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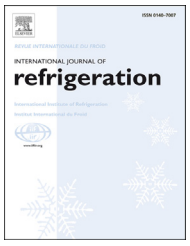

Highlights

- Alteration in the quality of fish was studied for 3 different temperatures.
- The influence of packaging materials on fish quality was also analysed.
- The current study helps to find the optimal storage conditions for Salmon.

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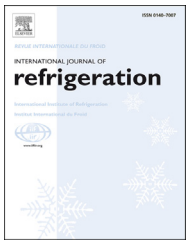

The influence of long-term storage, temperature and type of packaging materials on the quality characteristics of frozen farmed Atlantic Salmon (Salmo Salar)

Q2 E. Indergård [a], I. Tolstorebrov [b,*], H. Larsen [c], T.M. Eikevik [b]

[a] SINTEF Energy Research, Kolbjørn Hejes v 1D, Trondheim 7491, Norway
[b] Norwegian University of Science and Technology, Kolbjørn Hejes v 1B, Trondheim 7491, Norway
[c] NOFIMA, PB 210, N-1431 Aas, Norway

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ABSTRACT

The variations of biochemical, structural, sensory and microbiological characteristics of salmon were examined during the long-term frozen storage at −25 °C, −45 °C and −60 °C. The effects of four different types of packaging materials were studied as well. Lipid oxidation was measured by peroxide value (PV) and thiobarbituric acid reactive substances (TBARS). After 1 year of storage at −25 °C, the concentration of PV in red and white fish muscles increased from 1.26 to 1.82, and from 1.08 to 1.76, respectively. Formation of TBARS was higher in the red muscles than in the white, and reached a value of 14.04 (mg malondialdehyde (MDA) kg^{-1} of fish) after 1 year of storage at −25 °C. Decreasing the temperature to −45 °C inhibited PV and TBARS formation, but the use of the best packaging materials gave equally good results at −25 °C.

The concentrations of oxidation products were quite low for the storage conditions examined, which was reflected by the sensory analyses. The sensory analyses showed that salmon stored for 1 year at −25 °C maintained a level of quality comparable with fresh salmon.

The colour alteration was affected by the storage time. The storage at −60 °C reduced drip loss (2.0% from total mass) when compared with those of the higher storage temperatures, but other quality improvements were not significant.

* *Corresponding author*. Tel.: +47 96749192; fax: +47 73593580.
E-mail addresses: ignat.tolstorebrov@ntnu.no, i.tolstorebrov@gmail.com (I. Tolstorebrov).

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Nomenclature

CFU	Colony forming units
CVM	Computer vision method
HQL	High quality life
FFA	Free fatty acids
MDA	Malondialdehyde
PV	Peroxide value, meq of O_2 kg^{-1} of fat
TBARS	Thiobarbituric acid reactive substances, mg of malondialdehyde kg^{-1} of fish
ULT	Ultra-low temperature
a*	Redness, points
b*	Yellowness, points
h*	Hue, °
L*	Lightness, points

1. Introduction

The storage temperatures between −18 °C and −25 °C extend the shelf-life of fatty fish from 5 to 9 months (Bøgh-Sørensen, 2006). An optimal prolongation of the shelf-life[1] is considered to be an important goal of salmon freezing. This can be advantageous in allowing a more flexible selling strategy, especially for salmon, as market prices fluctuate during the year.

It is quite obvious that lowering the temperature increases fish stability. Previous studies showed that lowering the temperature from −22 °C to −40 °C significantly improved the quality and shelf life of fatty pelagic fish and salmon (Haugland, 2002). The temperature of −40 °C is critical because all freezable (free) water is frozen below this point, and only unfreezable (bounded) water remains in the system (Bøgh-Sørensen, 2006). Thus, the frozen storage of fish below −40 °C can be characterized as ultra-low temperature (ULT) storage. The application of ULT in the freezing of food and storage reduces the molecular mobility of water in the system and inhibits biochemical reaction (Champion et al., 2000). It was validated that ULT increases the shelf life of frozen salmon fillets in comparisons with the shelf-life in the range between −18 °C and −25 °C (Magnussen and Johansen, 1995). Some studies also reported the appearance of a glass transition at ULT, which indicates the best stability of frozen food. For different fish species, the glass transition occurs between −54.2 °C and −83.1 °C (Inoue and Ishikawa, 1997; Orlien et al., 2002; Rahman, 2009; Rahman et al., 2003; Sablani et al., 2007; Shi et al., 2009).

The best preservation temperature for fish can vary. For example, the optimal preservation of tuna was achieved at temperatures between −60 °C and −70° (Chow et al., 2004; Chow, 1988). But the preferred storage temperature for a high quality frozen product is far above that for other fish species. For example, for cod it is −40 °C, and for salmon fillets it is in the range between −45 °C and −60 °C (Magnussen and Johansen, 1995; Mørkøre and Lilleholt, 2007).

