



Research Article

Influence of Frozen Fish Muscle on Change of Quality

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Article History: Received: February 25, 2016 Revised: May 28, 2016 Accepted: June 26, 2016

ABSTRACT

Water in muscle is distributed throughout the tissue with approximately 90 % located intracellular and 10 % extra-cellular. The water is physically separated by cellular structures such as membranes, but as these are water permeable, the intra- and extra-cellular water can exchange, for example due to changes in osmotic pressure. Intra-cellular water or muscle water is thought to be distributed between different states or populations of water ('pools') characterized by differences in water mobility due to different degrees of binding or association to proteins. The quality of fish products after freezing and frozen storage is affected by factors such as fish species, temperature and handling before slaughter, slaughtering stress, the biological status of the fish, temperature of the pre-rigor storage, freezing rate, frozen storage temperature and time, temperature fluctuations, thawing procedure and prevention against oxidation (light and oxygen). If fish is frozen quickly, stored at low, non-fluctuating temperatures and thawed in the best way according to its rigor-state, the quality can be as good as or better than fresh fish stored for a few days at 0°C.

Key words: Muscle, Quality, Frozen, Protein

INTRODUCTION

Dehydration

Dehydration and the effect of inorganic salts the removal of water from solution as a result of ice crystal formation leads to dehydration of the cells (see section 4.4) and the intra-cellular protein molecules. The three-dimensional structure of proteins is stabilized by a network of hydrogen bonds and as many of these are water mediated, they will be disrupted when water is removed. This can result in an exposure of hydrophilic and hydrophobic regions which can interact with other exposed regions, either in the same or in adjacent proteins, resulting in aggregation (Sikorski, 1978; Shenouda, 1980). Increased solute concentration also affects protein denaturation and aggregation. Upon the freezing of fish muscle at -30 °C, potassium and sodium chlorides may form a solution of up to 7 % compared to about 0.5 % in the unfrozen muscle. These and other salt ions can interfere with secondary forces (electrostatic, van der Waals, hydrogen and hydrophobic) that stabilise the tertiary and quaternary structure of the proteins. At low ionic strength, many salts have a solubilising effect on proteins, but at higher ionic strength the inorganic salts compete for water with the hydrophilic groups. This may

result in a salting-out effect and decreased solubility of the protein (Sikorski *et al.*, 1976; Shenouda, 1980).

Unfrozen muscle water

Freezing is an effective way of preservation because the crystallisation of water results in a more concentrated solution and thus a lowering of the water activity in the food. Water associated with macromolecules, membranes and other ultra-structural elements in cells and tissues can, however, remain unfrozen at tens of degrees Celsius below the equilibrium freezing point of a bulk solution. Even in the presence of ice crystals, this water remains unfrozen due to a combination of the hydration effect, the presence of small solutes, very high viscosity and small dimensions between membranes and macromolecules (Wolfe *et al.*, 2002). It is therefore frequently named 'unfreezable' water, but in compliance with the recommendations of Franks (1986) and Wolfe *et al.* (2002), it is referred to as 'unfrozen water' throughout this thesis. The existence of an unfrozen water fraction in frozen foods is the main reason why chemical quality deteriorating processes occur in frozen foods. Several experimental values for the amount of unfrozen water in different muscle foods are reported in the literature: 9.5 % in cod muscle at -40 °C (Riedel, 1956), 11.0 % in haddock

muscle at -40 °C (Charm and Moody, 1966), 15.1 % in reindeer meat at -80 °C (Roos, 1986), 26.8 % in cod at -20 and -60 °C (Paper III), 31.0 % in fresh ground beef meat at -40 °C (Aktas *et al.*, 1997a) and 36.7 % in king fish at -90 °C (Sablani *et al.*, 2007). The relatively big differences between the reported values are probably due to differences between species and methodological differences.

Water binding, mobility and distribution

Water in muscle is distributed throughout the tissue with approximately 90 % located intracellular and 10 % extra-cellular (Schnepf, 1989). The water is physically separated by cellular structures such as membranes, but as these are water permeable, the intra- and extra-cellular water can exchange, for example due to changes in osmotic pressure. Intra-cellular water or muscle water is thought to be distributed between different states or populations of water ('pools') characterised by differences in water mobility due to different degrees of binding or association to proteins (Ruan and Chen, 1998). A small portion of the water molecules (less than 0.3 g water/100 g protein) are structurally bound to proteins and show a very different behaviour than that of bulk water (Schnepf, 1989; Isengard *et al.*, 2008). The main fraction of water interacts with proteins to different degrees and the physical retention of this water is dominated by the association with the myofibrillar structure (Schnepf, 1989). As described above the rotational mobility of water molecules is typically measured by relaxation NMR. Based on the relaxation curves, the number and size of different water pools can be determined by the use of different two- or three-way chemometric methods. Changes in the water distribution of a certain sample can indicate quality-related changes due to for example storage temperature or time (Jepsen *et al.*, 1999; Jensen *et al.*, 2002). In porcine meat, three different water pools have been identified, the fastest relaxing reflecting water tightly associated with macromolecules, the intermediate reflecting water located within highly organized protein structures and the slowest relaxing reflecting the extra-myofibrillar water containing the sarcoplasmic protein fraction (Bertram *et al.*, 2001). In fresh and pre-frozen cod, respectively, two to three and three (intact, minced and centrifuged cod) and four (minced cod) different water pools have been identified, the number depending on storage conditions (Andersen and Rinnan, 2002; Jensen *et al.*, 2002; Andersen and Jorgensen, 2004).

