the life of the fish. Fish lipids includes up to 40% of polyunsaturated fatty acids (PUFAs), as docosahexaenoic acid (DHA; 22:6*n*-3) and eicosapentaenoic (EPA; 20:5*n*-3)(Murray and Burt, 2001).

Fish is classified in three groups depending its lipid content:

- Lean fish, lipid content less than 5%. Examples of this group are: anchovy, bass, cod, grouper, hake, and atlantic or pacific halibut.
- Semi-fatty fish, lipid content between 5 and 10%. Examples of this group are: salmon, sea trout, sea bass and swordfish.
- Fatty fish, lipid content more than 10%,. Examples of this group are: dogfish, greenland turbot, herring, mackrel and black and European halibut.

1.1.1 Herring

Atlantic herring (*Clupea harengus*) (figure 4) is classified as fatty fish species.
It is available throughout the year, but the size and fat content varies a lot during a year. The fat content is at its peak in June and July, where it can reach about 20%. On the other hand the fat content dramatically drops down to 3-5 %, or even as low as 1% right after spawning by December/January before it starts to increase again. The size of the herring normally varies between 23 and 36 cm. The weight can vary from 100 to 400 grams (Stroud, 2001).

Figure 4 Atlantic herring (*Clupea harengus*)
Source: http://www.seafoodfromnorway.com/page?id=103&key=4307

The most important fishing areas are the North Sea, the Baltic Sea, and coastal waters of Britain, Norway, Iceland and Canada.

Herring is abundant and have a high content of omega-3-fatty acids; making it an ideal fish to be used as raw material. However due to the high lipid content it is important to take consideration of the lipid oxidation during the fish storage and processing (Undeland et al., 1999).

The quality of the herring depends on the season it was caught. There are three different categories:(Pedersen, 1989)

- Winter quality: the herring has a low fat content as well as roe and milt. It can be caught during November or December.
- Summer quality: fat content is from medium to high and the texture of the fat and lard is loose.

- Quality H: fat content is high and the texture of the fat and lard is hard. It can be caught from July to October.

Some of the products developed with herring are: (Ruiter, 1995, Doe, 2002, Pedersen, 1989)

- Maatjes: a dutch product made with herring caught in or at the end of feeding period, without roe or milt. The gibbed herring is placed in barrels in between layers of salt. Then the barrels are filled with 20% of brine. Barrels are stored at refrigeration temperature 2-4°.
- Kryddersild, sukkersalted sild, tidbits and gaffelbiter: typical Scandinavian sugar-salted herring product. Prepared during 18 months at low temperatures, the pickle contains spices as clove, cinnamon, nutmeg, ginger and Spanish hop.
- Herring Kippers; the herring are split, washed and soaked in brine in 80% brine, followed by smoking at 29˚C for 4 hours.
- Herring eggs are less expensive alternative caviar. Its preparation started separating the eggs from the ovaries at slaughtering. First eggs are washed in cold water. Then eggs are salted with fine salt (3 to 5% egg weight) or brined for 8 to 18 minutes at egg:brine ratio 1:3. At last, eggs are drained and packed.

1.1.2 Atlantic cod

Belonging to the gadoid family, Atlantic cod (*Gadus morhua*) is lean fish species (Figure 5). Atlantic cod can reach 150 cm length and 50 kg weight (Fahay et al., 1999). It is found all over the north Atlantic and Arctic oceans down to 450 meters depth. The most important fishing areas are the North Sea, the northern coast of Norway, Bear Island, the Barents Sea, Novaya Zemlya, Iceland and the Faroe Islands (Council, 2005, Waterman, 2001).

Figure 5. Atlantic cod (Gadus morhua).
Source: http://www.seafoodfromnorway.com/page?id=103&key=2505

The average chemical composition is about 78-80% water, 0.1-0.9% fat and 15-18% protein (Lauritzsen, 2004).

Products derivate from the cod are:

- Stockfish: fish is split lengthwise remaining joined in the tail, rinsed in fresh water and hang for drying for 1.5 -2 months at a relative humidity of 70-80% (Ruiter, 1995, Doe, 2002).
- Klippfish: fish is split, heavily salted and dried traditionally by sun and wind, nowadays by warm air driers (Ruiter, 1995, Doe, 2002).
- English cod: the cod is salted by Gaspé method. The final product has a salt concentration between 8-10%. It is sold in different presentations as fillets, blocks, crumbs, flanks and tails (Gallart-Jornet et al., 2003).
- Gutted cod with or without head. The cod is gutted manually and the head is either removed manually or by a machine (Federal Agency of Fisheries et al., 2009).

- Cod fillets with or without skin and bones. The process of filleting and removing the skin and bones can either be done manually or by a machine (Federal Agency of Fisheries et al., 2009).

1.2 The salting process

Evidence of the use of salt as a preservative has been found in Ancient Rome and Ancient Egypt. Ancient Egyptians called it "natron" *netjeri*, which means divine salts. It was used to prevent decomposition of dead bodies. In Ancient Rome, the importance of salt was so high that the settlement of the city was chosen in relation to the transportation route of the salt. In Ancient Rome people got paid with salt, hence the origin of the word "salary" (Albarracín et al., 2011).

The first signs of fish curing were found on the coast of Spain dating more than two thousand years ago. Fish was sundried by the ancient populations in Mesopotamia and Egypt. In order to enhance the efficiency of the preservation method, salting and smoking is usually combined with drying. Traditionally the drying is done by hanging the fish and exposing it to the sun.
The need for storing and preserving food made the development of technologies such as drying, salting and smoking necessary. Furthermore, geographical conditions, such as the production of salt in the Mediterranean area, made these preservation methods possible.

Fish is highly perishable, its water activity (a_w) is high (Gallart-Jornet, 2006). The efficiency of salting as a preservation method is related to the water availability. While salt penetrates the muscle, the concentration of solutes increases and water is displaced outside the tissue. This results in an alteration of the a_w and the water content (Horner, 1997).

Owing to the fact that water activity (a_w) and pH decrease in salting processes, spoilage is inhibited. Halophilic bacterial growth is inhibited when a_w is 0.75 corresponding to 25% salt concentration, whereas moulds are inhibited at a_w of 0.80 (Horner, 1997).

The stability of a salted fish is only reached when the flesh is saturated with salt. While klippfish is a fully salted fish, the salted herring is not. The composition of the fish, the size and the desired final product determines the suitable salting method. The concentration of solutes increases due to the fact that sodium chloride diffuses into the flesh by dialysis. The water diffuses outside the flesh by osmotic pressure between the brine and the flesh. The process continues until equilibrium is reached (Horner 1997).

1.2.1 Salting methods

The salting methods can be used singly or sometimes combined. For instance, klippfish is a product where brining and kench curing are parts of the process. Salted and dried herring is salted by brining.

- Kench curing:

Fish is opened out flat and layers of solid salt are placed between layers of fish. The brine obtained as a result of the extraction of moisture is drained away. It takes between 7 and 14 days for the salt to penetrate the muscle. Once or twice during the ripening period used salt is replaced by new salt (Lauritzsen, 2004). The complete kench curing takes more than 3 weeks. Kench curing is typically used in white fishes such as cod (Gallart-Jornet, 2006).

- Gaspé curing:

The fish is salted one week with solid salt, in between layers. Then the dry salt is removed and the fish gets immersed brine resulted from the exudates (Horner, 1997). Sun drying follows this process. Gaspé cured fish is highly demanded in the southeast of Spain. The cod salted this process is known as English cod, "bacalao inglés" (Gallart-Jornet, 2006).

- Blood pickle:

The fish is placed in dry salt and stay immersed in the salt-liquid fluid that comes from the salt and the exudation of the fish. As the skin and the fat under the skin represent a barrier to salt penetration only small fishes as herring or anchovies can be salted by this method without gutting (Horner 1997).

- Picklesalting:

Solid salt is placed between layers of fish, as in kench curing. But in this case, the brine, which is the product of the extracted fluids and the salt, is not drained away (Lauritzsen, 2004). Picklesalting takes place in tanks or wooden barrels (Gallart-Jornet, 2006). Absence of O_2 delays rancidity reactions, although rancidity flavor is desired (Horner, 1997). Fish that are usually salted by this method are fatty fishes such as herring, sardines, tuna and mackerel. The salt concentration is saturated and this is not affected by the exudates from the fish (Gallart-Jornet, 2006). The difference between blood pickle and picklesalting is the presence and absence of O_2.

- Injection salting:

Inserting automatic needles to the muscle ensures uniform salt concentration in the flesh. The needles inject brine, which penetrates from the open fillet surface down to the slim side of the fillet. Fresh cod that has been salt injected show an increase in salt concentration from 0.15% to 2-5%. This process can be used before kench curing, picklesalting or brining, until the flesh is salt saturated (Lauritzsen, 2004).

- Brine salting:

Fish is immersed into ready-made brine, a salt concentration usually between 18 and 25%. The final salt concentration depends on the time the fish has been immersed in the brine as well as the temperature of the brine (Lauritzsen, 2004). This technique is applied when salting is mainly used for flavoring purposes and preservation is acquired by another treatment such as smoking. A special characteristic of brine-salted fish is the glossy pellicle that is formed by the elution of soluble proteins (Horner, 1997).

- Vacuum osmotic dehydration (VOD)

A hydrodynamic mechanism (HDM) is created when porous food is immersed into an osmotic liquid at vacuum conditions, as a result of difference of pressure between the gas in the pores of the food and the environment. The pores are filled up with osmotic solution further than by ordinary osmotic dehydration causing a larger contact surface between the food and the osmotic solution (Fito, 1994).

1.2.2 Smoking process

Smoking is an old preservation method that probably began with the utilization of smoke coming from fire in open areas. The technique was evolving to the hanging of meat dried by smoke derived of smoldering smoky fire (Horner, 1997). In Europe consumption of smoked products increased in the 1990s. The most consumed smoked product is smoked salmon followed by trout and herring (Arvanitoyannis and Kotsanopoulos, 2011).

Smoked foods have an outer layer of condensed tar, phenols and aldehydes that help preventing spoilage and developing the characteristic flavor. Synergy between salt, smoke compounds and dehydration makes smoking process an efficient preservation technique (Horner, 1997).

- Hot smoking:

Fish is smoked between 70 and 80°C. Skipjack (*Katsuwonus pelamis*), Mussel (*M. galloprovincialis*), Mullet (*Mugil cephalus*) and Tilapia (*O. niloticus*), are some of the species processed with hot smoking (Arvanitoyannis and Kotsanopoulos, 2011). According to (Beltran and Moral, 1989) hot smoking leads to lipid oxidation. It is reflected in the loss of eicosapentaenoic and docosahexaenoic acids, as well as an increase of the peroxide number and thiobarituric acid value (TBA).

- Cold smoking:

The product is smoked at 30°C or less (Montero et al., 2003). The benefit of avoiding high temperatures is the preservation of nutritional contents, preventing a high degree of denaturation of proteins. Normally salting is combined with cold smoking, thus contributing to decrease the a_w.
Raw material species as: trout (*Salmo trutta*), Atlantic salmon (*S. salar L.)* and herring (*C. harengus*) are frequently used for cold smoked products (Arvanitoyannis and Kotsanopoulos 2011).

A variety of wood, sawdust, beams and shavings from different trees such as oak, beech, hickory or cherry are used to impart specific organoleptic characteristics

(Arvanitoyannis and Kotsanopoulos 2011). The use of wood makes the product to be smoked rather than be charred. Sawdust should be accompanied by a forced rapid air flow preventing that the sawdust burns instead of smolders (Horner, 1997).

Compounds derivated from smoking such as formic and acetic acid, carbonyl compounds such as fomaldehyde and methylglyoxal, and phenols are responsible to inhibit microbial growth (Ruiter, 1995). Some of these compounds such as carbonyls are also involved in browning reactions. The carbonyls may react with amino groups in proteins resulting in color compounds.

1.3 Effect of salting on water holdig capacity

Water-holding capacity (WHC) is defined as the ability of the protein to imbibe water and retain it against gravitational force (Damodaran, 2008).

According to (Nguyen et al., 2010), WHC increased significantly, from 88.6 to 99.3-99.6%, when salting cod loins in brine at concentrations of 6%, 15% and 18% (w/w) for 2 hours. However, WHC decrease significantly when cod loins were brined at 24% (w/w) for 14 hours.

The initial increase in WHC is explained due to the preference that molecules of water have to bind to anions (Cl^-). (Figure 6) At pHs slightly above the isoelectric point the net negative charge increase causing repulsive forces. Binding between salt ions and the charged part of the filaments cause larger inter-fibrillar spaces, allowing an increase of water absorption in the protein network. In the other hand, at pHs below the isoelectric point (Cl^-), the protein positive charge is neutralized, reducing the net charge and leading to swelling of the myofibrils, thus water absorption decreases (Albarracín et al., 2011, Thorarinsdottir et al., 2011b).

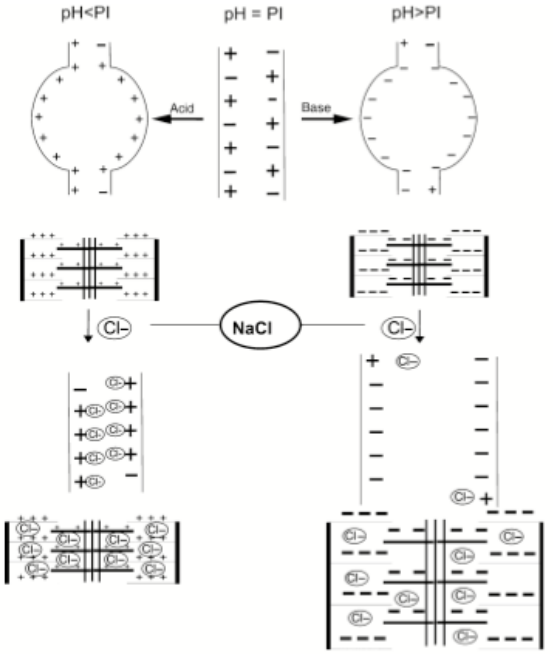

Figure 6 Interaction of Chloride anion in protein structure (Albarracín et al., 2011).

According to (Thorarinsdottir et al., 2011b) at ionic strength >0.15M salt ions bind to the filaments and the interfibrillar spaces become larger. While at concentrations >0.5M myofibirils swell due to the depolymerization of thick filaments. Then at higher salt concentration the WHC is reduced.