The main reason for the deterioration of quality in fatty fish is the oxidation of lipids. It causes changes in taste, colour, texture/structure, and nutritional value, and also leads to the formation of toxic compounds (Hansen et al., 2004). The oxidation rate of fat in fish relies on Product/Process/Packing factors, which include: the initial fish composition (including FFA content), rigour state of fish, handling, type and rate of freezing, type of packaging material, and storage temperature (Aubourg et al., 2002; Brendeng et al., 1991; Hyldig et al., 2012). Because of these reasons, studies devoted to the stability of salmon and other fatty fish vary significantly.

For example, "post-rigour" salmon (stored at −60 °C) showed lower PV and FFA concentrations during long-term storage experiments (8 meq O_2 kg^{-1} of fat and 1.4% oleic acid after 8 month) than fish stored at −30 °C (14.1 meq O_2 kg^{-1} of and 2.2% oleic acid after 8 months). But an organoleptic comparison gave a small difference between the two storage conditions (Gormley et al., 2002). On the other hand, a study of salmon stored from −13 °C to −35 °C showed the appearance of fish oil flavour after 6 months, irrespective of the fat content and storage temperature (Andersen and Steinsholt, 1992).

There are two main types of lipid oxidation in food systems: enzymatic and non-enzymatic oxidation. The first can occur mostly at the site of the oil–water interface, where an enzyme's active center is moving towards the fat droplet, micelle or membrane. The second type is induced by the presence of oxygen (Brockerhoff, 1974; St. Angelo et al., 1996). The accumulation of free fatty acids (FFA) during the frozen storage of fish allows us to conclude that enzymes remain active both at standard and ULT freezing temperatures (Aubourg et al., 2007; Aubourg and Medina, 1999; Gormley et al., 2002; Rodríguez et al., 2007). In such cases, when the concentration of the substrate for oxidation is high, the availability of oxygen in the package will strongly affect the oxidation of the fatty products during frozen storage. The concentration of oxygen is controlled by the barrier properties of the packaging material and by the packaging method. Thus, vacuum packaging improved the sensory scores of silver salmon steaks stored at −18 °C (Yu et al., 1973). A previous study on frozen herring stored at −18 °C showed that the herring vacuum-packaged in film, with a low oxygen barrier, had a shelf-life of 10 months, while the herring packaged in films with a high oxygen barrier had a shelf-life of 15 months (unpublished results). The quality of the herring was analyzed by chemical (TBARS) and sensory analyses.

A sensory evaluation of the vacuum-packaged salmon stored at −18 °C and −60 °C concluded that the HQL[2] (High

[1] The "Shelf-life of fish" is more often used as an informal abbreviation for the Practical Storage Life, when fish is acceptable for consumption and retains its characteristic properties Bøgh-Sørensen, 2006. Recommendations for the Processing and Handling of Frozen Foods, 4th ed. International Institute of refrigeration, Paris, France.

[2] HQL is defined as the time between freezing of the initially high quality product and the moment when, by sensory assessment, a statistically significant difference (often $P < 0.05$) from the initial high quality (immediately after freezing) can be established (Bøgh-Sørensen, 2006).

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Quality Storage Life) for salmon fillets is between 90 and 120 days at −18 °C, and between 160 and 230 days stored at −60 °C (Brendeng et al., 1991).

It is apparent that the ULT storage of fish requires larger investments and higher running costs than traditional frozen storage, thus industry has obvious problems applying ULT to fish, with the exception of Bluefin Tuna. But the proper combination of storage temperatures and packaging materials can improve the long-term frozen storage of salmon.

The scope of the actual study is devoted to the investigation of safe and economically efficient ways for maintaining an acceptable quality of frozen salmon during long-term storage. The development of some quality parameters of pre-rigour gutted Atlantic Salmon (Salmo Salar) was investigated during long-term frozen storage at the traditionally applied temperature of −25 °C, and two ULT temperatures (−45 °C and −60 °C). Four packaging materials with different oxygen permeability were in the current study. Hence, the relationship between the product's quality and combinations of both temperature and packaging materials were investigated. The storage temperature of −25 °C and open LDPE bags, which are widely used in salmon freezing, were considered to be a reference experiment.

2. Materials and methods

2.1. Raw material

A total of 150 pieces of frozen farmed salmon (Salmo Salar) (from 5 to 6 kg weight each) were delivered by "Nova Sea AS" Lovund, Norway. The pre-rigor gutted salmon were frozen in a blast freezer (freezing temperature −40 ± 1 °C), glazed and packed at the factory. The temperature in the middle of the fish bodies was −25 ± 1 °C. The tails were removed before packaging. The packaged samples were stored at −25 ± 1 °C at the production site, and then were delivered by ordinary frozen transportation to the research institute 5 days after the processing. Additional studies on the storage of salmon fillets at the ULT showed that a frozen salmon could be intermediately stored at −25 °C for 40 days before reducing the core temperature to −45 °C and −60 °C, without reducing the extended shelf life at ULT (Haugland, 2002).