Frozen storage

Frozen storage offers a means of preserving fish; however, during frozen storage, quality is lost due to a deterioration of texture, flavour and colour, especially after long periods of storage, when poor freezing practices are employed or when the initial fish quality is low. The main problem is the change of texture which reduces consumer acceptability (Sikorski *et al.*, 1976; Shenouda, 1980). Texture changes are the result of denaturation of muscle proteins, particularly those in the myofibrillar fraction (Haard, 1992). For these reasons, different methods of measuring protein denaturation have been used to follow textural deterioration. Among them, protein solubility is one of the most often chosen, because of its

simplicity and relatively good correlations with textural characteristics (Shenouda, 1980; De Koning & Mol, 1991).

Quality

The quality of fish products after freezing and frozen storage is affected by factors such as fish species, temperature and handling before slaughter, slaughtering stress, the biological status of the fish, temperature of the pre-rigor storage, freezing rate, frozen storage temperature and time, temperature fluctuations, thawing procedure and prevention against oxidation (light and oxygen) (Sorensen *et al.*, 1995; Sigholt *et al.*, 1997; Erikson *et al.*, 1997; Kristoffersen *et al.*, 2006; Nielsen and Jessen, 2007). If fish is frozen quickly, stored at low, non-fluctuating temperatures and thawed in the best way according to its rigor-state, the quality can be as good as or better than fresh fish stored for a few days at 0 °C (Cappeln *et al.*, 1999). For optimally handled cod, the quality remains as high as for fresh cod for one month at -30 °C. The fish is still suitable for consumption after one year, though the characteristic frozen storage flavour starts to develop after approximately three months. Fatty species, such as trout, are stable during frozen storage and are still suitable for consumption after 18 months, though only of high quality up to six months. Due to a high content of poly unsaturated fatty acids (PUFA), fatty species are susceptible to lipid oxidation, which results in rancid taste and odour, if not packed in an oxygen-free atmosphere (Nielsen and Jessen, 2007). In lean fish, protein denaturation causes textural and functional changes in the fish muscle, whereas oxidative lipid degradation results in the characteristic cold-store flavour.

Factors affecting protein changes

Several factors are of importance in relation to the protein changes occurring during frozen storage: ice crystal formation, dehydration, increased concentration of salts in the unfrozen water pools, changes in lipids and fatty acids, lipid oxidation, enzymatic breakdown of trimethylamine oxide (TMAO) and interactions between these factors. Protein denaturation in frozen muscle has been reviewed by several authors (Love, 1970; Sikorski *et al.*, 1976; Shenouda, 1980; Mackie, 1993).

Denaturation of muscle proteins

Denaturation of muscle proteins has been attributed to different factors, fish species being one of them. Basically, fish species can be divided into two groups based on the possession or not of trimethylamine oxide demethylase (TMAOase), which degrades trimethylamine oxide (TMAO) to dimethylamine (DMA) and formaldehyde (FA) during iced and/or frozen storage. The enzyme is mostly found in the viscera and red muscle of fishes belonging to gadoid species (Mackie & Thomson, 1974; Hebard *et al.*, 1982; Haard, 1992). During frozen storage, a strong relationship between the production and accumulation of FA and the deterioration of texture in muscle of this group of fish has been found (Dingle *et al.*, 1977; Gill *et al.*, 1979; Matthews *et al.*, 1980; Parkin and Hultin, 1982a; Kelleher *et al.*, 1981; Jahncke *et al.*, 1992). Although FA might react with proteins during frozen storage, it is still not clear how FA accelerates protein

denaturation (Ang & Hultin, 1989). Hake, fish belonging to the Order Gadiformes, are gaining importance as fisheries, as cod are increasingly being depleted (Morrow, 1992). These species represent the main high-quality white-fleshed fish in some European countries, such as Spain (Whitaker, 1980), where they are very often sold in the frozen state. It is also an important source of derived frozen products. There are some data on storage properties of frozen South Atlantic hake, *Merluccius hubbsi* and *M. gayi* (Almandos *et al.*, 1984; Ciarlo *et al.*, 1985) *M. bilinearis* (Hiltz *et al.*, 1976; Licciardello *et al.*, 1980), *M. productus* (Crawford *et al.*, 1979) and *M. capensis* (De Koning & Mol, 1991). Also, it has been reported that there are changes in some sensory and chemical parameters in European hake (*Merluccius merluccius*) during ice and frozen storage (Pérez-Villareal & Howgate, 1987, 1991). However, there are no data on changes in muscle protein solubility of frozen stored European hake.