1.4 Effect of salting in lipid and protein oxidation

Reactive oxidative species (ROS) lead to lipid and protein oxidation. ROS include oxygen radicals such as superoxide anion (O_2-), hydroxy (HO-), peroxy (ROO-), alkoxy (RO-) and hydroperoxy (HOO-) radicals. Nonradical derivates of oxygen, as hydrogen peroxide (H_2O_2), ozone (O_3) and singlet oxygen ($^1O_2$-), are also ROS (Choe and Min, 2005). Oxidative reactions, as protein and lipid oxidation happen simultaneously. As a matter of fact, peroxyl radicals results of lipid oxidation abstract hydrogen atoms from the protein molecule, leading to a protein with a free radical. Similar radical mediated chain reactions occurs in both lipid and protein oxidation (Lund et al., 2011).

1.4.1 Lipid oxidation

The oxidation of lipids results in alterations in flavor (rancidity), color, and loss of nutritional value such as reductions in omega-3 fatty acids and some vitamins, and formation of potentially harmful substances.

The main form of lipid oxidation is by a reaction between free radicals and oxygen in the presence of initiators (metal, light, heat) that results in the formation of hydroperoxides (Andersen et al., 2007). Hemoglobin leads to initiation of lipid and protein oxidation (Christensen et al., 2011). Thus fish that are not bled or rinsed before salting is more susceptible to oxidation due to the presence of heme proteins found in the blood.

Oxidation occurs in three main steps:

I. Initiation: formation of fatty acid radicals

An alkyl radical ($L^{\bullet}$) is formed due to the abstraction of hydrogen (H) from a fatty acid (L), reaction 1. Initiators (In) react by binding a hydrogen atom from an unsaturated

lipid leading to formation of a free radical(McClements and Decker, 2007). ROS and other prooxidants such as transition metals, ionizing radiation or light and elevated temperature are responsible for initiation of oxidation reactions (Damodaran, 2008).

$$In\bullet + LH \rightarrow InH + L\bullet \qquad (1)$$

II. Propagation: fatty acid radical reaction.

An oxygen molecule reacts binding to the fatty acid radical leading to formation of peroxyl-fatty acid radical ($LOO^{\bullet}$, see reaction 2). Covalent bonds of unsaturated fatty acids are weak and susceptible to react with the peroxyl radicals. The reaction between unsaturated fatty acids and peroxyl radicals leads to the formation of fatty acid hydroperoxyl (LOOH, see reaction 3) and fatty acid radical (McClements and Decker, 2007).

$$L^{\bullet} + O2 \rightarrow LOO^{\bullet} \qquad (2)$$

$$LOO^{\bullet} + LH \rightarrow LOOH + L^{\bullet} \qquad (3)$$

III. Termination: the combination of two fatty acid radicals leads to the formation of nonradical products.

Reactions between peroxyl and alkoxyl radicals take place under atmospheric conditions while reactions between alkyl radicals lead to the formation of fatty acid dimers under low oxygen levels (McClements and Decker, 2007).

$$LOO\bullet + LOO\bullet \rightarrow LOOL + O2 \qquad (4)$$

$$\bullet L\bullet + LOO\bullet \rightarrow LOOL \qquad (5)$$

$$\bullet L\bullet + L\bullet \rightarrow LL \qquad (6)$$

Schiff base (RCH=NR) type products get formed as a result of a reaction between carbonyl compounds (specially aldehydes) and amine groups (Tironi et al., 2007). Polymerization by the rearrangement of Schiff base products results in dimers and

complex high molecular brown marcomolecules, known as melanoids (Pokorny, 1981). Non-enzimatic browning develops yellow-brown pigments (Thanonkaew et al., 2007).

1.4.2 Effects of salting on lipid oxidation.

A relatively low salt concentration affects lipid oxidation has a prooxidant effect. (Andersen and Skibsted, 1991) reported that the prooxidant effect is shown at 1% salt concentration in pork parties. Rhee, Smith et al. (1983) reported increase in lipid oxidation (TBARS) in ground beef with 0.5, 1,2 and 3% NaCl in samples refrigerated for 3 or 6 days. According to (Osinchak et al., 1992), NaCl accelerates lipid oxidation catalyzed by the soluble fraction of fish muscle. Lipid oxidation increases with increasing salt content (Rhee, 1999).

Salt alters the structural integrity of membranes leaving lipid molecules more accessible to react with ROS or with other prooxidants. According to Rhee and Ziprin (2001) NaCl promotes lipid oxidation, consequently metmyoglobin formation and discoloration in raw meat. When oxymyoglobin, MbFe(II), is oxidized this results in the formation of metmyoglobin, MbFe(III) and H_2O_2. The reaction between MbFe(III) and H_2O_2 results in formation of ferrylmyoglobin, MbFe(IV), a catalyst of lipid oxidation. (Chen et al., 1992), reported that in the absence of lipid, NaCl is also able to oxidize myoglobin, suggesting that NaCl primarily induces MbFe(IV), secondarily MbFe(IV) catalyzes lipid oxidation and then free radicals from lipid oxidation promote pigment oxidation (Rhee, 1999).

Transition metal ions such as copper and iron, found as salt impurities and in the fish muscle (Lauritzsen, 2004) are major prooxidants in muscle foods. (Osinchak et al., 1992) reported that lipid oxidation in mackerel press juice was higher in samples with iron, copper and salt than in samples with just NaCl.

1.4.3 Protein oxidation (Pox)

ROS are involved not only in lipid oxidation; they also take part in protein oxidation. The mechanisms of protein oxidation are very similar to the lipid oxidation. ROS abstract a hydrogen atom leading to a protein with a free radical (P•). In the presence of oxygen, protein carbon centered radical reacts to a peroxyl radical (POO•). Due to its

reactivity such a peroxyl radical abstracts a hydrogen atom from another molecule leading to the formation of alkyl peroxide (POOH•) (Lund et al., 2011, Martins et al., 2000).

Protein oxidation reactions are caused by irradiation, light exposure, metal catalysis and peroxydation. Protein oxidation (Pox) results in the formation of protein carbonyls, loss of sulfhydryl groups and protein cross-linking (Figure 7). In processed food, protein oxidation leads to changes in protein hydrophobicity reducing the WHC and altering the texture of the product.

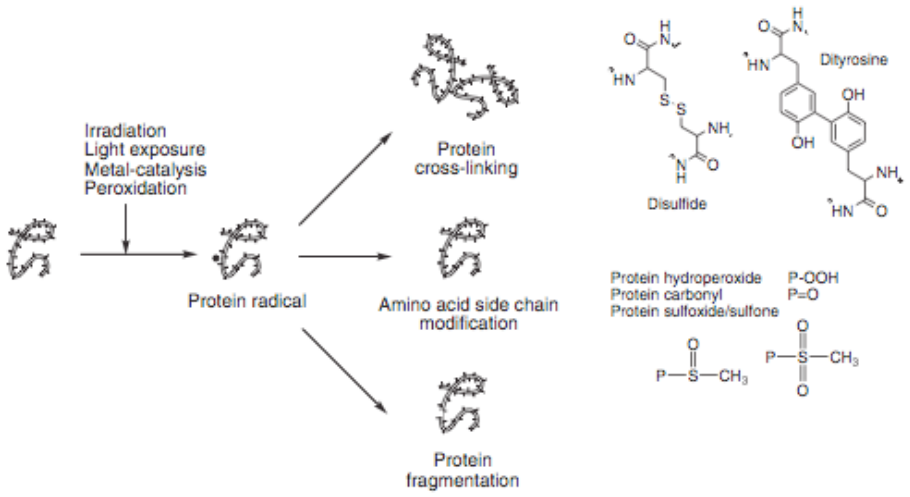

Figure 7. Scheme of protein oxidation and its consequences (Lund et al., 2011).

Metal catalyzed reactions oxidize amino acids such as arginine, lysine and proline into carbonyl residues. Amino acids such as cysteine or methioine react in cross-linking or sulfur derivatives (Lund et al., 2011).

Heme proteins (myoglobin and hemoglobin) initiate Pox. Hydrogen peroxide (H_2O_2) reacts with metmyoglobin (Mb(FeIII)) whichhas accumulated in the muscle after slaughter, leading to formation of perferrylmyoglobin and ferrylmyoglobin. Pox can also be catalyzed by non-heme iron and other transition metals in the presence of H_2O_2.

In the presence of ferric iron (Fe^{3+}) and H_2O_2, myofibrillar proteins get oxidized, resulting in formation of a semialdehyde and ferrous iron (Fe^{2+}). Ferrous iron in the

presence of H_2O_2 continues the oxidative reaction degrading amino acids into hydroxyl radicals.

On the other hand, the formation of sulfydryl groups is due to the reaction between H_2O_2 and the thiol groups contained in amino acids, such as cysteine. The results of the oxidation of thiol groups are sulfenic acid (RSOH), sulfinic acid (RSOOH) and disulfide cross-links (RSSR) (Lund, Heinonen et al. 2011).

1.5 Browning reactions

A non- enzymatic browning reaction was first studied in 1912 by the French chemist Louis Maillard. Since then there has been numerous studies on these reactions. In Maillard reaction a reducing sugar bonds with an amino group (from a protein or an amino acid) to produce a glycosylamine, which rearranges to form the Amadori rearrangement product (ARP). Whether the ARP is further degradated, depends on the pH of the system. Furfural or hydroxymethylfurfural is formed when pH is below 7. Whereas acetol, pyruvaldehyde or diacetyl are formed at pH higher than 7. Further reactions occurs because of the highly reactivity of this compounds. A reaction between dicarbonyl compounds and amino acids, is forming aldehydes and a-aminoketones, that is called Stecker degradation. The formation of melanoids, brown nitrogenous polymers, results from subsequent reactions like cyclisations, dehydrations, retro-aldolisations, rearrangements and isomerisations (figure 8)(Martins et al., 2000).

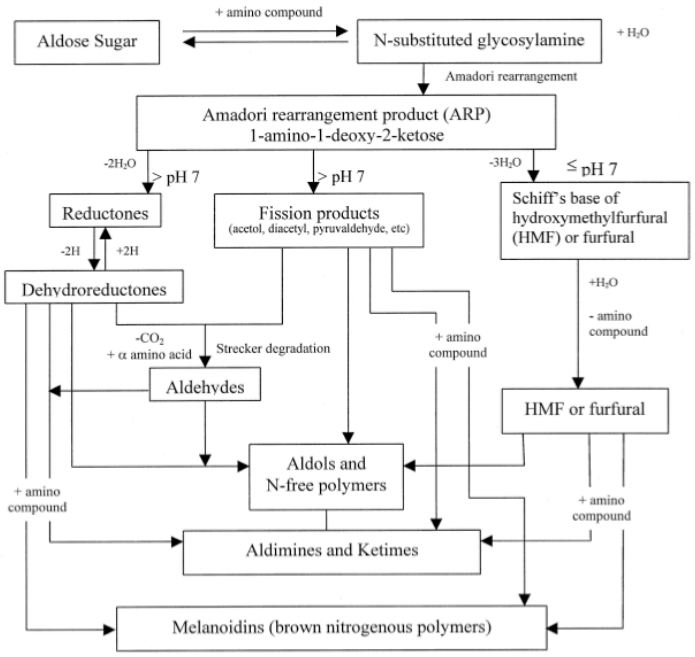

Figure 8 Scheme of Maillard reactions (Martins et al., 2000).

Sulfur containing groups, products of lipid oxidation, are also involved in Maillard reactions. They participate in the formation of long chain sulfur- containing heterocyclic compounds in the Maillard reaction (Ikan, 1996).

Lipid and protein oxidation products yield to products similar to melanoids, products of the Maillard reaction. However, the difference between then other is the solubility. Melanoids are water-soluble while the brown pigments products of oxidation have a chloroform-metanol soluble fraction and insoluble fraction.

2 Materials and Methods

2.1 Samples

2.1.1 Salted Herring

Herring samples were obtained from SINTEF Fisheries and Aquaculture. Herring was kept frozen (-28°C) until brining was done. Herring brined in sucrose were kept frozen between 5 to 21 days, whereas herring brined in glucose and xylose were kept for 2 months.

Salted herring were prepared as described in the flowchart (figure 9)

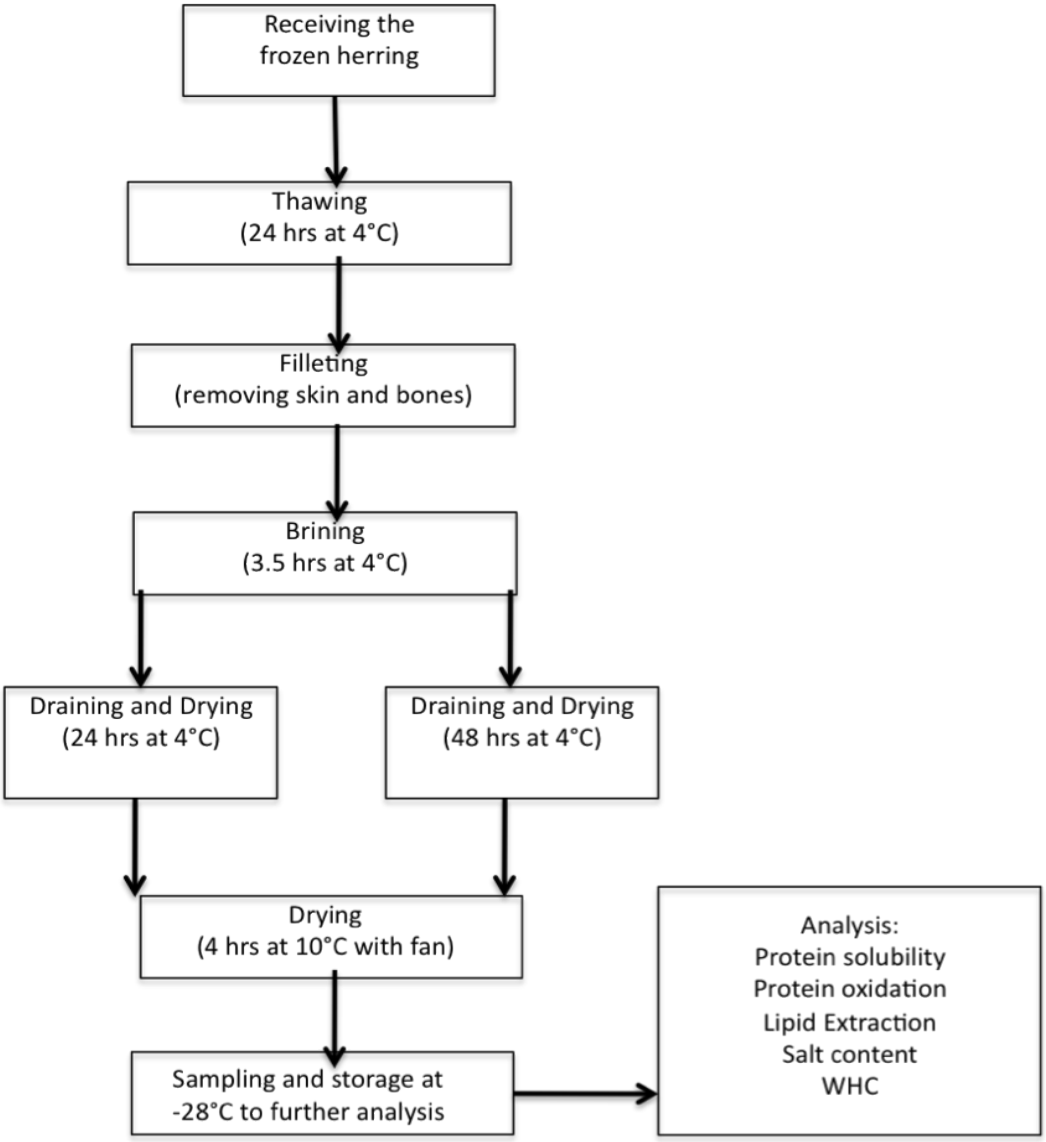

Figure 9 Experimental flowchart for salted and dried herring

Different brines were tried in order to find out their effect on the protein and lipid oxidation. All the brines contained 81.08% of water and 10.81% of salt. The other compounds were as describe in table 1.