The samples were divided into 10 groups by type of packaging material and storage temperature, and stowed into 3 freezing chambers with temperatures −25 ± 1 °C (4 types of packaging material), −45 ± 2 °C (4 types of packaging material) and −60 ± 2 °C (2 types of packaging material), respectively. The quality parameters, as described in the paragraphs below, were studied after 16, 98, 203 and 375 days. Such large intervals were chosen based on the high resistance of salmon lipids to oxidation due to the presence of natural antioxidants, like astaxanthin. Post-rigor salmon fillets packed in LDPE bags showed the continuous increasing of PV up to 10.3 meq O_2 kg^{-1} of fat during 30 weeks of frozen storage at −10 °C (Refsgaard et al., 1998). For farmed trout the same trends of low rates of fat oxidation at freezing temperatures were found (Baron et al., 2007; Jensen et al., 1998).

This process was carried out in exactly the same way for all of the samples to reduce the influence that thawing might have on product quality. The following procedure was used. The samples were placed in refrigerated sea water at 8 °C until a core temperature of 0 °C was reached (Haugland, 2002). This method helped to obtain the same rate of thawing for the fish. Thereafter, the samples were placed into a chilling room at 2 ± 1 °C to equilibrate the product temperature. The quality analyses were performed the same day.

In order to characterize the samples, their water holding capacity (WHC), pH, colour, gaping score, and microbiological activity were analyzed, in addition to the rancidity of both the red and white muscles. Samples for analyzing were taken from the middle part of the fish.

2.2. Packing materials

Several packaging materials are available for the packaging of whole frozen salmon. The following materials were used in the investigation:

i.) 60 μm Low Density Polyethylene (LDPE bags) bags, which are used today as the main packaging material for frozen salmon, and are considered to be the reference material in experiments. Bags were supplied by the Tommen Gram (Levanger, Norway). The O_2 barrier was low (3300 ml O_2 m^{-2} day^{-1} 23 °C), and the pouches were open at one end (the poorest O_2 protection).

ii.) Vacuumed 70 μm polyolefin bags (Polyolefin-vac), which have barrier properties similar to those of the reference material (2900 ml O_2 m^{-2} day^{-1} at 23 °C), but the O_2 was removed from the headspace by vacuum- packaging.[3] Bags were supplied by the Tommen Gram (Levanger, Norway).

iii.) 65 μm vacuumed bags of PA/PE-laminate (PA/PE-vac) with a medium O_2 barrier (65 ml O_2 m^{-2} day^{-1} at 23 °C). Bags were supplied by the Wipak (Nastola, Finland).

iv.) 55 μm shrinkable bags of barrier laminate (Barrier bags) with a high O_2 barrier – which is assumed to be the best O_2 protection (12 ml O_2 m^{-2} day^{-1} at 23 C°). Bags were supplied by the CFS (Holland).

Both the LDPE bags and the storage temperature −25 °C were used as a control experiment (or reference experiment). Such packing does not protect against oxygen penetration inside the fish tissues. A combination of the highest temperature of storage (in the current experimental series) and the packaging material with the lowest oxygen barrier characteristics resulted in high lipid oxidation and other quality degradation in the salmon in this study. When the industry became interested in an extension of the shelf-life of frozen fish, the attention was focused on the influence of packaging material with higher barrier properties on the salmon quality at the end of the storage time (days 203 and 375). The storage temperature of −60 °C was not applied to barrier bags, because this type of packaging is expensive, and will probably not be demanded by industry in the near future.

2.3. Colour measurement methods

The muscles in a fish are represented by two main groups: white and red muscles. The white muscles of salmon have a

[3] Vacuum degree was 99.5% for all vacuumed bags.

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colour between pink and orange, due to the presence of natural pigments like astaxanthine. The initial concentration of pigments is influenced by feed composition and other factors. The red muscles, which are situated along the skin in the middle of the body and along the fins, have a brown colour. In this study only the colour development of the white muscles relative to storage conditions was the subject of interest.

The colour was measured with Computer Vision Method (CVM) and by Spectroscopy, both in CIE L*, a*, b* colour space. Fillets measured with CVM were illuminated in a light box with a PixeLINK digital camera. The images were filtered from noise and their colour was calibrated. Only the red-coloured area of the fillet was analyzed, and all other areas were blended off (Erikson and Misimi, 2008). Determining the fillet colour by spectroscopy was done by an X-Rite® 948/968 instrument. Each fillet was measured at 10 different point spreads over the area of the fillet, and the average value was calculated. The measured points involved both the white muscles and the light fat structure in between them.

CIE L*, a*, b* is a rectangular, 3 - dimensional colour space, based on an opponent colour theory, where L* is the lightness axis (0 refers to black and 100 refers to white colour), a* is the green/red (pink) axis (positive values express redness), and b* is the yellow/blue axis (positive values express yellowness) (Schubring, 2009).