Changes in functional properties caused by protein changes

Freezing and frozen storage may, as described above, result in denaturation and aggregation of especially myofibrillar proteins resulting in products with reduced WHC and increased drip loss upon thawing causing a hard, dry and fibrous fish product with altered colour and reduced juiciness (Sikorski *et al.*, 1976; Shenouda, 1980; Barroso *et al.*, 1998). The main changes are reported to occur in myosin light-chain, but actin and actinin also degrade during frozen storage (Careche *et al.*, 1998; Saeed *et al.*, 1999; Saeed and Howell, 1999; Kiran Jasra *et al.*, 2001; Badii and Howell, 2002b; Schubring, 2005; Kjaersgard *et al.*, 2006b). Some of the changes reported are increases in β -sheet at the expense of α -helix structure (Herrero *et al.*, 2004). As the main part of muscle water is located within the myofibrillar structure, changes in this typically result in reduced WHC. Numerous studies have shown a relationship between decrease in protein extractability and increased toughness of fish. Protein solubility and extractability are often used to characterize the degree of protein denaturation during frozen storage. Increased protein aggregation results first in an increased protein insolubility in salt solutions and thereafter in unextractability in sodium dodecyl sulphate (SDS) and SDS plus β -mercaptoethanol. Storage temperature and time have great impact on the degree of protein denaturation and many authors have shown a relation between storage temperature, time as well as a combined effect and degree of protein denaturation or muscle toughness. Protein changes or changes in texture are reported to be higher after storage at -10 to -20 °C compared to -30 °C, temperatures below -30 °C are less studied (Licciardello *et al.*, 1982; Chapman *et al.*, 1993; Herrero *et al.*, 2004).

MATERIALS AND METHODS

This article is review and the aims of quality-related changes in frozen fish muscle. The experiment 1 was conducted by Hulya and Kayisoglu (2008). Whiting, gray mullet and anchovy fish which were caught in the Marmara Sea were used in this study. The length and weight of fish species were determined during the

pretreatments. The length of whiting was found between 24-28 cm, gray mullet was 25-33 cm, and anchovy was 11-15 cm. The weights of the fish species were between 113-158 g, 157-188 g, and 11-16 g respectively (Hulya and Kayisoglu 2008). Mean oil content of fish species were 2.06% for anchovy, 0.85% for gray mullet and 0.56% for whiting. The fish were processed immediately after catching, placed in crushed ice and stored in plastic boxes and transferred to the laboratory for freezing. Each fish species was divided in to three lots, one lot was for the whole (W), the second lot was for gutted (G), and the third lot was for fillet. Fish lots were frozen in blast freezer (Armfield – Blast and Fluid Bed Freezer) at -40°C. They were then packed into plastic refrigerator bags and stored at -26 ±2°C in freezer (Williams HS 1 BCBF) (Hulya and Kayisoglu 2008). For each specified time, two bags from each treatment were removed for examination. Cooking test was applied on sensorial analysis of chilled fish. In sensorial analysis a scale of 0-3 were used to determine for color, smell, muscle, structure (chewing) and taste of fish. Determination of pH values in all three fish species was carried out by using Hanna 8014 model pH meter (Hulya and Kayisoglu 2008). Indication of total Volatile Base Nitrogen (TVB-N) content was determined and evaluated as described by kjeldahl distillation mechanism. The method was based on water vapor distillation and separation of volatile base. Fixing of first amine compound, Trimethylamine (TMA) values, which are the results of changes seen in fish, were determined by following the procedure explained (Hulya and Kayisoglu 2008). The indication principle of thiobarbituric acid value (TBA) was based on malonaldehyde which is the result of dissatisfied fatty acid oxidation, and red colour which is the result of heating by thiobarbituric acid. This was determined by using kjeldahl distillation system and spectrophotometer (Hitachi UV/Vis). Peroxide value (PV) indication was done by audiometric Wheeler method. For determination of PV value of fish, firstly oil of fish was obtained according to method and mean oil contents were found from calculation of these values (Hulya and Kayisoglu 2008).