Table 1.Overview of the brine preparation

Brine	Ingredients
Brine 1	8.1% Sucrose
Brine 2	8.1% Sucrose, Fe^{2+} 1ppm
Brine 3	8.1% Sucrose, Fe^{2+} 3ppm
Brine 4	8.1% Sucrose, Cu^{2+} 1ppm
Brine 5	8.1% Sucrose, Cu^{2+} 3ppm
Brine 6	8.1% Glucose
Brine 7	8.1% Glucose, Cu^{2+} 3ppm
Brine 8	8.1% Xylose
Brine 9	8.1% Xylose, Cu^{2+} 3ppm

Stock solutions of 5000 ppm of Fe^{2+} and Cu^{2+} were prepared with $FeCl_2$ and $CuSO_4$ in water, respectively. Stock solutions with transition metals were prepared just before starting the brining.

Water content, water-holding capacity, salt content, protein solubility, carbonyl groups, SH-groups, lipid extraction, peroxide value and browning intensity were determined in the samples brined in sucrose solution. For the samples brined in glucose and xylose water content, water-holding capacity, carbonyl groups and browning intensity were determined.

Fillets were minced just before the analysis.

2.1.2 Fully salted cod

Cod fillets were bought in the local market, "Fiskehallen", in Trondheim. It was reported that the fish was caught two days before it was bought (24.01.12-26.01.12). Samples were prepared according to the flow chart (figure 10).

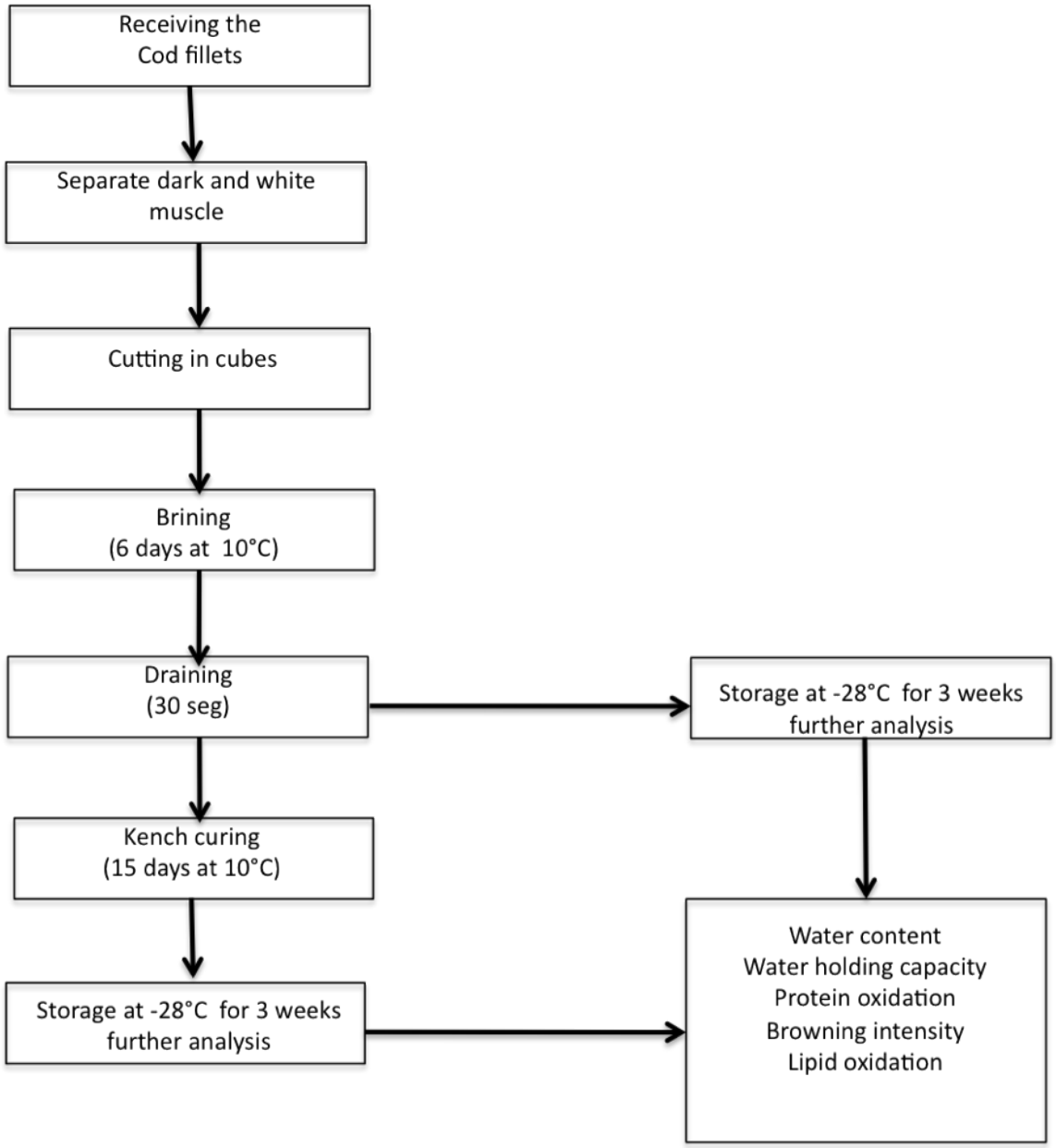

Figure 10 Experimental flow chart for fully salted cod

Brining followed by kench curing was done with the Atlantic cod (Gadus morhua). Fish were cut in cubes in order to increase the surface of contact between salt and fish, as done by (Lauritzsen et al., 1999).

All the brines contained 74% of water and 26% of salt. The other compounds were as describe in table 2.

Table 2 Overview of the brine preparation

Brine	Ingredients
Brine 1	Normal brine
Brine 2	Hemoglobin 5ppm
Brine 3	Hemoglobin 5 ppm, Cu^{2+} 5ppm
Brine 4	Cu^{2+} 5ppm

The fillet cubes were kench cured for 15 days with solid NaCl in a weight ratio of 1:2, salt:fish.

Water content, water-holding capacity, carbonyl groups, browning intensity and TBARS were determined after brining and after kench curing.

2.2 Water content

The samples were dried at 105°C for 24 hours. The difference in weight of the sample after and before drying was defined as the water content. (Choe and Min, 2005)

2.3 Water holding capacity (WHC)

This method determines the ability of the muscle to retain water after the application of mechanical force. WHC was determined with the method described by (Eide et al., 1982). The minced samples were centrifuged with a centrifugation force of 210 g for 5 minutes. The determination was done in four replicates and the WHC was expressed as the amount of water retained in percentage of the original water in the sample.

2.4 Salt content

Salt content was determined with the Volhard method (AOAC, 1990). In order to determine the concentration of chloride ions in a solution, back titration with potassium thiocyanate was used. The first point was to make a precipitate of silver chloride by the addition of an excess volume of silver nitrate to the sample. (Reaction 1) Then the ferric indicator was added. The sample solution is titrated with potassium thiocyanate. As the silver ions reacts with the thiocyanate ions to form a silver thiocyanate precipitate, the

solution remains pale yellow (Reaction 2). When all the silver ions have reacted, the most minimal excess of thiocyanate reacts with Fe^{3+} forming a red-brownish complex. (Reaction 3)

$Ag^+ + Cl^- \rightarrow AgCl$ (1)

$Ag^+ + SCN^- \rightarrow AgSCN$ (2)

$Fe^{3+} + SCN^- \rightarrow [FeSCN]_2$ (3)

The difference between the number of moles of silver that react with the thiocyanate with the total moles of silver nitrate added to the sample solution, determines the concentration of chloride ions.

2.5 Protein solubility: water and salt soluble proteins

In order to determine protein solubility, the proteins were extracted by a modification of the methods of (Anderson and Ravesi, 1968) and (Licciardello et al., 1982) described by (Hultmann and Rustad, 2002). In a cold room (+4°C), 4 grams of fish were homogenized for 30 seconds with 80 ml of Bis Tris buffer (0.05M Bis Tris, pH 7). To obtain the water soluble fraction the volume was made up to 100 mL with Bis Tris Buffer after centrifugation (4°C, 20 minutes at 8000 3 g). The precipitate was homogenized for 10 seconds with 80ml of Bis Tris buffer with KCl (0.05M Bis Tris, 0.6M KCl, pH 7.0) and centrifuged as above. To obtain the salt soluble the volume was made up to 100mL. Extraction procedure was done once on each sample.

The amount of protein was determined following the BioRad protein essay using bovine serum albumin as a standard (Bradford, 1976). The analyses were run in triplicate and the absorbance was measured at 595 nm wavelengths.

2.6 Determination of protein oxidation

In order to determine the oxidation of protein carbonyls and sulfhydryls groups were determined, as these groups are products of protein oxidation Carbonyl groups was determined by the method of (Morzel et al., 2006), and SH-groups determination by the method of (Ellman, 1959), modified by (Sompongse et al., 1996).

2.6.1 Determination of carbonyl groups in fish products

The sample (2 g) was homogenized on ice with 10 mL of Fish buffer (50 mM trisbuffer, 1mM EDTA and 5% SDS at pH 7,4). 200 μL of the homogenate was pipetted out into 4 Eppendorf tubes (one blank and three parallels). The remaining homogenate was centrifuged at 12 000 rpm for 10 minutes, to separate the water-soluble fraction. 500 μL of the water-soluble fraction was pipetted out into another 4 Eppendorf tubes (one blank and three parallels). To all the samples in Eppendorf tubes, 50 μL of trichloroacetic acid (TCA) 100% was added, mixed with the wirlmixer for 10 seconds and centrifuged at 12 000 rpm and 4°C for 4 minutes. The supernatants were decanted. To the blank samples 500 μL of 2M HCl and to the other samples 500 μL of 10 mM 2,4-Dinitrophenylhydrazine (DNPH) in 2M HCl were added. All the samples were placed in a dark room for one hour. To all the samples 50 μL of TCA 100% was added, mixed with the wirlmixer for 10 seconds and centrifuged at 12 000 rpm and 4°C for 4 minutes. The supernatants were decanted. The samples were washed with 1mL ethanol/ethylacetate (1:1) followed by mixing on a whirlmixer for 10 seconds and centrifugation at 12 000 rpm for 4 minutes. The washing process was repeated twice, so pellets were washed 3 times in total. The pellets were dissolved in 1 mL of saturated Guanidine hydrochlorid-solution and left in refrigeration overnight. The next day the samples were left at room temperature for an hour. The samples were mixed on a whirlmixer and centrifuged at 12 000 rpm and 20°C for 10 minutes. The blank samples were read at 280 and 370 nm with saturated guanidine hydrochloride as blank. The parallels samples were measured at 370 nm with saturated guanidine hydrochloride as blank.

2.6.2 Determination of SH groups

Two solutions were made:

- Solution 1: 7.88g of Tris-HCl [0.2 M] buffer with pH 6.8 that contains 120g of urea [8 M], 5g SDS [2%] and 0.73g EDTA [10 mM] with adjusted volume of 250 mL distilled water.
- Solution 2: 0.05g of DNTB [0.1%] into 1.54g of Tris-HCl [0.2M] with adjusted volume of 50 mL distilled water.

A mixture was made with 9 mL of solution 1 and 1 mL of water-soluble extract. 4 mL was pipetted out from this mixture into two tubes respectively and 0. 4mL of solution 2 was added to each tube. The tubes were incubated at 40°C for 25 minutes. The absorbance was determined at 412 nm with buffer used to extract proteins as blank.

2.7 Lipid extraction

Lipid was extracted by a modification of the Bligh and Dyer method. (Bligh and Dyer, 1959)

10 g of the sample was homogenized with 10 mL distilled water, 20 mL of chloroform and 40 mL of methanol during 2 minutes. 20 mL of chloroform were added and homogenized for 30 seconds. 20 mL of distilled water were added and homogenized for 30 seconds. The homogenate was centrifuged for 10 minutes at 9000 rpm. The chloroform phase was pipetted out. 1mL of the chloroform phase was pipetted into a tube pre weighted. The chloroform was evaporated under a stream of N_2 at 40°C. After evaporation of the chloroform, the sample was cooled down and weighed again.

Samples were placed in glass containers and labeled. Samples were kept frozen at -28°C for 1 week before analysis of lipid oxidation. The chloroform was evaporated under a stream of N_2 at 40°C just before lipid oxidation analysis.

2.8 Determination of lipid oxidation

2.8.1 Peroxide value determination

Peroxide value (PV) was determined by thiocyanate method according to *Ueda, Hayashi et al (1986)* and IDI standard 174A (1991) reference. The principle of this method is that hydroperoxides in the lipids oxidizes from Fe(II) to Fe(III). Fe(III) reacts with ammonium thiocyanate forming a red complex with absorption maximum at 500 nm.

- Analysis of the sample:

The lipid extract was dissolved in iso-hexan to a concentration of at least 20 mg/mL. 10 mL of 95% ethanol were mixed with 200 μL sample. 200 μL of 30% ammonium

thiocyanate and 200 μL of Fe(II) solution were added. The solution was mixed for 15 seconds at a whirlmixer. After 2 minutes and 45 seconds absorption was read at 500 nm against a cuvette with 95% ethanol.

- Analysis of the blank:

All the steps of the analysis of sample were followed. Instead of using the sample, 200 μL pure iso-hexane were added. The same iso-hexane was used for the preparation of the sample and the blank.