Changes in a* and b* values could be analyzed simultaneously by using the Hue value. Hue is an attribute of a visual sensation according to which an area appears to be similar to one of the perceived colours: red, yellow, green, and blue, or to a combination of two of them. It is measured in degrees from 0 to 360°, and can be calculated from a* and b* values by equation (1) (Carter, 2004).

$$h^* = \tan^{-1}\left(\frac{b^*}{a^*}\right) \quad (1)$$

2.4. Water holding capacity

The water-holding capacity of a fish is described as the ability of the muscles to retain water even though external pressures are applied to them. An absorbent method was chosen, which consisted in applying a 1 kg weight for 60 s on a 1.0 g sample that had been placed between two filter papers (Schleicher & Schuell 597, Ø = 90 mm). The samples were weighed before and after this procedure. A low water loss indicated that the structure and cells in the muscles were well preserved.

2.5. Rancidity determination methods

The rancidity of the salmon muscle was determined by TBARS and PV, and measured in 3 fillets from each test group. Fat used for the determination of the PV value was extracted using the rapid method for lipid extraction (Bligh and Dyer, 1959). The samples of fat were kept frozen with chloroform at −85 ± 2 °C for 2 weeks before analyses. The chloroform was evaporated with N_2 at +40 °C just before analyses.

The PV (meq O_2 kg^{-1} of fat) was measured by the ferric thiocyanate method of the International Dairy Federation (ISO/IDF, 2006) as modified by Ueda et al. (1986). The hydroperoxides in the lipids oxidize Fe(II) to Fe(III). Fe(III) then reacts with ammonium thiocyanate, forming a red complex where the optical density is measured with an absorption maximum at 500 nm.

The TBARS, mg of MDA kg^{-1} of fish, were determined in tissues by the method developed by Ke et al. (1984). The tissues were digested in low pH media and then separated by the application of reflux distillation. The 2-thiobarbituric acid was mixed with a distillate, and TBARS were measured by spectrophotometric measurement with an absorption maximum at 538 nm.

2.6. pH measurement

The muscle's pH was measured directly in the white muscle by a shielded glass electrode (WTWSen-Tix 41) connected to a portable pH-metre (Metron 80). During the measurements, the instrument was frequently calibrated using pH 4.01 and pH 7.00 buffers. To obtain consistent results, the electrodes were cleaned frequently. The pH was measured in 3 different places on 6 fillets from each test group.

2.7. Gaping score and drip loss determination methods

The evaluation of gaping is a method for characterizing texture changes. Gapes are fractures of fish flesh after filleting. The deep gapes over a large area of the fillet indicate low quality. Gapes were judged by the visual inspection of 6 fillets from each test category. There is no standardized method for determining the gaping score. For this work it was evaluated by measuring the area of the gapes (10^{-3} m^2) on each fillet where they occurred, but not their depths.

Drip loss represents the juice and water loss of the fish after defrosting. It was calculated as the difference between the weights of the fish before long-term frozen storage and after thawing.

2.8. Microbiology

The total colony forming units (CFU, method NMKN96) were measured. After thawing, 2 cubes (approx. 40 × 40 × 30 × 10^{-3} m, with skin on one side) were taken from the fish and placed in aseptic bags. Microbiological activity was measured on the first day after thawing, and after 4, 5 and 6 days stored at +4 °C. This was done to check the stability of defrosted salmon in the retail chain, and to investigate the growth rate of microorganisms. Before analyzing, the skin was removed and the muscle homogenized. In total, 6 parallels from each test category were analyzed.

2.9. Sensory evaluation

The sensory evaluation was performed using a trained sensory panel at Nofima AS, using descriptive sensory profiling (ISO, 1985). The sensory panel consisted of 10 selected assessors (ISO, 1993), and the analyses took place in a custom-built sensory laboratory (ISO, 1988). Prior to the analyses, the panel was trained in the detection and intensity of each of the defined attributes. The salmon was cut into 2 cm slices and packaged in vacuum pouches labelled with 3-digits and assessor numbers. Each assessor received the same section of

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the salmon for all the samples. The salmon samples were cooked in a steam cooker at 80 °C for 8 min. The samples were served in duplicate in the coded plastic bags, and each assessor individually opened the bag for an immediate registration of sample odour. The samples were served in a randomized order (totally 48 samples for each assessor: 6 variants × 4 parallels × 2 servings). Water and crackers were served for cleansing the palate between samples. The intensity of twenty sensory attributes were evaluated, and attribute intensities were recorded on a continuous non-structured computer system scale for the direct recording of data (Compusense five, ver. 4.6, Compusense Inc., Guelph, Canada). Each assessor evaluated the samples at an individual speed and recorded the results, and the computer transformed the responses into numbers between 1 = low intensity, and 9 = high intensity. The sensory intensities for each sample of salmon were obtained by averaging the individual intensities for the 10 sub-samples.