The experiment 1 was conducted by Gang (2013). This project was undertaken in two parts as shown in Figure 1. The first part (A) was to map ambient temperature profile and fluctuation in different fish processing areas and in fillets of two fish species: saithe and redfish. The second part (B) entailed evaluation of quality changes of fresh fillets as influenced by holding time before packaging. Two experiments (I and II) were conducted in part B. In experiment I, the drip loss and temperature of fish fillets during holding time (up to 2.5 hours) at different temperatures (10±2°C, 16±2°C, 22±2°C) were evaluated. Two different species, saithe and redfish fillets were used. The fillets varied size and weight as illustrated in Figure 1. In experiment II, only saithe fillets were used. After holding different times (0, 1, 2 hours), the fillets were packed and transported for evaluation of quality and shelf life changes during chilled storage (2±2°C). Drip loss, cooking yield, colour, texture, sensory, microbiological, and pH changes were followed for 13 days. The raw material used was saithe (*Pollachius virens*) and deep sea redfish (*Sebastes mentella*) caught by a trawler at 63.12°N, 24.34°W, on 8-9 February 2014.

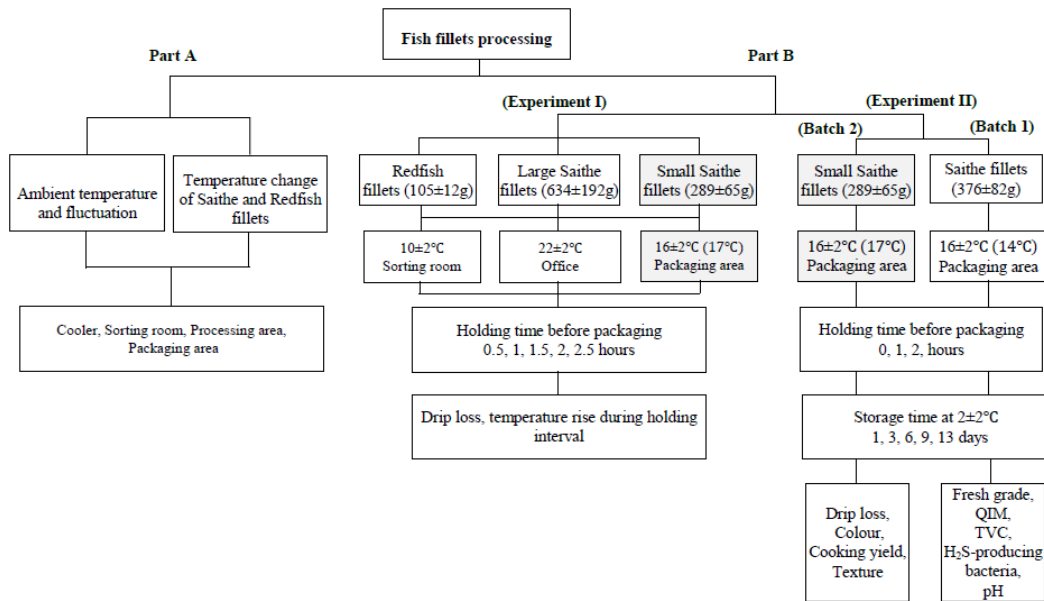
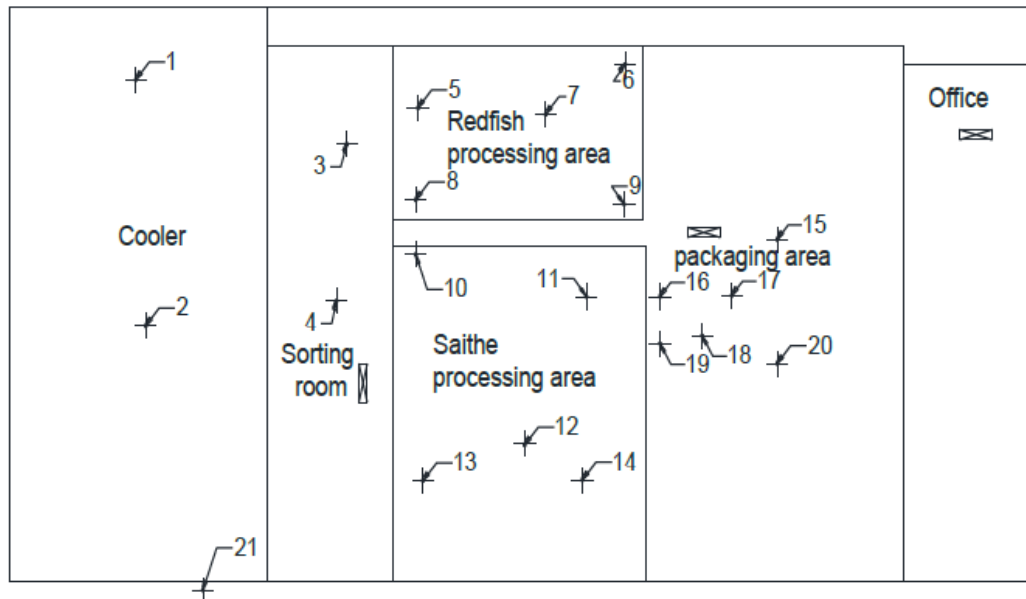