- Standard graph based on Fe(II)

10 mL of 95% ethanol were placed into 5 test tubes respectively. 200 μL of iso-hexane were added to each tube. The tubes were also included the following:

1) 200 μL of Fe(III)
2) 150 μL of Fe(III) + 50 μL of 3.5% HCl
3) 100 μL of Fe(III) + 100 μL of 3.5% HCl
4) 50 μL of Fe(III) + 150 μL of 3.5% HCl
5) 200 μL of 3.5% HCl

200 μL of 30% ammonium thiocyanate solution were added and mixed for 15 seconds. After 2 minutes and 45 seconds the absorption was read at 500 nm.

2.8.2 Thiobarbituric acid (TBARS)

Thiobarbituric acid quantification is an index of lipid peroxidation. Thiobarbituric acid reacts with malondialdehyde, a product of lipid peroxidation, resulting in a fluorescent product.

TBARS were determined by the method described by Dulavik. (Dulavik et al., 1998)
A homogenate was made with 8 gr of minced sample, 30 ml of TCA 10%, 0.1% propylgallate and 0.1% EDTA. The homogenate was placed in a boiling bath for 30 min and centrifuged at 1000 rpm for 15 min. 1ml of the supernatant was pipetted into an Eppendorf tube with 1 ml of 2-thiobarbituric acid (0.6% water). The Eppendorf tube was placed in a boiling bath for 30 min. The sample was cooled down in ice water.

Absorbance was measured at 532 nm. A standard curve was made with 1,1,3,3 tetraethoxypropane (10-2 nmol/ml).

2.9 Browning intensity:

Browning intensity were determined by the method described by (Azad Shah et al., 2009) 1.25 g of the sample were homogenized in 7 ml of cold 7% TCA for 1 minute. The sample was centrifuged at 4000 rpm for 20 minutes and filtered through glass wool. The precipitate was homogenized in 7 ml of cold TCA for 30 seconds. The homogenization, centrifugation and filtration processes were repeated twice. The volume after the last filtration was adjusted to 25 ml. Absorbance was measured at 420 nm (A_{420}) to express browning intensity (A_{420}/g) of the salted herring (Azad Shah et al., 2009) .

2.10 Statistical Analysis

Analysis of variance ANOVA was used to find significant difference in WHC determination as well as in lipid oxidation in fully salted cod samples.

3 Results and Discussion

3.1 Salted and dried herring

3.1.1 Water content and water holding capacity.

Table 3 shows the percentage of water content and water holding capacity in the different herring samples. Water content of samples dried for 1 day was between 46 and 70%. While water content of samples dried for 2 days was between 36 and 66%. The presence of different solutes, as sugar and metal ions, affects by osmosis the water content, and the denaturation of proteins affects the water holding capacity. Thus the difference of the water content and water holding capacity is explained due to alteration in the structure of the protein.

Table 3. Average and standard deviation of water content and water holding capacity of the herring brined in different solutions and dried for one and two days.

	Water content		WHC	
Samples	**average (%)**	**stdev (%)**	**average (%)**	**stdev (%)**
Raw herring	69.89	2.26	85.33	2.93
Sucrose brine 1 day dried	67.44	0.21	98.11	0.75
Sucrose brine 2 days dried	63.59	0.19	98.75	0.27
Sucrose brine + Fe 1ppm 1 day dried	63.07	1.23	97.37	0.79
Sucrose brine + Fe 1ppm 2 day dried	59.79	6.64	93.23	2.84
Sucrose brine + Fe 3ppm 1 day dried	69.46	1.06	98.46	0.56
Sucrose brine + Fe 3ppm 2 day dried	65.48	1.69	98.90	0.35
Sucrose brine + Cu+2 1ppm 1 day dried	68.79	0.24	95.85	0.75
Sucrose brine + Cu+2 1ppm 2 day dried	58.36	1.70	96.83	1.88
Sucrose brine + Cu+2 3ppm 1 day dried	64.30	2.69	96.26	0.54
Sucrose brine + Cu+2 3ppm 2 day dried	63.17	0.97	96.08	0.22

Glucose brine 1 day dried	47.07	7.26	93.49	1.94
Glucose brine 2 day dried	41.96	2.20	89.94	1.93
Glucose brine + Cu2+ 3ppm 1 day dried	54.17	14.32	95.17	1.57
Glucose brine + Cu2+ 3ppm 2 days dried	49.67	0.29	94.15	3.02
Xylose brine 1 day dried	45.87	0.93	90.64	3.03
Xylose brine 2 days dried	44.13	0.27	89.76	2.76
Xylose brine + Cu2+ 3ppm 1 day dried	46.11	0.98	96.67	1.53
Xylose brine + Cu2+ 3ppm 2 days dried	36.25	2.93	85.94	3.61

Fillets of raw herring were used as a control. As mentioned in chapter 1.3, low ionic strength of the NaCl induces a change in the protein structure, which helps to retain water in the protein structure increasing the water holding capacity demonstrated by the WHC in the brined samples. Water holding capacity increases with increasing salt concentration in the muscle phase (Gallart-Jornet et al., 2007, Thorarinsdottir et al., 2011b). Transition metals in the brine induce a lower water content and lower water holding capacity than in the samples brined in the normal brine (10.81%NaCl, 8.1% sucrose, and 81.08% water). According to (Puolanne and Halonen, 2010) as the salting out coefficient increases, hydrophobicity increases leading to lower capacity to binding with water.

The difference between the samples with the highest and the lowest water holding capacity was 12.8%. The sample that showed highest water holding capacity (98.9%) was the sample brined in sucrose in addition to 3ppm of Fe^{3+} and dried for 2 days. The sample with lowest water holding capacity (85.9%) was the sample brined with xylose in addition to 3ppm of Cu^{2+} and dried for 2 days.

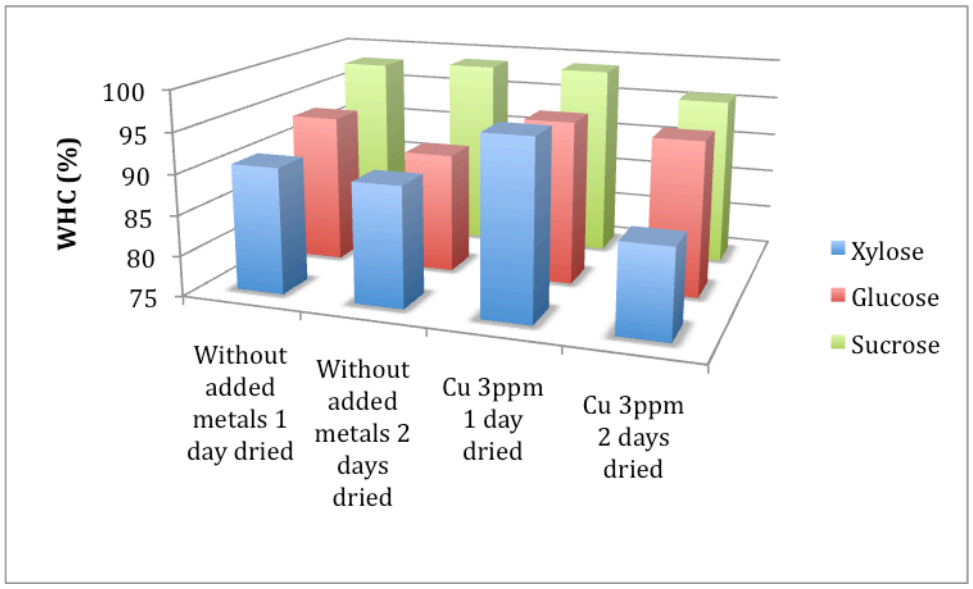

Figure 11 Comparison of WHC between the herring samples brined with addition of different sugars and copper

The difference in water content and water holding capacity showed in samples brined with different types of sugars shown in figure 11. Sugar protects the protein against denaturation can explain the difference in water holding capacity in the samples brined in different sugars. This can be explained due to the cryoprotective effect of sugar against protein denaturation. It has been reported that sugar and polyols are cryoprotective against protein denaturation and retain protein functionality in fish muscle (Park, 1994). (Sultanbawa and Li-Chan, 1998) observed that sucrose:sorbitol blends (4%:4%) increased protein extractability, as well as water holding capacity in cod surimi.

3.1.2 Protein solubility and salt concentration

The presence of salt and metal ions affects the protein solubility and structure. However, the presence of transition metals also affected the solubility of the proteins (figure 12).

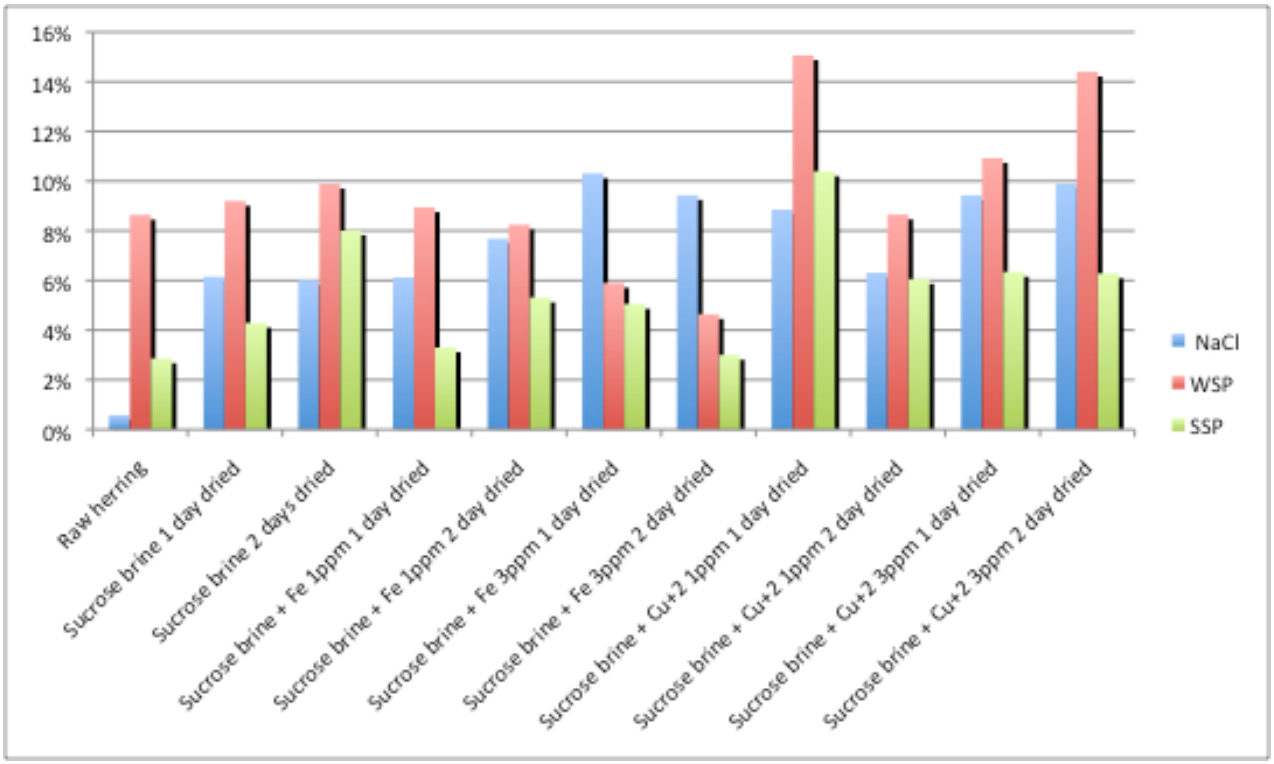

Figure 12 Salt concentration, amount of salt soluble and water-soluble proteins in herring. Values are given in percentage of dry weight.

In all the samples, solubility of SSP and WSP increased in comparison with raw samples. In relation to dry weight, the sample that exhibits a higher increase in sarcoplasmic proteins (WSP) was the brined in 1 ppm Cu^{2+} and dried for 1 day. Water holding capacity is also affected by the changes in the solubility of myofibrillar proteins or proteins soluble at high ionic charge (SSP) and sarcoplasmic or proteins soluble in water (WSP). Myofibrills hold 85% of the water in muscle cells (Huff-Lonergan and Lonergan, 2005).

Samples with added transition metals to the brine showed a higher salt concentration than the samples without transition metals (figure 12). A higher SSP and WSP values were observed in the samples brined with Cu^{2+}, due to the higher salt diffusion that leads to stronger protein aggregation. It can be observed a higher salt, SSP and WSP

percentage in the samples that were dried two days compared to one day. Appendix A and B. show the detailed results of salt content and protein solubility, respectively.

(Thorarinsdottir et al., 2002) point out that at concentrations over 5% NaCl attraction between water molecules and salt ions is produced resulting in a decrease in moisture in the protein that results in a decrease in WHC. A reduction in repulsive forces and an interaction between the hydrophobic regions results in protein aggregation, precipitation and a decrease in the solubility. Thus the decrease in the SSP is related to the increase in salt content.

3.1.3 Protein oxidation

The presence of carbonyl groups indicates protein oxidation. A decrease in free thiol groups (SH-) also indicates protein oxidation. Table 4 shows the content of carbonyl groups in the homogenate and water -soluble fraction as well as the content of free thiol groups. Thiol groups were determined only in samples brined with sucrose.

Table 4 Carbonyl groups and thiol groups amount in herring samples. Values are given in relation to wet weight.

Samples	Carbonyl uM/g in the homogenate	Carbonyl uM/g water soluble fraction	Thiol groups [nmol/L]
Raw herring	0.203	0.110	0.002
Sucrose brine 1 day dried	0.101	0.059	0.001
Sucrose brine 2 days dried	0.226	0.132	0.0014
Sucrose brine + Fe 1ppm 1 day dried	0.140	0.191	0.0015
Sucrose brine + Fe 1ppm 2 day dried	0.313	0.105	0.0017
Sucrose brine + Fe 3ppm 1 day dried	0.331	0.106	0.0018
Sucrose brine + Fe 3ppm 2 day dried	0.359	0.172	0.0009
Sucrose brine + Cu+2 1ppm 1 day dried	0.230	0.168	0.0012
Sucrose brine + Cu+2 1ppm 2 day dried	0.459	0.107	0.0014

Sucrose brine + Cu+2 3ppm 1 day dried	0.616	0.125	0.0018
Sucrose brine + Cu+2 3ppm 2 day dried	0.924	0.308	0.0023
Glucose brine 1 day dried	0.328	0.099	
Glucose brine 2 day dried	0.416	0.111	
Glucose brine + Cu2+ 3ppm 1 day dried	0.215	0.157	
Glucose brine + Cu2+ 3ppm 2 days dried	0.525	0.141	
Xylose brine 1 days dried	0.320	0.064	
Xylose brine 2 days dried	0.222	0.080	
Xylose brine + Cu2+ 3ppm 1 days dried	0.124	0.019	
Xylose brine + Cu2+ 3ppm 2 days dried	0.131	0.019	

Carbonyl groups were determined in the homogenate and in the water-soluble fraction. Determination in the homogenate gives a total overview of the carbonyl groups in the samples. Thus the presence of carbonyl groups was higher in the homogenate than in the water-soluble fraction. Carbonyl groups were also higher in samples dried for 2 days compared with samples brined with the same solution and dried for one day (Figure 13).