2.10. *Statistical analysis*

The analysis of variance (ANOVA: single test and two-factor test with replication) was applied to analyse the effects of time, temperature and type of packaging material on quality parameters. The difference was considered significant at $p < 0.05$.

3. Results and discussion

3.1. *Rancidity development in fish muscles*

PV in reference fish showed 1.08 (0.23)[4] meq O_2 kg^{-1} of fat for white muscles, and a relatively higher value of 1.26 (0.09) meq O_2 kg^{-1} of fat for red muscles at the 16th day. During frozen storage a slow increasing of PV with time for both red and white muscles was observed, and the maximum value at the end of the storage period was 1.76 (0.17) and 1.82 (0.17) meq O_2 kg^{-1} of fat for white and red muscles, respectively, see Table 1. No statistical difference between PV in white and red muscles was found at 203 and 375 days ($p > 0.05$), in spite of its higher initial content in red muscles.

The application of Polyolefin-vac, PA/PE and Barrier packing materials decreased the PV formation in white and red muscles during storage at −25 °C when compared to those stored in LDPE bags ($p < 0.05$). The decreasing of the storage temperature for salmon packed in LDPE bags to −45 °C resulted in the inhibition of PV formation for white and red muscles ($p < 0.05$) in comparisons with references. At the same time, the positive influence of the packaging materials became negligible for storage temperatures below −25 °C. No statistically significant difference in PV was found between samples stored at −45 °C and packed in LDPE, PA/PE or barrier bags ($p > 0.05$).

Decreasing the storage temperature from −45 °C to −60 °C for fish packed in Polyolefin-vac and PA/PE bags did not show any statistical significance in the inhibition of PV formation in red muscles at the 375th day of storage ($p > 0.05$). For white muscles, the PV formation after one year was even higher at −60 °C, than at −45 °C ($p < 0.05$). This confirms previous investigations of PV formation in salmon fillets, where the beneficial effect of storage at −60 °C was negligible in comparison with storage at −45 °C (Magnussen and Johansen, 1995).

Salmon packed in LDPE bags stored at −45 °C, and salmon packed in other packaging materials stored at −25 °C (Polyolefin-vac, PA/PE-vac, and Barrier), did not show any statistical difference in the formation of PV in the white meat ($p > 0.05$). This was true for the red meat as well, except when packed in a Polyolefin-vac package. This leads to the conclusion that the application of vacuum packaging, and using materials with higher barrier properties, has an effect on PV formation at −25 °C similar to that of storing the fish at −45 °C in traditional packaging (LDPE).

The secondary oxidation product of the lipids measured by TBARS was, after 16 days of storage, at levels of 1.29 (0.41) and 4.32 (1.25) mg MDA kg^{-1} fish, for white and red muscles, respectively. Further storage at the reference conditions did not result in a noticeable increase until 203 days ($p > 0.05$), Table 2. A substantial increasing of TBARS was detected at the 375th day of storage ($p < 0.05$); oxidation was significantly higher for the red muscles than for the white ($p < 0.05$), and reached 14.04 (1.70) and 2.21 (0.39) mg MDA kg^{-1}. This was due to the difference in fat content between the two types of muscles. For red muscles, the fat content reached up to 50% of the wet basis, while for white muscles it was around 15% of the wet basis. It should be mentioned, that the lipid composition varied little between the different parts in farmed

Table 1 – Development of PV (meq O_2 kg^{-1} of fat) in frozen salmon against storage time and type of packaging material.

Storage time, days	Type of packaging material	Storage temperature, °C		
		−25 °C	−45 °C	−60 °C
16	LDPE bags	1.08 (0.23)	n/a	n/a
		1.26 (0.09)		
98	LDPE bags	0.92 (0.20)	0.76 (0.14)	n/a
		1.34 (0.20)	0.96 (0.15)	
230	LDPE bags	1.66 (0.28)	1.04 (0.27)	n/a
		1.53 (0.17)	1.01 (0.19)	
	Polyolefin-vac	1.28 (0.21)	0.93 (0.06)	0.75 (0.04)
		1.20 (0.25)	1.21 (0.20)	0.76 (0.19)
	PA/PE-vac	0.89 (0.12)	1.06 (0.04)	0.90 (0.26)
		0.82 (0.08)	1.25 (0.22)	0.81 (0.03)
	Barrier bags	1.05 (0.06)	n/a	n/a
		0.96 (0.21)		
375	LDPE bags	1.76 (0.17)	1.18 (0.24)	n/a
		1.82 (0.17)	1.13 (0.19)	
	Polyolefin-vac	1.37 (0.22)	0.74 (0.13)	1.52 (0.05)
		1.50 (0.13)	1.34 (0.25)	1.20 (0.23)
	PA/PE-vac	1.49 (0.20)	1.30 (0.25)	1.60 (0.28)
		0.95 (0.20)	1.08 (0.32)	1.31 (0.23)
	Barrier bags	1.30 (0.30)	1.30 (0.20)	n/a
		1.13 (0.21)	1.21 (0.18)	

Values represent PV – above the line in the white muscles, below the line in the red muscles. Standard deviation is shown in the brackets.