Fig. 1: Experimental design diagram of project



1: Inner cooler, 2: outer cooler, 3: redfish sorting, 4: saithe sorting, 5: redfish beheading, 6, 7: redfish filleting, 8: redfish trimming, 9: redfish pre-chilling, 10: saithe beheading, 11, 12: saithe filleting, 13, 14: saithe trimming, 15: saithe loin packaging, 16, 17, 18, 19: redfish packaging, 20: saithe loin packaging, 21: outside of plant

Fig. 2: Location of loggers (No.1-21) in the fish processing plant, for monitoring of ambient temperature in different areas.

Only saithe was bled and gutted onboard and stored with slurry before processing, while the redfish were stored whole in crushed plate ice. The fish was processed on 13 February 2014 in an Icelandic fish processing plant. Additional saithe (batch 1) was caught using trawler at 63.13°N, 24.98°W, on 16 January 2014. Before processing (21 January 2014), the saithe was stored in slurry ice 5 days. Whole fillets without skin were collected from filleting and skinning machine in the fish processing plant, marked with numbered plastic tags. In the experiment I, the three type of fillets collected on 13 February 2014 were large saithe fillets (634±192 g), small saithe fillets (289±65 g) and redfish fillets (105±12 g). After temperature and weigh determination, six fillets (n=6) of each trial group

were placed in a plastic box and placed in different temperature areas (sorting room, 10±2°C, packaging area, 16±2°C, office, 22±2°C) for holding time up to 2.5 hours. Temperature loggers were inserted inside saithe loins. The redfish fillets were thinner, therefore the loggers were put below the fillets in each box to monitor temperature changes during the holding time. Ambient temperature and relative humidity during experiment I was measured with HoBo U12 temperature and relative humidity loggers from Onset Computer Corporation (Bourne, MA, USA). Weight of each fillet was measured again for drip loss every 30 minutes.

In experiment II, two batches of saithe fillets processed on different dates (21 January 2014 and 13

February 2014) were collected. The sample were split into three trail groups with holding time of 0, 1, 2 hours before packaging in plant, respectively. After holding time, the samples were weighed and packed, then put into expanded polystyrene box (400×264.5×135mm). Every box contained 6 fillets with an ice mat (120 g) on top and an adsorbing pad at the bottom. The boxes were transported to the laboratory (Matís ohf in Reykjavik) within 30 min. The boxes were stored in a cold storage room at $2\pm 2^{\circ}\text{C}$ sampled on days 1, 3, 6, 9 and 13. Ambient and fillets temperature during storage was measured with temperature loggers (IButton DS1922L, USA). The procedure for each batch is described in following paragraphs. Twenty one loggers were hung from the ceiling of plant, 2-2.5m from floor (Figure 2). The mapping ambient temperature at different fish processing areas for about 72 hours, with 10 minutes sampling intervals, i.e. of the cooler (used for storage of raw material), sorting room (used for size grading and gutting of fish before fillets processing), processing and packaging area of both species, as well as outside of plant. The temperature loggers (IButton DS1922L, USA) had an accuracy of $\pm 0.0625^{\circ}\text{C}$ from -10°C to 65°C . The temperatures of fish were determined at different processing stage, the measure point and processing flow of redfish and saithe is shown in Figure 3. The handheld thermometer (Testo 926, Germany) with an accuracy of $\pm 0.3^{\circ}\text{C}$ from -50 to $+400^{\circ}\text{C}$ were used to measure whole fish and fillets. Three whole redfish and saithe were sampled respectively from top of the tub, where the fish stored with crushed ice and slurry ice in the cooler. The thin sharpened probe of thermometer was inserted from the side of dorsal fin into fish flesh, with at least 75 to 100 mm depth. When fillets ($n = 3-5$) were measured, the temperature-sensitive element at the end of the probe was inserted from side into the thickest part of fillets. In experiment I (at plant), drip loss (%) was determined as the weight loss of fillets (large saithe, small saithe and redfish) over holding time at $10\pm 2^{\circ}\text{C}$, $16\pm 2^{\circ}\text{C}$ and $22\pm 2^{\circ}\text{C}$. The fillets ($n = 6$) of each trial group, were weighed directly after filleting and skinning. The fillets were again at 0.5 hour holding intervals until 2.5 hours. In the experiment II (during storage), drip loss (%) was determined as the weight loss during storage time (up to 13 days). The fillets ($n = 6$) were weighed before packaging (after the holding time) and after each storage period. The difference in weight (g) was divided by the initial weight of the product (g) and expressed as g/g%.