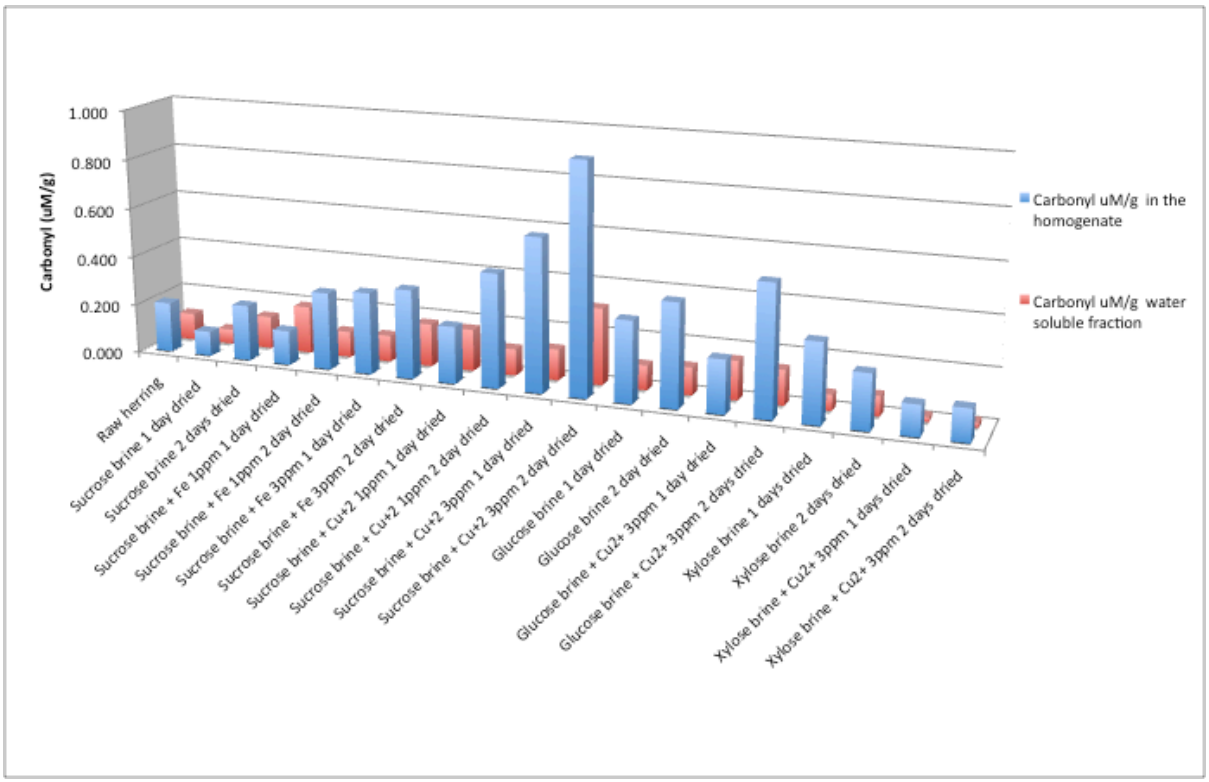

Figure 13. Carbonyl groups found in the homogenate and in the water-soluble fraction in salted and dried herring samples wet weight

Carbonyl groups in the homogenate and browning intensity values in the samples brined with sucrose were linearly correlated (figure 14). Significant correlation ($p<0.05$) was found between the browning intensity and carbonyl groups. Appendix C and E show detailed results of carbonyl groups and browning intensity determination.

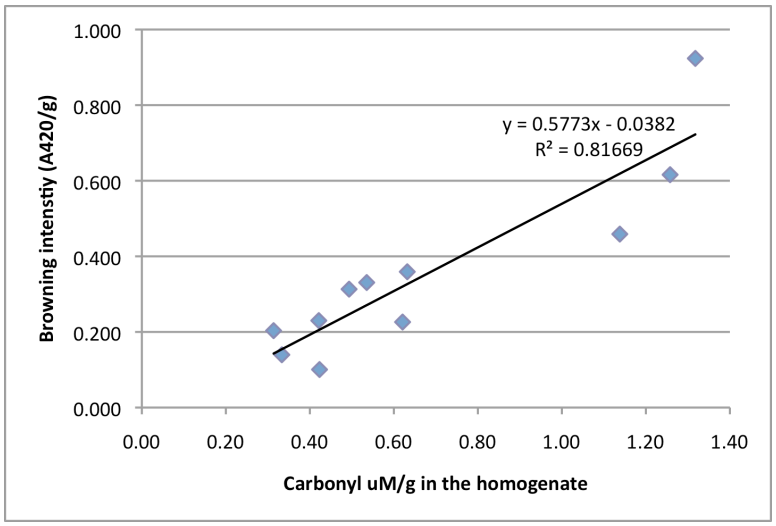

Figure 14. Correlation between browning intensity and carbonyl group values in the samples brined with sucrose.

However carbonyl groups are products of protein oxidation, but they can also be used a substrate in browning reactions such as the Maillard reaction. In the case of the samples brined with glucose and xylose, the amount of carbonyl groups was similar to the amount found in raw herring. A higher browning intensity in such samples indicates that the carbonyl groups (resultant from protein oxidation) were used as substrate in browning reactions.

SH groups were determined in samples brined with sucrose. Higher content of oxidative products, as carbonyl groups and SH-groups, were found in samples dried 2 days than in samples brined with the same solution and dried for one day. The samples dried for 2 days were more exposed to the oxygen, thus probably more susceptible to oxidation. Table 19 in Appendix D, shows SH values. Due to the fact that metals work as prooxidants it was expected that samples with transition metals were more oxidized than samples without any transition metal. It is well known that H_2O_2, which is formed in cells and accumulated postmortem, can oxidize the thiol group of cysteine (Harel and Kanner, 1985, Lund et al., 2011). It can explain why in some samples the content of SH groups were not the expected.

3.1.4 Lipid oxidation

Lipid extraction of the samples was done in order to determine later the lipid oxidation. Table 5 shows the content of lipid extraction in the herring brined in sucrose solution and dried for 1 and 2 days

Table 5 Overview of the lipid content of the samples.

Sample	Lipid content (g lipid/ 100g sample)
Raw herring	17.1 %
Sucrose brine 1 day dried	9.9 %
Sucrose brine 2 days dried	13.5 %
Sucrose brine + Fe 1ppm 1 day dried	14.1 %
Sucrose brine + Fe 1ppm 2 day dried	18.1 %
Sucrose brine + Fe 3ppm 1 day dried	10.9 %
Sucrose brine + Fe 3ppm 2 day dried	14.4 %

Sucrose brine + Cu+2 1ppm 1 day dried	18.9 %
Sucrose brine + Cu+2 1ppm 2 day dried	19.2 %
Sucrose brine + Cu+2 3ppm 1 day dried	15.6 %
Sucrose brine + Cu+2 3ppm 2 day dried	13.3 %

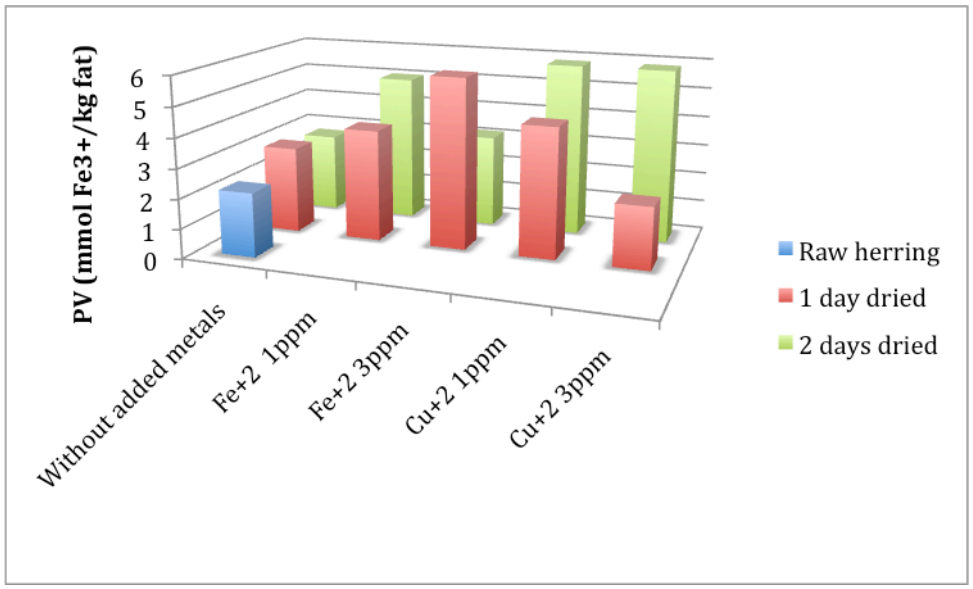

Figure 15 Overview of the PV(mmol Fe3+/kg fat) of different herring sucrose brined samples .

Since unsaturated fatty acids react with oxygen, it was expected that PV were higher in samples dried for two days. It also was expected that samples containing metals show an increase in PV, since metals contribute to accelerate oxidation. Results can be observed in figure 15. The peroxide radicals, primary products of lipid oxidation, react abstracting hydrogen atoms from proteins yielding to protein oxidation. Resulting in the formation of volatile, nonvolatile and polymeric secondary oxidation products. Secondary oxidation products include aldehydes, ketones, alcohols, hydrocarbons, volatile organic acids and epoxy compounds (Shahidi and Zhong, 2005).

Lipid oxidation is also related to Maillard reaction. Aldehydes, secondary products from lipid oxidation, react when cysteine is present to the formation of long-chain sulfur heterocyclic compounds (Ikan, 1996).

3.1.5 Browning intensity

Browning intensity was measured in all samples. In order for the Maillard reaction to occur it is required carbohydrates in presence of an amino group. Such products can be formed with the resulting compounds of protein oxidation and lipid oxidation as well as the sugar from the brine as the carbohydrate source. Table 6 shows the results of browning intensity of herring samples.

Table 6. Browning intensity (A420/g) of herring samples.

Sample	Browning intensity (A420/g)
Raw herring (white tissue)	0.314
Raw herring dark tissue)	0.518
Sucrose brine 1 day dried	0.423
Sucrose brine 2 days dried	0.621
Sucrose brine + Fe 1ppm 1 day dried	0.333
Sucrose brine + Fe 1ppm 2 day dried	0.493
Sucrose brine + Fe 3ppm 1 day dried	0.535
Sucrose brine + Fe 3ppm 2 day dried	0.632
Sucrose brine + Cu+2 1ppm 1 day dried	0.421
Sucrose brine + Cu+2 1ppm 2 day dried	1.138
Sucrose brine + Cu+2 3ppm 1 day dried	1.258
Sucrose brine + Cu+2 3ppm 2 day dried	1.318
Glucose brine 1 day dried	0.778
Glucose brine 2 day dried	1.333

Glucose brine + Cu2+ 3ppm 1 day dried	1.384
Glucose brine + Cu2+ 3ppm 2 days dried	1.480
Xylose brine 1 days dried	1.520
Xylose brine 2 days dried	1.650
Xylose brine + Cu2+ 3ppm 1 days dried	1.650
Xylose brine + Cu2+ 3ppm 2 days dried	1.744

It was expected a higher browning intensity in samples with addition of transition metals. Thus transition metals works as prooxidants and oxidation helps to the development of color. Also because, while more oxidation, more oxidation products that can be used in Maillard reaction leading a more intense color.

Large differences in browning intensity between the samples brined with glucose and xylose were found (Figures 16, 17 and 18). Appendix F shows detailed results of browning intensity.

Figure 16 Herring fillets salted in sucrose solution and dried for 1 day.

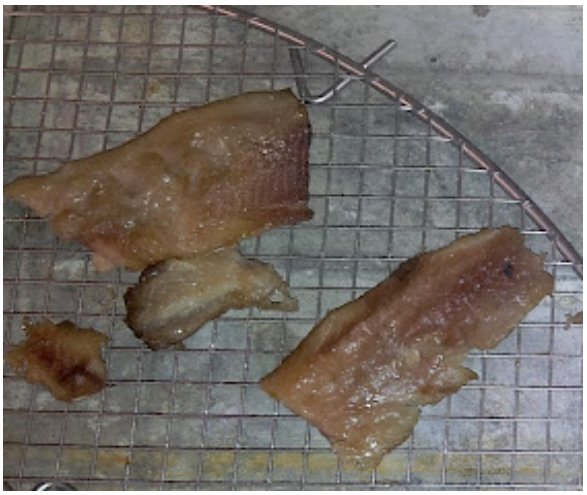

Figure 17 Herring fillets salted in glucose solution and dried for 1 day.

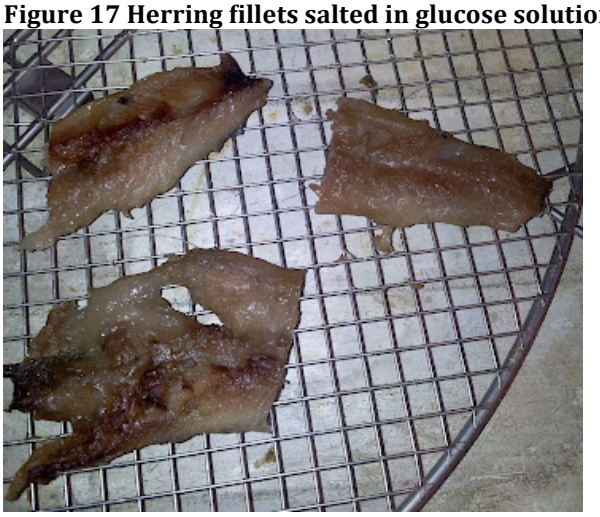

Figure 18 Herring fillets salted in xylose solution and dried for 1 day.