[4] Standard deviation introduced in the brackets.

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Atlantic salmon, and its influence on oxidation was considered to be insignificant (Refsgaard et al., 1998a). But red muscles contain high amounts of pro-oxidants like myo/hemoglobin and metal ions, and undergo oxidation more intensively than white muscles.

The beneficial influence of packaging material on TBARS formation in white muscles was detected on the 375th day of storage at −25 °C ($p < 0.05$), but the difference between the values obtained for Polyolefin-vac, PA/PE and Barrier bags, was statistically insignificant ($p > 0.05$). The decreasing of temperature to −45 °C and −60 °C also inhibited the TBARS formation in the white muscles, at the same time the influence of the packaging materials was negligible for the red and the white muscles ($p > 0.05$). The benefit of frozen storage of the salmon at −60 °C, in comparison with storage at −45 °C, was not detected ($p > 0.05$).

Comparisons of the TBARS for the salmon packed in LDPE bags and stored at −45 °C, with salmon packed in the other packaging materials stored at −25 °C (Polyolefin-vac, PA/PE-vac, and Barrier), did not show any statistical difference for PV in the white and the red meat ($p > 0.05$) in the end of the storage time. This leads to the conclusion that the application of the packaging materials with higher barrier properties in combination with the vacuum techniques had an effect on TBARS formation at −25 °C similar to that of storage at −45 °C in traditional packaging (LDPE).

Table 2 – Development of TBARS (mg MDA kg^{-1}) in the frozen salmon against the storage time and the type of packaging material.

Storage time, days	Type of packaging material	Storage temperature, °C		
		−25 °C	−45 °C	−60 °C
16	LDPE bags	1.29 (0.41) 4.32 (1.25)	n/a	n/a
98	LDPE bags	1.37 (0.40) 4.67 (1.10)	1.16 (0.21) 3.90 (1.15)	n/a
230	LDPE bags	1.45 (0.32) 4.68 (1.30)	1.24 (0.37) 3.96 (1.19)	n/a
	Polyolefin-vac	1.30 (0.43) 4.32 (1.79)	1.25 (0.46) 3.74 (1.20)	1.27 (0.22) 3.96 (1.00)
	PA/PE-vac	1.51 (0.43) 3.60 (1.79)	1.31 (0.21) 3.60 (0.6)	1.64 (0.23) 4.68(0.75)
	Barrier bags	1.40 (0.43) 3.60 (1.57)	n/a	n/a
375	LDPE bags	2.21 (0.39) 14.04 (1.70)	1.40 (0.48) 3.88 (1.70)	n/a
	Polyolefin-vac	1.04 (0.48) 11.46 (1.71)	1.27 (0.22) 7.56 (1.77)	1.86 (0.24) 12.62 (2.08)
	PA/PE-vac	1.73 (0.31) 10.44 (2.24)	1.62 (0.24) 12.44 (2.52)	1.55 (0.42) 10.24 (2.87)
	Barrier bags	1.51 (0.34) 11.68 (1.60)	1.48 (0.22) 12.17 (1.40)	n/a

Values represent TBARS – above the line in the white muscles, below the line in the red muscles. Standard deviation is shown in the brackets.

For white and red muscles, the PV did not reach significant levels, and thus a critical decrease in quality was not expected. Previous studies concluded that PV of 4.4 meq O_2 kg^{-1} did not influence the sensory response (Fagan et al., 2003). The average level of the PV value in salmon in this study was not higher than 1.82 meq O_2 kg^{-1} of fat for red muscles and 1.75 meq O_2 kg^{-1} of fat for white.

The variation of the packaging materials did not show any significant improvement in the inhibition of fat oxidation at temperatures lower than −25 °C. It was also difficult to point out the best packing material for the salmon stored at −25 °C, due to the insignificant difference between the results for white muscles ($p > 0.05$).

The observed events could have the following explanation. First, the fish were protected by a packaging material and a glaze layer (fresh water ice). A penetration of oxygen through the ice was not detected by previous studies, so the diffusion was possible only through the intercrystalline brine layer (Hemmingsen, 1953). In the case of LDPE bags the ice layer became thin and less dense with the storage time due to a sublimation process. At the temperatures below −25 °C, The sublimation process went more slowly at the temperatures below −25 °C, due to lower vapour pressure above the ice surface; thus the glaze layer maintained its protective properties for a longer time and influenced the PV and TBARS formation. Alternatively, the application of the ULT results in small fractures of the glaze layer, which increases oxygen permeation through the ice (Flink and Goodhart, 1978).