RESULTS AND DISCUSSION

In the experiment I was conducted by Hulya and Kayisoglu (2008). Changes in sensory evaluation of storage fish species at -26°C were given in Table 1. Throughout the storage period there were decreases and significant changes ($P<0.05$) in all sensorial criteria. Average values showed that highest decreases were in smell (2.41) and then taste (2.48) among sensorial criteria. Colour quality (2.54) was found to be higher than the other quality criteria (Table 1) (Hulya and Kayisoglu (2008). Namulema *et al.* [1990] also reported that there was no significant difference in texture, taste, appearance and overall acceptability, but off-flavours developed

during storage period in the frozen Nile perch at -27°C for a period of 12 weeks. In our research, color, smell and taste criteria of whiting were determined higher than other species. However maximum muscle and structure value were found in gray mullet (Hulya and Kayisoglu (2008).. Anchovy was showed to be significant decline all sensorial criteria during the time of frozen storage ($p < 0.05$). According to treatments that were carried out at fish species, maximum mean colour value was found in fillet while maximum smell and muscle values found in gutted fish (Table 1). Lakshmanan *et al.* [1990] reported that from 36 to 44 weeks frozen samples whole and fillet were still acceptable quality but showed loss of characteristic flavours and texture in rock cod (*Epiplatys* spp.) at -20°C . Our study showed similar results and end of ninth month treatments were acceptable quality, but there was significant decline ($P>0.05$) in all sensorial quality (Hulya and Kayisoglu (2008). Tokur *et al.* [2006] reported that the scores did not exceed acceptable levels of filleted Trout (*Oncorhynchus mykiss*) during frozen storage (-18°C) for 12 month. Differences may be result from differences between fish species. Köse *et al.* [2001] determined that acceptable quality of anchovy only lasted three months of frozen storage at -18°C . This study important because it shows that decline of storage temperature provides extent of storage time of fish. According to storage period, a decline was observed in the pH values of fish species. Differences between the species and treatments were significant ($p < 0.05$) in terms of pH value (Hulya and Kayisoglu (2008). Suarez *et al.* [2002] reported that storage time caused significant differences in pH value of frozen sardines (*Sardinella aurita*). Simeonidou *et al.* [1997] were found that the pH values significantly different in frozen whole and fillet of horse mackerel (*Trachurus trachurus*) and similar with us. The highest pH value was obtained in whiting comparing with other species. However, when the treatments were compared, the highest pH value was found in fillets (6.56) and the lowest pH (6.50) value was found gutted fish (Table 2, Fig. 2). Olgunoglu *et al.* [2002] found that pH value increased from 6.80 to 7.02 in frozen pike perch fillets at -20°C throughout 7 months, however, Lakshmanan *et al.* (1990) reported that there was a decline in pH after 36 weeks in whole, gutted and fillet frozen rock cod (*Epinephelus* spp.) at -20°C . Catfish muscle pH after frozen storage for various times was not significantly different from fresh muscle pH [Eun *et al.* 1994].

In our research, a slightly decrease was observed in fish species throughout 9 months storage. Similarly, Tokur *et al.* [2006] found that the pH value of filleted Trout decreased significantly during frozen storage ($p < 0.05$). TVB-N is used for determination of the spoilage level of fish during the storage period (Oehlenschläger 1981). The level of 35 mg/100 g has been considered the upper limit, above which fishery products are considered spoiled (Ludoff and Meyer 1973, Schormüller 1969). TVB-N values of three fish species increased during the storage period. According to the TVB-N values, the differences between the fish species were significantly important ($P<0.05$) whereas treatments did not show significant differences. During the storage period, according to TVB-N value whiting kept its freshness better than gray mullet and anchovy (Table 2). The lowest

Table 1: Comparison of three fish species in terms of sensory evaluation

Criteria	Fish species	T	Storage period, month						Means	
			0	2	4	7	9	mean	mean	mean
1 Colour	whiting	3	4	5	6	7	8	9	10	11
		W	3.00	2.91	2.70	2.58	2.09	2.66 a	2.58 a	Whole: 2.53 b
		G	3.00	2.76	2.71	2.32	2.06	2.57 d	-	Gutted: 2.51 c
		F	3.00	2.96	2.74	2.14	2.01	2.57 d	-	Fillet: 2.59 a
		W	3.00	3.00	2.34	2.26	2.01	2.52 e	-	-
		G	3.00	3.00	2.50	2.13	1.98	2.52	-	-

LSD values (P<0.05), storage period: 0.001, species: 0.001, treatment: 0.002.