(Kwak and Lim, 2004) reported different degree of browning intensity with different sugars; it was reported in the following order xylose > arabinose > glucose > maltose > fructose. Comparing the traditional method and ingredients (sucrose brine dried 1 day) with glucose brine, the browning intensity is almost doubled and with xylose brine is tripled. Coinciding with Kwak et al (2004) the browning intensity is higher with the xylose than with the glucose. The fact that a higher intensity of browning was found in the samples containing metals also coincides with the study of Kwak. The presence of metal ions has a larger effect on samples brined in sucrose than in samples brined in glucose or xylose.

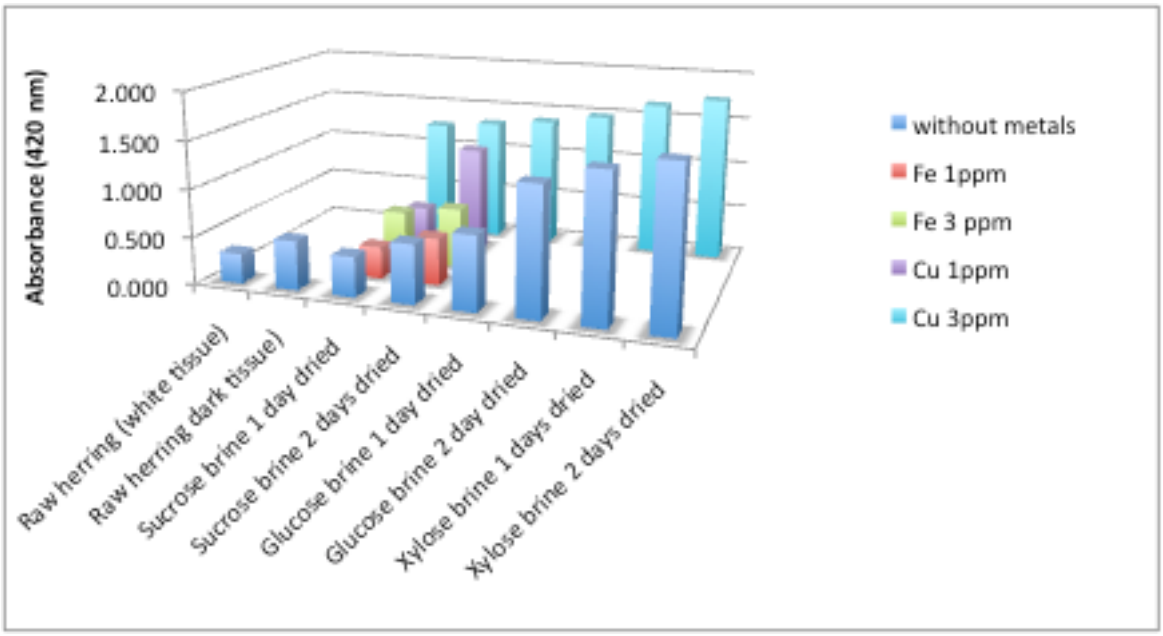

Figure 19. Browning intensity of herring samples brined in different sugars with and without metal ions.

Figure 19 shows the increase shown by the presence of 3ppm Cu^{2+} was of 112% in the sample brined in glucose and dried for 2 days. (Figure 19) However, the mentioned increase of 80% in browning intensity in Fe^{2+} and Cu^{2+} samples in Kwak's *et al* study has been observed in samples heated for 3 hrs. (Kwak and Lim, 2004) It should be consider an alternative use of xylose or glucose in order to get a golden color.

3.2 Fully salted cod

3.2.1 Water content and water holding capacity

Water content and water holding capacity in cod samples were determined and are shown in table 7.

Table 7. Average and standard deviation of water content and water holding capacity of cod cubes, raw, brined and kench cured.

	Water content		WHC	
	Average (%)	Stdev (%)	Average (%)	Stdev (%)
Raw DM	81.50	0.20	72.00	7.80
Raw WM	81.10	0.40	71.40	1.90
After brining				
DM Normal brine	72.30	0.20	74.10	2.70
DM Heme	70.70	0.20	75.30	4.90
DM Heme + Cu2+	71.00	0.50	82.10	1.30
DM Cu2+	71.80	0.80	83.00	3.70
WM Normal brine	70.30	0.30	78.00	3.90
WM Heme	71.60	0.20	80.60	2.40
WM Heme + Cu2+	71.40	0.10	77.40	3.60
WM Cu2+	72.00	0.00	77.00	5.90
After kench curing				
DM Normal brine	52.60	0.10	86.20	4.10
DM Heme	52.10	3.80	83.70	2.60
DM Heme + Cu2+	57.10	0.70	85.70	6.40
DM Cu2+	49.40	4.50	88.50	2.50
WM Normal brine	53.10	2.50	89.10	0.60
WM Heme	49.90	1.00	87.50	4.40
WM Heme + Cu2+	50.60	0.40	81.10	3.00
WM Cu2+	51.20	0.70	88.60	1.50

Water content decreased from 81.5% in raw dark muscle to 49.4% in dark muscle samples with 5ppm of Cu^{2+} and kench cured. In white muscle, water content decreased from 81.1% in raw muscle to 49.9% in samples with 5ppm of heme and kench cured. Results observed in figure 3.2.1.1. The larger reduction in water content in kench cured samples is explained due to an increase in the ionic strength that leads to an increase in hydrophobity. According to (Thorarinsdottir et al., 2011b), salting out and aggregation occurs at high concentration (>1M). During the salting out of proteins muscle fibers shrink allowing the enlargement of inter-cellular space resulting in swelling of the myofibrils. (Thorarinsdottir et al., 2011a) Therefore being more susceptible to oxidation.

Analysis of variance test were used to find significant difference between the WHC of the brined samples and between the kench cured samples (Appendix H). No significant difference was found between brined and kench cured respectively (Appendix H, table 25 and 26). However, there was significant difference ($p < 0.05$) between the two groups (Appendix H table 24).

3.2.2 Protein oxidation

Carbonyl groups are indicators of protein oxidation. It was determined the carbonyl content after the brining and the kench curing in the dark and white muscle samples (table 8).

Table 8 Carbonyl determination in the samples after brining and after kench curing.

	Carbonyl uM/g in the homogenate	Carbonyl uM/g in the water soluble fraction	Carbonyl uM/g in the homogenate	Carbonyl uM/g water in the soluble fraction
Raw DM	0.136	0.103		
Raw WM	0.214	0.022		
	After brining		**After kench curing**	
DM Normal brine	0.038	0.046	0.054	0.069
DM Heme	0.027	0.066	0.098	0.062
DM Heme + Cu2+	0.045	0.081	0.072	0.074
DM Cu2+	0.028	0.072	0.075	0.041
WM Normal brine	0.071	0.013	0.048	0.025
WM Heme	0.035	0.027	0.063	0.034
WM Heme + Cu2+	0.036	0.021	0.117	0.074
WM Cu2+	0.044	0.04	0.108	0.084

Results in table 8 show higher carbonyl content in samples treated with heme and Cu^{2+}, agreeing with the fact that both acted as prooxidant. Higher carbonyl content was found in samples brined with both, 5ppm of heme and 5ppm of Cu^{2+} respectively. It was expected to have higher protein oxidation in samples with heme and transition metals, due to the fact that heme and transition metals acted as prooxidants. Protein oxidation was expected to be higher after the kench curing.

It was also expected to have higher carbonyl content after the kench curing due to a higher protein aggregation (Lund et al., 2011).

In figures 9 and 10 showed the carbonyl content in the homogenate and in the water-soluble fraction.

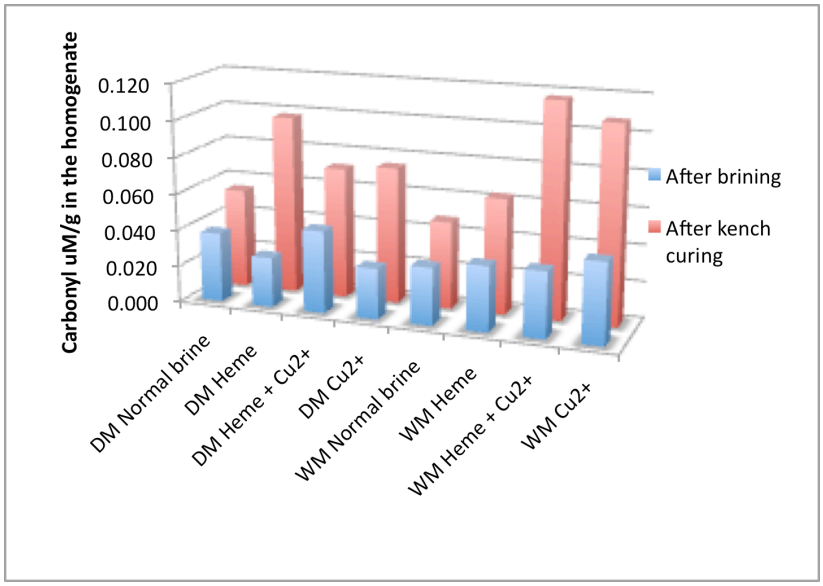

Table 9 Carbonyl groups found in the homogenate in the dark muscle cod samples

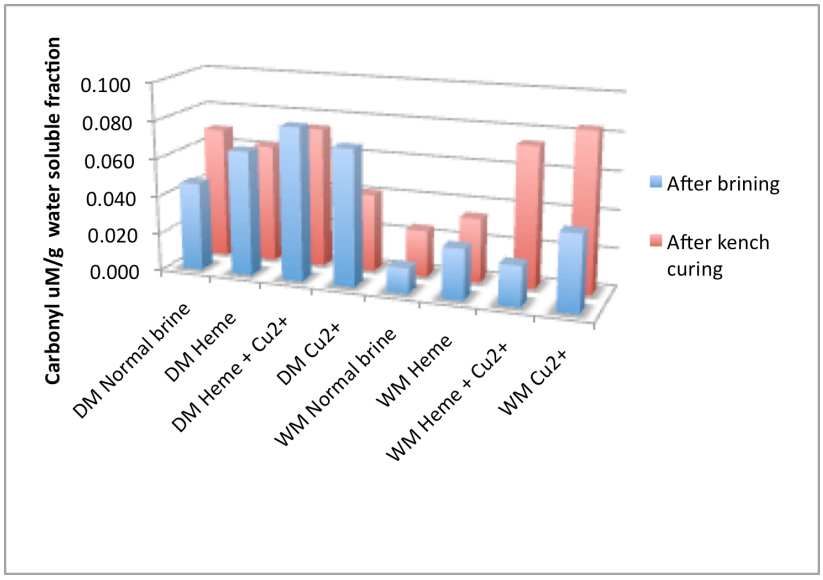

Table 10 Carbonyl groups found in the homogenate in the white muscle cod samples

Since dark muscle has a higher content of lipid and heme proteins it was expected higher carbonyl content in the dark muscle samples. After brining the sample with higher carbonyl groups was the dark muscle with heme, while after kench curing the sample with higher carbonyl groups was the white muscle with heme and Cu^{2+}. This can be explained due to

higher protein availability to oxidation in the white muscle. The content of carbonyl groups after brining was higher in dark muscle than in white muscle. Then after kench curing the increase of carbonyl groups in the dark muscle was small. Whereas in white muscle both samples the content of carbonyl groups almost tripled in samples containing Heme and Cu^{2+} and double in samples containing Cu^{2+}. This results coincide with (Lauritzsen et al., 1999) that reported that even discrete quantity of copper and heme residue of blood acted as a prooxidant affecting the development of discoloration.

3.2.3 Lipid oxidation

The thiobarbituric acid test (TBARS) was used to detect oxidation in lipids. Table 11 shows the results. Equation for the determination of TBARS is shown in Appendix G, as well as detailed results.

Table 11 Overview of TBARS found in fully salted dark and white muscle of cod.

	TBARS (nmol/g)	TBARS (nmol/g)
Raw DM	3.787	
Raw WM	3.498	
	After brining	**After kench curing**
DM Normal brine	6.149	6.612
DM Heme	7.436	6.521
DM Heme + Cu2+	8.093	10.148
DM Cu2+	8.334	9.066
WM Normal brine	4.156	4.528
WM Heme	5.662	6.271
WM Heme + Cu2+	6.096	9.412
WM Cu2+	7.641	6.977

Dark and white muscle of raw cod was included as a control. The differences between the samples were significant ($p>0.01$). Appendix J shows the calculation for the analysis of variance.

After kench curing protein in fish samples are expected to be highly denaturated and more exposed to react with ROS and others prooxidants. It was observed higher TBARS content in samples after kench curing, as it was expected (Table 11). It is also remarkable the difference between the TBARS content in white muscle and in dark muscle. Since dark muscle has a higher content of unsaturated lipid as well as heme proteins a higher content of TBARS was expected. Heme proteins as well as transition

metals are prooxidants and therefore higher content of TBARS was found in samples with both prooxidants. Synergy of prooxidants is shown after kench curing, it could be explained due to in kench curing samples are exposed to oxygen facilitating oxidation.

3.2.4 Browning intensity

Browning intensity was determined. Table 12 shows the results of browning intensity observed in the fully salted cod samples.

Table 12 Overview of browning intensity in dark and white fully salted cod muscle.

	Browning intensity (A420/g)	**Browning intensity (A420/g)**
Raw DM	0.446	
Raw WM	0.369	
	After brining	**After kench curing**
DM Normal brine	0.377	0.477
DM Heme	0.548	0.566
DM Heme + Cu2+	0.611	0.630
DM Cu2+	0.497	0.500
WM Normal brine	0.200	0.298
WM Heme	0.200	0.248
WM Heme + Cu2+	0.136	0.209
WM Cu2+	0.169	0.260

Products of lipid and protein oxidation as carbonyl and amino compounds react resulting in browning. As (Shah et al., 2009) cited, fatty fish is more prominent to be involved in such reactions than lean fish; due to a high amount of polyunsaturated fatty acids that are susceptible to oxidation. Higher browning intensity was observed in samples after kench curing. It can be explained due to an increase in the protein and lipid oxidation that left more radicals able to react. Browning intensity was higher in dark muscle than in white muscle. It can be explained due to high content of heme pigments as well as unsaturated lipid in dark muscle, thus more susceptible to oxidation Higher browning intensity was registered in samples with heme coinciding with the results obtained by (Lauritzsen, 2004) that residues of viscera of blood can contribute to discoloration.

4 Conclusion

This study first concentrated on the factors that influence development of color in salted herring. It has been appreciated that products of lipid and protein oxidation can react in the formation of melanoids. The use of different sugars in the brine causes a more intense color. The browning intensity was in the following order xylose > glucose > sucrose. According to the results carbonyl groups and browning intensity are directly correlated when sucrose is used in brining. The prooxidant effect of transition metals was independent of the brine used. The effect of the cooper as a prooxidant is higher than the iron.