Second, the penetration of oxygen for packaging materials was measured at standard conditions, which are quite different from real frozen storage. An O_2 transmission rate of packaging material depends significantly on the temperature. For example, PA/PE bags showed an O_2 transmission rate of 0.51 ml O_2 pkg^{-1} day^{-1} at +23 °C, and 0.01 ml O_2 pkg^{-1} day^{-1} at −25 °C, and for other packaging materials the dependence was similar (Larsen, 2004). In this way, the O_2 penetration rate could be quite low, and comparable for different packaging materials at −25 °C and below. At the same time, a decreasing temperature, a high humidity, and packaging conditions can cause micro-damage to a bag's structure. That can increase the O_2 permeability and impair the advantages of the selected packaging material (Demorest, 1992; Siracusa, 2012).

In general, a temperature of −25 °C was suitable for the inhibition of PV and TBARS formation during frozen storage. This effect was significantly increased by the application of vacuum packaging technology, irrespective of the type of packaging material.

3.1.1. pH

The reference salmon showed pH at 6.09 (0.11) just after freezing. Normally it has a pH of 6.4–7.0 after slaughtering, depending on stress and size. The decreasing of pH is associated with the defrosting process, and can be explained by the ATP degradation and lactate accumulation during the freezing, frozen storage, and thawing processes (Cappeln et al., 1999). The same results were obtained before for salmon were defrosted after short-term frozen storage: the pH dropped to 6.1 (Einen et al., 2002).

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At the 98th day the pH of salmon, stored at reference conditions, increased to 6.27 (0.11), Fig. 1A. The same trend had been previously obtained for mackerel stored for 12 months at −18 °C. Such a process was explained by the enzymatic degradation of the muscle content (Ciarlo et al., 1985; Simeonidou et al., 1997).

After the 98th day, the pH value decreased, and reached 5.93 (0.12) at day 375, which can be explained by the increase of PV and TBARS during frozen storage.

However, neither the storage temperature nor the packaging materials affected the alteration of pH during storage time ($p > 0.05$).

3.2. Water holding capacity

A high water loss after thawing may indicate a larger number of cells bursting during freezing and frozen storage. This gives a reduced yield and a loss in quality after thawing (Hemmingsen, 2002). The weight losses observed in this study were relatively uniform, and varied from 3% to 5% throughout the whole storage period independently of the storage temperature or the packaging technology. The weight losses were low in comparisons with the previous data for thawed salmon, which showed a loss of up to 7% (Haugland, 2002).

The WHC decreased slightly with time in the case of the reference conditions, from 97.0% to 94.6% ($p < 0.05$). The largest decrease of WHC was measured between the 203rd and 374th days of storage. A storage temperature of −45 °C stabilized the WHC at 96.1% ($p < 0.05$), and there were no statistically significant differences among the packaging materials for any storage temperatures ($p > 0.05$).

3.3. Gaping and drip loss

While the gaping score and the drip loss were affected by the storage temperature ($p < 0.05$), no influence of packaging materials was observed ($p > 0.05$). Up to the 203rd day of storage the gaping score was quite low (below 10^{-3} m^2 per fillet), and the positive influence of ULT was not detectable ($p > 0.05$), Fig. 1B. In the end of the storage period, the products stored at −25 °C had the highest gaping score, ranging from 1.7 to 2.7 10^{-3} m^2 per fillet, while the gaping score for the products stored at −45 °C and −60 °C varied from 1.09 to 1.6×10^{-3} m^2 per fillet. A reduction of the storage temperature from −45 °C to −60 °C did not show a statistically significant improvement of the gaping score ($p > 0.05$).

The drip loss was determined only in the vacuum packed bags, due to the immersion of the fish into water during the first stage of defrosting. No real decreasing of drip loss with the application of the PA/PE-vac and the Polyolefin-vac bags had been found for any observed temperatures of storage ($p > 0.05$) The highest drip loss 3.7 (0.12) % was obtained at −25 °C. Drip loss was reduced to 2.53 (0.37) % at −45 °C and to 2.0 (0.52) % at −60 °C for polyolefin-vac bags. At the same time, the glaze layer just after freezing was equal to 1.75 (0.25) %.

3.4. Colour

The colour of the salmon flesh was examined by two methods. Both of them used the L*a*b* colour scale, with a different procedure of measurements. In the case of the Computer Vision Method, one can detect changes in the colour of the muscle fibres. The second method determined the average