Table 2: Changes in pH, TVB-N, TMA, TBA and peroxide values of three fish species during

Criteria	Fish species	T	Storage period, month						Means	
			0	2	4	7	9	mean	mean	mean
1 pH	whiting	3	4	5	6	7	8	9	10	11
		W	6.94	6.93	6.76	6.81	6.85	6.86 a	-	Whole: 6.52 b
		G	6.94	6.70	6.71	6.82	6.92	6.83 b	-	Gutted: 6.50 c
		F	6.94	6.80	6.73	6.77	6.83	6.80 c	-	Fillet: 6.56 a
		W	6.49	6.49	6.24	6.34	6.28	6.37	6.40 b	-
		G	6.48	6.22	6.32	6.76	6.27	6.33 h	-	-

LSD values (p < 0.05), storage period: 0.09, species: 0.007, treatment: 0.007

TVB-N value. (17.23 mg/100 g) was determined in whiting, the highest value in anchovy (22.55 mg/100 g). Anchovy showed more increase than the other species at 9th month of storage period. According to TVB-N value, while whiting and gray mullet were very good quality, anchovy was within acceptable limit end of storage period (Hulya and Kayisoglu (2008). Pons-Sanchez- -Cascado et al. [2005] determined the levels of TVBN values for fresh anchovies ranging from 3.79 \pm 0.30 mg/100 g to 13.7 \pm 0.46 mg/100 g and lower our findings. Lakshmanan *et al.* (1990) found that after 52 weeks, TVB-N values (18.2 mg/100 g) in fillet rock cod (*Epinephelus* spp.) frozen at -20°C were the lowest from the all frozen fish (29.4 mg/100 g). Our results were similar with findings of Simeonidou *et al.* [1997]. Kemal *et al.* (1996) reported that TVB-N increased from 5.60 to 27.20 mg/100 g in frozen hilsa fish at -20°C throughout 75 days. The other researchers stated that TVB- -N value was 4.19 at the beginning but increased to 14.90 at the end of 7th month of storage in pike fish at -18°C (Olgunoglu et al. 2002). However, in this study, a relatively smaller increase (from 15.81 mg/100 g average value to 24.83 mg/100 g) determined as to this finding. This lower increasing in TVB-N value may result from lower storage temperature (Hulya and Kayisoglu, 2008).

In the experiment 2 was conducted by Gang (2013). Temperature mapping of the fish processing plant was performed on 16 January 2014, for 9 hours. The ambient temperature profiles of cooler (used for storage of raw material) and sorting room (used for size grading and gutting of fish before fillets processing) are shown in Figure 5. The temperature of coolers was between 3°C and 5°C. Meanwhile, inhomogeneous temperature distribution also was observed in inner and outer of cooler (Gang , 2013). The raw fish can kept in this temperature 0-5 days before processing, according to the investigation. The range of temperature fluctuation was larger in the sorting room compared to cooler, or 7 to 11°C. The temperature also tended to increase more during the day in the sorting room. The temperature in the processing areas fluctuated within the range of 13 to 18°C. It was 15-17°C in the redfish processing area. The highest temperature (17°C) occurred at the position near the beheading

machine. Temperatures of other position of redfish filleting was similar for all loggers (Gang , 2013). Compared with redfish processing area, the average temperature of saithe processing was lower. The ambient temperature of saithe processing areas fluctuated within the range of 13 to 17°C. Maximum and minimum temperature in this area appeared in position of trimming (loggers No 14 and 13). Lower temperature of No 13 was observed probably due to closed to sorting room, where the temperature was lower. The time of wide fluctuation was about 8:30 am, 10:15 and 13:20. The maximum amount of fluctuation was more than 2°C (No 13) in the trimming area for saithe. The highest temperature (18.7°C, No 15) was measured within the packaging area as shown in Figure 8. The average temperature of this area was about 17°C. Maximum temperature difference between the different measurements positions was more than 3°C. The fluctuation range of each position was around 2°C. The time of temperature peaks (approximately 10:15 and 13:15) was similar to what was observed in the processing area. Redfish and saithe temperature before and at different processing stages, are shown in Figure 9 and 10 respectively. The temperature of raw material (cold storage) was close to 0°C, for both species. Larger standard deviation was observed for saithe temperature than redfish. This was due to differences in cooling medium (slurry for saithe and crush ice for redfish) between the species and position of fish in the tub (Gang, 2013).