The second part of the study was focused on the factors that influence discoloration on fully salted cod. The minimal presence of impurities as cooper in the salt or heme residue of blood in the fish acted as prooxidant in protein and lipid oxidation leading to the formation of color compounds. Higher oxidation and discoloration was found in dark muscle than in white muscle. Thus in order to get a non discolored fully salted fish is important to take into consideration the purity of the salt and even of the water and also to clean as good as possible the fish from blood and viscera.

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Appendix A. Salt content equation and results

Equation to calculate the salt content:

$$\%NaCl = \frac{(ml\ AgNO_3 - ml\ NH_4SCN) * N * 58.45\ g/mol * 200\ ml * 100}{g\ (sample) * 20\ ml * 1000}$$

Where:
N = Normality of the titrate agent

Table 13. Salt content of the different herring samples.

Type of sample	Average of Salt content % (wet weight)	Average of Salt content % (dry weight)	stdev
Raw herring	0.17	0.57	0.083
Sucrose brine 1 day dried	2.00	6.15	0.097
Sucrose brine 2 days dried	2.19	6.01	0.067
Sucrose brine + Fe 1ppm 1 day dried	2.26	6.12	0.328
Sucrose brine + Fe 1ppm 2 day dried	3.09	7.68	0.089
Sucrose brine + Fe 3ppm 1 day dried	3.15	10.31	0.329
Sucrose brine + Fe 3ppm 2 day dried	3.26	9.43	0.393
Sucrose brine + Cu+2 1ppm 1 day dried	2.76	8.85	0.140
Sucrose brine + Cu+2 1ppm 2 day dried	2.63	6.31	0.306
Sucrose brine + Cu+2 3ppm 1 day dried	3.37	9.43	0.081
Sucrose brine + Cu+2 3ppm 2 day dried	3.64	9.90	0.471

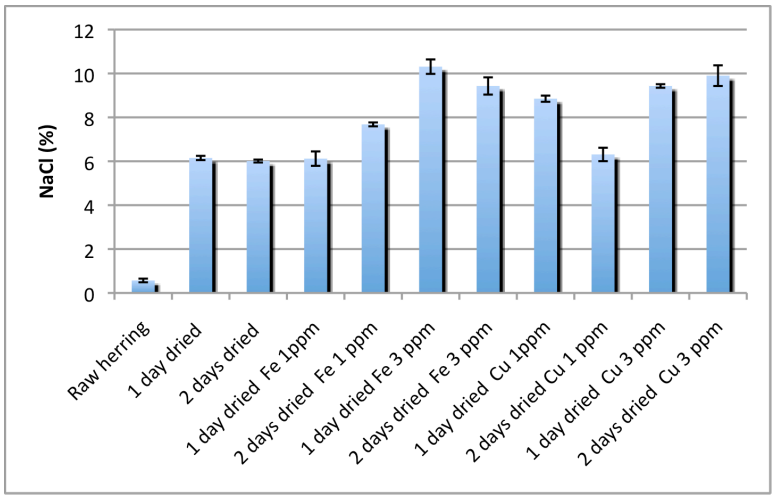

Figure 20. Salt content of different herring samples (dry weight %)

Appendix B. Calculations for the determination of protein solubility

The Biorad method was used to the determination of protein in extracts. Standard solutions to the determination of the standard curve were prepared with Bovine serum albumin.

Table 14 Overview of concentration of Biorad standard curve.

Standard	Blank	1	2	3	4	5
µl standard	0	20	40	60	80	100
µl water	100	80	60	40	20	0

Table 15 Absorbance of the standard curve, for all samples except Cu^{3+} treated samples.

Standard	mg/ml	OD_{595}			Average
0	0	0.000	-0.001	-0.008	0
1	0.2	0.272	0.223	0.241	0.245
2	0.4	0.495	0.502	0.503	0.500
3	0.6	0.633	0.624	0.679	0.645
4	0.8	0.839	0.818	0.837	0.831
5	1	0.954	0.978	0.967	0.966

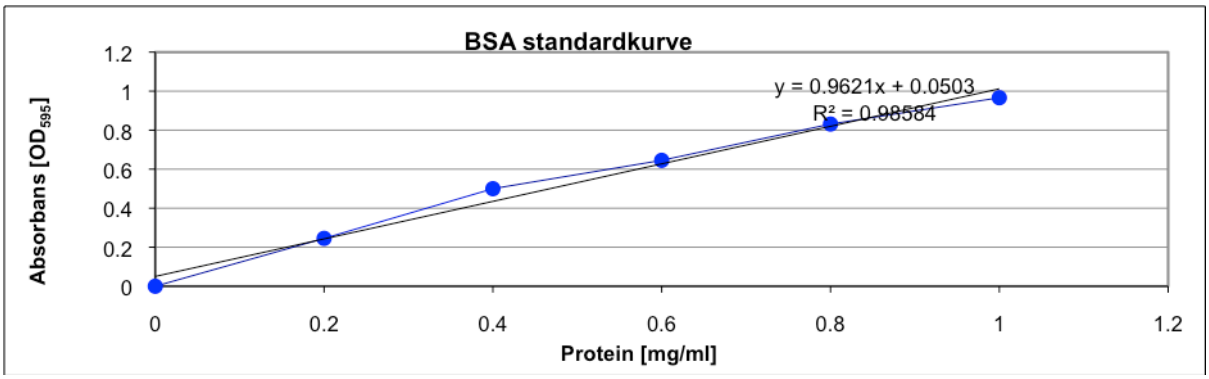

Figure 21 Standard curve for determination of protein solubility of all samples except Cu^{3+} treated samples.

Table 16 Absorbance of the standard curve for samples where Cu^{3+} was added.

Standard	mg/ml	OD_{595}			Average
0	0	0	0.009	-0.004	0
1	0.2	0.164	0.149	0.147	0.153
2	0.4	0.371	0.385	0.39	0.382
3	0.6	0.545	0.556	0.552	0.551
4	0.8	0.777	0.644	0.686	0.702
5	1	0.841	0.828	0.758	0.809

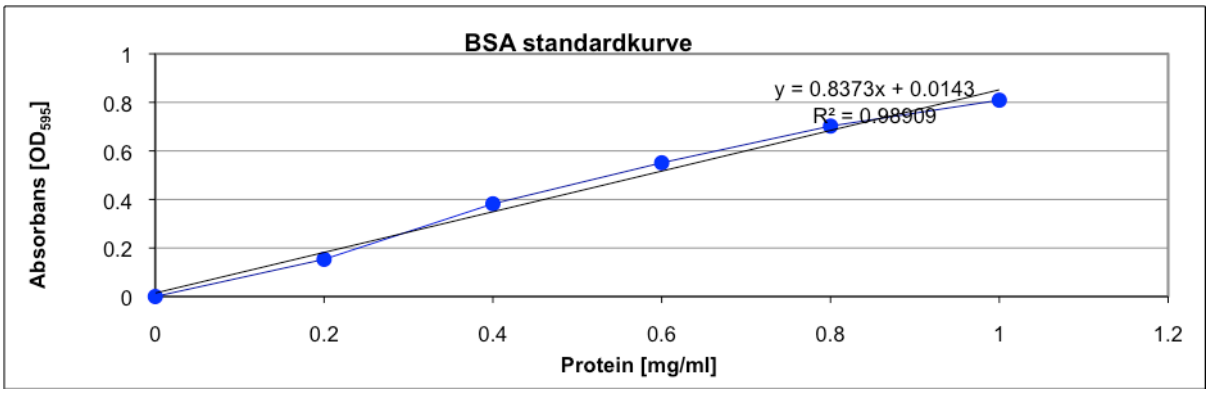

Figure 22 Standard curve for determination of protein solubility of samples where Cu^{3+} were added.

Table 17 Overview of the protein concentration in the different extracts.

Sample	Water soluble protein in the extract (% wet weight)	Water soluble protein in the extract (%dry weight)	Salt soluble protein in the extract (% wet weight)	Salt soluble protein extract [% dry weight]
Raw herring	2.59%	8.64%	0.86%	2.86%
1 day dried	3.03%	9.20%	1.40%	4.29%
2 days dried	3.55%	9.89%	2.92%	8.01%
1 day dried Fe^{2+} 1ppm	3.25%	8.94%	1.22%	3.30%
2 days dried Fe^{2+} 1ppm	3.25%	8.25%	2.12%	5.31%
1 day dried Fe^{2+} 3ppm	1.84%	5.90%	1.54%	5.05%
2 days dried Fe^{2+} 3ppm	1.57%	4.64%	1.04%	3.00%
1 day dried Cu^{2+} 1ppm	4.44%	15.06%	3.24%	10.39%
2 days dried Cu^{2+} 1ppm	3.63%	8.65%	2.52%	6.05%
1 day dried Cu^{2+} 3ppm	3.93%	10.92%	2.26%	6.34%
2 days dried Cu^{2+} 3ppm	5.32%	14.40%	2.31%	6.28%

Appendix C. Carbonyl group determination equation and results

Equation to the calculation of carbonyl group

$$\text{Carbonyl} = \frac{\text{Abs sample} * V}{Mo * v * W}$$

Where:

Abs sample= absorbance of the sample from either homogenate or supernatant at 370nm

V= total sample volume

Mo = molar extinction coefficient

v = volume taken out = 0.2 and 0.5 mL for the homogenate and the supernatant respectively

W = weight of the sample

Table 18 Overview of the carbonyl groups in the different samples

Samples	Carbonyl uM/g homogenate	Carbonyl uM/g supernatant
Raw herring	0.203	0.110
Sucrose brine 1 day dried	0.101	0.059
Sucrose brine 2 days dried	0.226	0.132
Sucrose brine + Fe 1ppm 1 day dried	0.140	0.191
Sucrose brine + Fe 1ppm 2 day dried	0.313	0.105
Sucrose brine + Fe 3ppm 1 day dried	0.331	0.106
Sucrose brine + Fe 3ppm 2 day dried	0.359	0.172
Sucrose brine + Cu+2 1ppm 1 day dried	0.230	0.168
Sucrose brine + Cu+2 1ppm 2 day dried	0.459	0.107
Sucrose brine + Cu+2 3ppm 1 day dried	0.616	0.125

Sucrose brine + Cu+2 3ppm 2 day dried	0.924	0.308
Glucose brine 1 day dried	0.328	0.099
Glucose brine 2 day dried	0.416	0.111
Glucose brine + Cu2+ 3ppm 1 day dried	0.215	0.157
Glucose brine + Cu2+ 3ppm 2 days dried	0.525	0.141
Xylose brine 1 days dried	0.320	0.064
Xylose brine 2 days dried	0.222	0.080
Xylose brine + Cu2+ 3ppm 1 days dried	0.124	0.019
Xylose brine + Cu2+ 3ppm 2 days dried	0.131	0.019

Appendix D. Thiol content equation and results.

$$\text{Thiol content (nmol/L)} = \frac{(\text{Abs sample} - \text{Abs blank}) * 1.1 * 10}{C * 1000 * 0.1 * W}$$

Where:
C= molar extintion coefficient
W= weight of the sample

Table 19 Overview of thiol content in samples of salted and dried herring.

Samples	Thiol groups [nmol/L]
Raw herring	0.0020
Sucrose brine 1 day dried	0.0010
Sucrose brine 2 days dried	0.0014
Sucrose brine + Fe 1ppm 1 day dried	0.0015
Sucrose brine + Fe 1ppm 2 day dried	0.0017
Sucrose brine + Fe 3ppm 1 day dried	0.0018
Sucrose brine + Fe 3ppm 2 day dried	0.0009
Sucrose brine + Cu+2 1ppm 1 day dried	0.0012
Sucrose brine + Cu+2 1ppm 2 day dried	0.0014
Sucrose brine + Cu+2 3ppm 1 day dried	0.0018
Sucrose brine + Cu+2 3ppm 2 day dried	0.0023

Appendix E. Browning intensity calculation

Equation to the calculation of browning intensity

$$\text{Browning intensity} = \frac{\text{Abs sample}}{\text{Weight of the sample}}$$

Table 20 Overview of browning intensity of herring samples

Sample	Weight of the sample	Absorbance at 420 nm	Browning intensity (A420/g)
Raw herring (white tissue)	1.3135	0.412	0.313665778
Raw herring (dark tissue)	1.3777	0.713	0.517529215
Sucrose brine 1 day dried	1.3026	0.551	0.423000154
Sucrose brine 2 days dried	1.2308	0.764	0.620734482
Sucrose brine + Fe 1ppm 1 day dried	1.257	0.419	0.333333333
Sucrose brine + Fe 1ppm 2 day dried	1.2464	0.615	0.493421053
Sucrose brine + Fe 3ppm 1 day dried	1.2085	0.647	0.535374431
Sucrose brine + Fe 3ppm 2 day dried	1.2501	0.79	0.631949444
Sucrose brine + Cu+2 1ppm 1 day dried	1.2201	0.514	0.421276945
Sucrose brine + Cu+2 1ppm 2 day dried	1.2567	1.43	1.137900851
Sucrose brine + Cu+2 3ppm 1 day dried	1.2547	1.578	1.257671156
Sucrose brine + Cu+2 3ppm 2 day dried	1.2309	1.622	1.317734991
Glucose brine 1 day dried	1.3785	1.072	0.777656873
Glucose brine 2 day dried	1.2799	1.706	1.332916634
Glucose brine + Cu2+ 3ppm 1 day dried	1.2585	1.742	1.384187525

Glucose brine + Cu2+ 3ppm 2 days dried	1.3537	2.004	1.480387087
Xylose brine 1 days dried	1.3358	2.031	1.520437191
Xylose brine 2 days dried	1.319	2.176	1.649734647
Xylose brine + Cu2+ 3ppm 1 days dried	1.2931	2.134	1.650297734
Xylose brine + Cu2+ 3ppm 2 days dried	1.2941	2.257	1.744069237

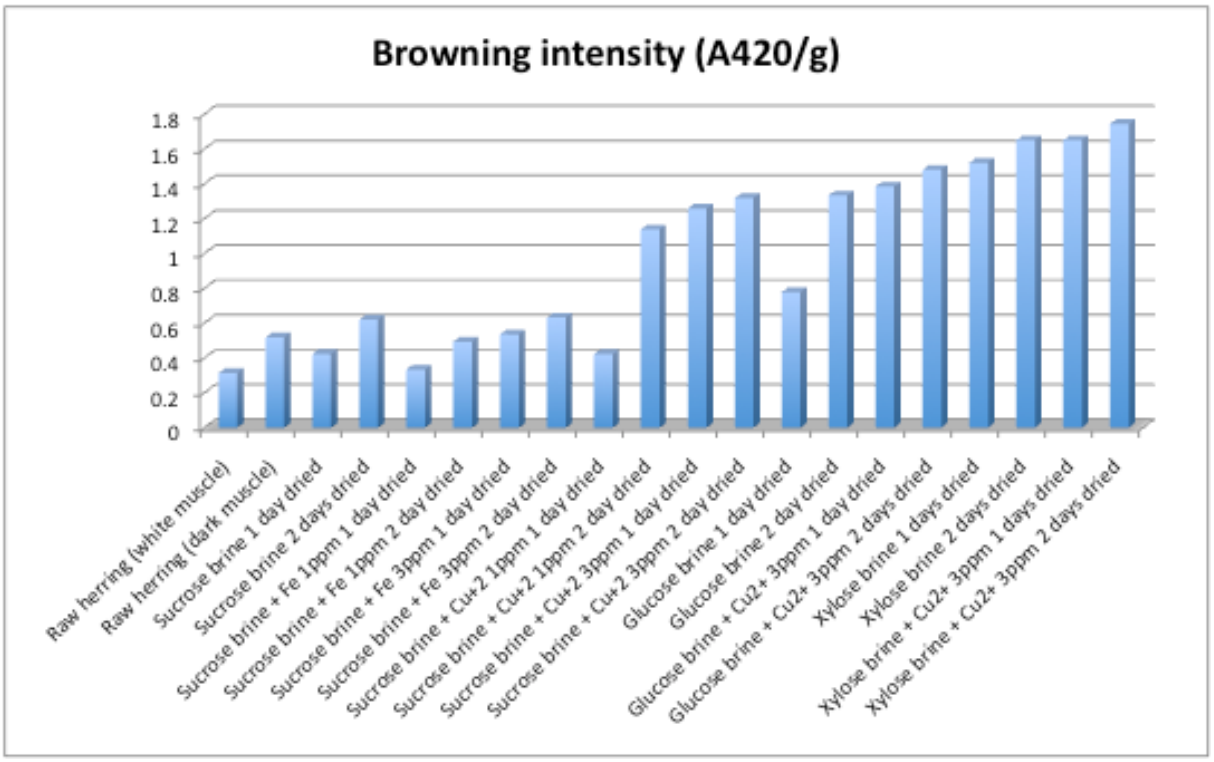

Figure 23 Overview of browning intensity in salted and dried herring samples.

Appendix F. Determination of total lipid content.

$$\text{g lipid/ 100 g sample} = \frac{a * b * 100}{c * v}$$

Where:

a = grams of evaporate fat
b = volume of chloroform added
c = volume of evaporated chloroform
v = weight of the sample

Table 21 Overview of the lipid content of the samples.

Sample	Lipid content (g lipid/ 100g sample)
Raw herring	17.1 %
Sucrose brine 1 day dried	9.9 %
Sucrose brine 2 days dried	13.5 %
Sucrose brine + Fe 1ppm 1 day dried	14.1 %
Sucrose brine + Fe 1ppm 2 day dried	18.1 %
Sucrose brine + Fe 3ppm 1 day dried	10.9 %
Sucrose brine + Fe 3ppm 2 day dried	14.4 %
Sucrose brine + Cu+2 1ppm 1 day dried	18.9 %
Sucrose brine + Cu+2 1ppm 2 day dried	19.2 %
Sucrose brine + Cu+2 3ppm 1 day dried	15.6 %
Sucrose brine + Cu+2 3ppm 2 day dried	13.3 %

Appendix G. Peroxide value determination and standard curves

Analysis of standards containing from 0 to 20 μg Fe^{3+}.

Equation for determination of peroxide value

$$\text{meq peroxide } O_2/\text{kg} = \frac{(\text{Abs sample} - \text{Abs blank}) * L * V}{55.845*S*0.1*2}$$

where :

L= slope of the standard curve

V= volume of iso-hexane used for dissolving lipid (mL)

55.845= molar weight of iron (g/mol)

S= amount of lipid (g)

0.1 = volume of the sample dissolved in iso-hexane that was added to ethanol (mL)

½ =correction factor

Table 22 Absorbance of standard curve

Std	I	II	III	average	μg Fe^{3+}
1	0.017	0.012	0.009	0.013	0
2	0.212	0.215	0.229	0.219	5
3	0.319	0.334	0.326	0.326	10
4	0.464	0.456	0.442	0.454	15
5	0.531	0.527	0.517	0.525	20

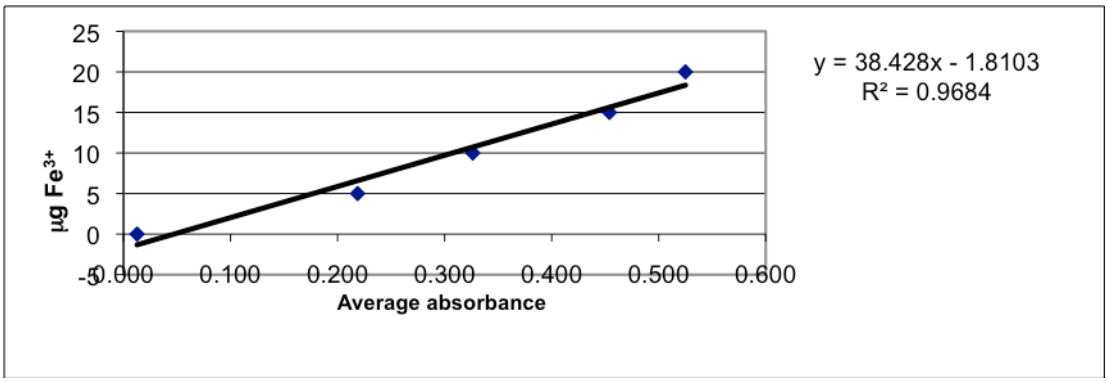

Figure 24 Standard curve for peroxide value determination.

Table 23 Absorbance at 500 nm and calculation of peroxide values of lipid extracts

Sample	1	2	average	PV (mmol Fe3+/kg fat)
Raw herring	0.163	0.231	0.197	2.162659402
Sucrose brine 1 day dried	0.384	0.223	0.3035	2.930649246
Sucrose brine 2 days dried	0.265	0.288	0.2765	2.69513236
Sucrose brine + Fe 1ppm 1 day dried	0.316	0.298	0.307	3.784653953
Sucrose brine + Fe 1ppm 2 day dried	0.397	0.403	0.4	5.011300689
Sucrose brine + Fe 3ppm 1 day dried	0.401	0.433	0.417	5.767091738
Sucrose brine + Fe 3ppm 2 day dried	0.318	0.286	0.302	3.13623422
Sucrose brine + Cu+2 1ppm 1 day dried	0.347	0.37	0.3585	4.390497768
Sucrose brine + Cu+2 1ppm 2 day dried	0.495	0.45	0.4725	5.841842749
Sucrose brine + Cu+2 3ppm 1 day dried	0.264	0.183	0.2235	2.097439341
Sucrose brine + Cu+2 3ppm 2 day dried	0.49	0.456	0.473	5.849010654

Appendix H. Analysis of variance ANOVA for WHC in cod samples

Table 24 Analysis of variance ANOVA of the WHC of all cod samples

Analysis of Variance (One-Way)

Summary

Groups	*Sample size*	*Sum*	*Mean*	*Variance*
DM Normal brine	4	2.96555	0.74139	0.00075
DM Heme	4	3.01217	0.75304	0.00244
DM Heme + Cu2+	4	3.28285	0.82071	0.00017
DM Cu2+	4	3.31966	0.82991	0.00134
WM Normal brine	4	3.1182	0.77955	0.00153
WM Heme	4	3.2254	0.80635	0.00055
WM Heme + Cu2+	4	3.09756	0.77439	0.0013
WM Cu2+	4	3.08109	0.77027	0.00346
K DM Normal brine	4	3.44862	0.86216	0.00171
K DM Heme	4	3.34746	0.83686	0.00069
K DM Heme + Cu2+	4	3.42966	0.85742	0.00411
K DM Cu2+	4	3.53923	0.88481	0.00064
K WM Normal brine	4	3.5643	0.89108	0.00004
K WM Heme	4	3.50162	0.87541	0.00197
K WM Heme + Cu2+	4	3.2438	0.81095	0.00091
K WM Cu2+	4	3.54427	0.88607	0.00024

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*p-level*	*F crit*
Between Groups	0.14856	15	0.0099	7.24867	0.	2.1979
Within Groups	0.06558	48	0.00137			
Total	0.21414	63				

Table 25 Analysis of variance ANOVA of the WHC of brined cod samples

Analysis of Variance (One-Way)

Summary

Groups	*Sample size*	*Sum*	*Mean*	*Variance*
DM Normal brine	4	2.96555	0.74139	0.00075
DM Heme	4	3.01217	0.75304	0.00244
DM Heme + Cu2+	4	3.28285	0.82071	0.00017
DM Cu2+	4	3.31966	0.82991	0.00134
WM Normal brine	4	3.1182	0.77955	0.00153
WM Heme	4	3.2254	0.80635	0.00055
WM Heme + Cu2+	4	3.09756	0.77439	0.0013
WM Cu2+	4	3.08109	0.77027	0.00346

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*p-level*	*F crit*
Between Groups	0.02811	7	0.00402	2.78382	0.02866	3.02233
Within Groups	0.03463	24	0.00144			
Total	0.06274	31				

Table 26 Analysis of variance ANOVA of the WHC of brined cod samples

Analysis of Variance (One-Way)

Summary

Groups	*Sample size*	*Sum*	*Mean*	*Variance*
K DM Normal brine	4	3.44862	0.86216	0.00171
K DM Heme	4	3.34746	0.83686	0.00069
K DM Heme + Cu2+	4	3.42966	0.85742	0.00411
K DM Cu2+	4	3.53923	0.88481	0.00064
K WM Normal brine	4	3.5643	0.89108	0.00004
K WM Heme	4	3.50162	0.87541	0.00197
K WM Heme + Cu2+	4	3.2438	0.81095	0.00091
K WM Cu2+	4	3.54427	0.88607	0.00024

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*p-level*	*F crit*
Between Groups	0.0215	7	0.00307	2.38071	0.05338	3.02233
Within Groups	0.03096	24	0.00129			
Total	0.05245	31				

Appendix I. TBARS determination standard curve

Table 27 Absorbance of standard curve

standard					
rør #	nmol/ml	OD_{532}			average
0	0	0	0.001	-0.001	0
1	2	0.058	0.057	0.056	0.057
2	4	0.059	0.058	0.058	0.058
3	6	0.06	0.059	0.059	0.059
4	8	0.061	0.06	0.061	0.061
5	10	0.062	0.063	0.062	0.062

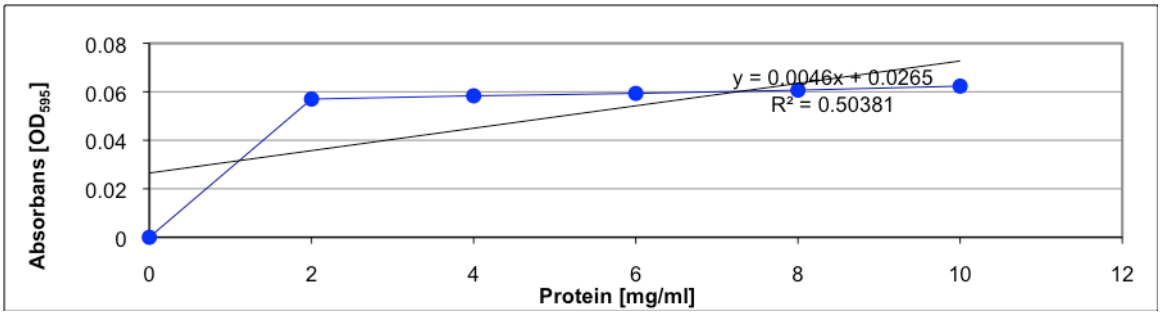

Figure 25 Standard curve for TBARS determination.

Appendix J. Analysis of Variance ANOVA for TBARS determination

Table 28 Analysis of variance ANOVA of the TBARSC of dark muscle of cod brined samples

Analysis of Variance (One-Way)

Summary	Dark muscle brined samples			
Groups	*Sample size*	*Sum*	*Mean*	*Variance*
DM Normal brine	6	36.895	6.14917	0.34844
DM Heme	6	44.61466	7.43578	0.08941
DM Heme + Cu2+	6	48.55648	8.09275	2.48993
DM Cu2+	6	50.00643	8.33441	0.74786

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*p-level*	*F crit*
Between Groups	17.25853	3	5.75284	6.26051	0.00359	4.1134
Within Groups	18.37821	20	0.91891			
Total	35.63675	23				

Table 29 Analysis of variance ANOVA of the TBARSC of white muscle of cod brined samples

Analysis of Variance (One-Way)

Summary	White muscle brined samples			
Groups	*Sample size*	*Sum*	*Mean*	*Variance*
WM Normal brine	6	24.93419	4.1557	0.13853
WM Heme	6	33.9691	5.66152	0.16338
WM Heme + Cu2+	6	36.5786	6.09643	0.21728
WM Cu2+	6	45.84554	7.64092	0.87063

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*p-level*	*F crit*
Between Groups	37.01009	3	12.3367	35.50597	0.	4.1134
Within Groups	6.94908	20	0.34745			
Total	43.95918	23				

Table 30 Analysis of variance ANOVA of the TBARSC of dark muscle of cod kench cured samples

Analysis of Variance (One-Way)

Summary	Dark muscle kench cured samples			
Groups	*Sample size*	*Sum*	*Mean*	*Variance*
K DM Normal brine	6	39.67082	6.6118	0.24358
K DM Heme	6	39.12541	6.5209	0.68896

K DM Heme + Cu2+	6	60.88983	10.1483	3.27214
K DM Cu2+	6	54.39621	9.06604	0.57915

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*p-level*	*F crit*
Between Groups	59.01811	3	19.6727	16.44932	0.00001	4.1134
Within Groups	23.91917	20	1.19596			
Total	82.93728	23				

Table 31 Analysis of variance ANOVA of the TBARS of white muscle of cod kench cured samples

Analysis of Variance (One-Way)

Summary White muscle kench cured samples

Groups	*Sample size*	*Sum*	*Mean*	*Variance*
K WM Normal brine	6	27.16567	4.52761	0.56812
K WM Heme	6	37.62421	6.2707	0.43578
K WM Heme + Cu2+	6	56.47255	9.41209	0.50317
K WM Cu2+	6	41.86496	6.97749	0.43681

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*p-level*	*F crit*
Between Groups	73.79038	3	24.59679	50.61402	0.	4.1134
Within Groups	9.71936	20	0.48597			
Total	83.50974	23				