The temperature of saithe after beheading was similar to the temperature in raw material. During filleting and skinning of both fish, the temperature started to increase. It increased by 1°C for saithe and 2 °C for redfish. Higher temperature rises in redfish fillets was found probably due to smaller size of redfish fillets compared to saithe and led to heat transfer faster. The higher standard deviation in temperature of redfish fillets was presumably caused by inequality in fillet size. After filleting, the redfish was chilled by liquid ice, resulting in lower temperature until packaging. However, the temperature of saithe fillets started to increase after trimming due to high environmental temperatures, and no pre-cooling was applied order to maintain low temperature like redfish, as mentioned above (Figure 3). The average temperature of

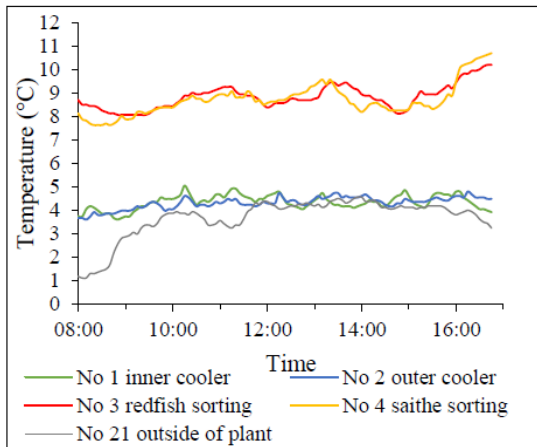


Fig. 3: Ambient temperature profiles for coolers (storage of raw material), sorting room (size grading and gutting) and outside of the plant (Gang, 2013).

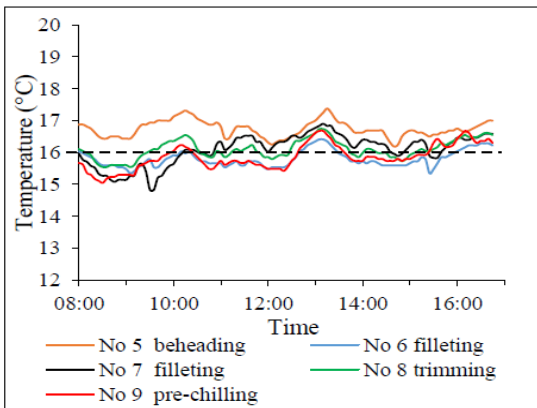


Fig. 4: Ambient temperature profiles for redfish processing areas (beheading, filleting, trimming and pre-chilling with liquid ice) (Gang, 2013).

saithe loins at packaging was up to 2.5°C. The temperature of some loins was even higher than 3°C, the maximum temperature measured was 3.8°C. The temperature changes during holding of large saithe fillets, which were placed on different temperature area are shown in Figure. The temperature rise was fast during the first 2 hours. Higher ambient temperature led to faster temperature increases of fillets (Gang, 2013). When the fillets were kept at 10±2°C, the temperature of fillets increased to ambient temperature from 2°C. The average increase was 3°C per/ hour (°Ch-1). The rate of temperature changes reached 5°C/h-1 for fillets held at 16±2°C and 22±2°C. After 1.5 hours holding, the temperature of fillets held at 10±2°C, became relatively steady for the rest of the holding time. On the other hand, the temperature of fillets kept at higher ambient temperature (16±2°C, 22±2°C) kept increasing, up to final measurement.

As shown in, there was an equal increase in temperature (5.5-6°C) of three kinds of fillets, during first one hour holding at 16±2°C. However, it was observed that the redfish fillets had a higher reading (14.6°C) after the first hour, than large (11°C) and small (12°C) saithe fillets. For the remaining holding time, the temperature of

fillets equilibrated with ambient temperature. This means that if fillets are exposed in packaging area for 1-1.5 hours without any heat protection, the temperature of fillets will close to ambient temperature. The drip loss increased more rapidly in redfish fillets compared to small and large saithe, during the 1 to 1.5 hour time frame (Gang, 2013). When comparing the different fillet types at each holding condition, a significant difference ($p < 0.01$) between fillets all groups held in at 22±2°C and 16±2°C, was observed. After 2.5 hours at 22±2°C, the drip loss of redfish, small and large saithe fillets reached 7.13%, 4.8% and 3.3%, respectively. The drip of redfish almost kept at an even speed from beginning, whereas both size groups of saithe fillets started to lose weight after 1 hour. Unlike the fillets kept in 22±2°C, the process of drip loss at 16±2°C had higher homogeneity for the three fillet types. Also, fillets began to lose weight more rapidly after longer holding time (1.5 hours vs. 1 hours at 22±2°C). The maximum drip of redfish and small saithe was 4.7% and 3.4% after 2.5 hours held in packaging area. Drip at low environmental temperatures (10±2°C) accelerated after 0.5 hours. Drip rate of redfish was faster than of saithe ($P < 0.05$) (Gang, 2013).

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