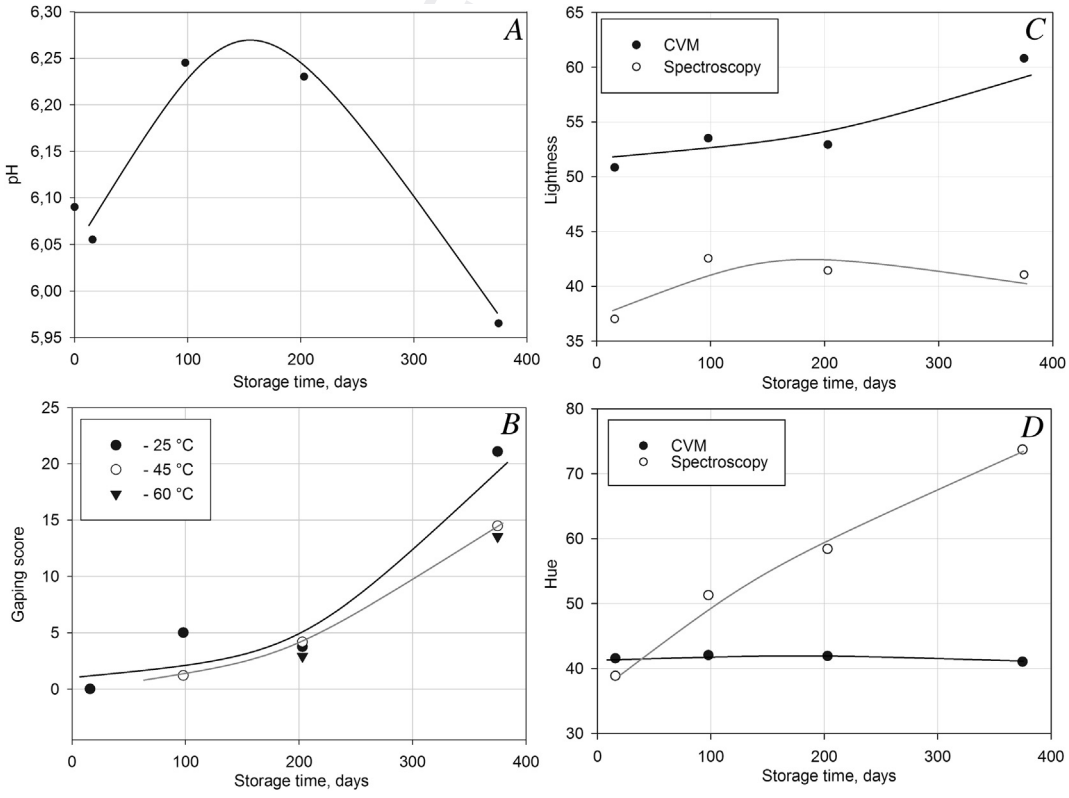

Fig. 1 – General trends for different quality parameters of frozen salmon. A– pH, B – gaping score, C – Lightness, D – hue.

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colour of the surface. The lightness of the reference salmon determined by the CVM increased from 49.5 (2.3) points at the 16th day to 60 (2.1) points at the 375th day, Fig. 1C. The influence of the storage temperature and the packaging materials was insignificant ($p > 0.05$). The lightness of the whole fillet determined by the Spectroscopy was lower. It also increased from 35 (2.97) points at the 16th day to 40.47 (1.75) points at the 375th day. The influence of the storage temperature and the type of packaging material were not statistically significant ($p > 0.05$).

The Hue of the reference fish measured by the CVM was stable with respect to the storage time ($p > 0.05$); 41.57 (2.4)° at the beginning, and 40.41 (2.76)° at the end of the storage, Fig. 1D. The influence of the storage temperature and the packaging materials was not detected ($p > 0.05$). The oxidation of lipids was probably not developed enough to induce changes in the colour of the fibres.

The hue of the whole reference fillet determined by the Spectroscopy showed significant alteration with the storage time after 203 days of storage ($p < 0.05$). It increased with the time from 39.97 (3.1)° at the 16th day to 73.18 (2.23)° at the 375th day. The influence of the storage temperature and type of packaging materials was not detected ($p > 0.05$).

Such an increase of the Hue means that the average colour of fillet changed from the red to the yellow range. In our study, the average colour of the salmon was considered to be more orange at the end of the frozen storage period.

Previous studies of the colour of frozen salmon (Spectroscopy) showed the simultaneous increase of a fillet's red and yellow intensity with the storage time, and that the Hue angle was stable at a value of 54 (2.0)° during the whole period of storage at −45 and −60 °C (Magnussen and Johansen, 1995).

Since the white muscles (myomeres) did not go through significant changes in colour, the observed changes could be explained by the evolution of the colour, size and shape of the diametric myoseptums during frozen storage. As soon as the myoseptums of the salmon become white or yellow colour, they could influence the fillet colour significantly.

3.5. *Sensory analyses*

The results from the sensory analyses are presented for some selected attributes in Fig. 2. It shows that neither the packaging material nor the freezing temperature had any evident effect on the salmon quality after 375 days of storage, and no significant differences were found between the samples for any of the attributes in the sensory analyses ($p > 0.05$). The salmon in the open LDPE bags (the packaging material used today) had an even lower intensity of rancid flavour than three of the other packaging materials. The low intensity of the rancid odour and flavour was in accordance with the measurements of the relatively low TBARS and PV-values after 375 days of storage. The salmon stored for one year had generally low intensities of rancid odour and flavour, and was comparable to the quality of fresh salmon stored on ice for three days, analysed in a previous project (Rødbotten et al., 2009). An intensity of 2–3 for rancid odour and flavour is difficult to detect, even by a trained sensory panel, and will usually not be detected by consumers.

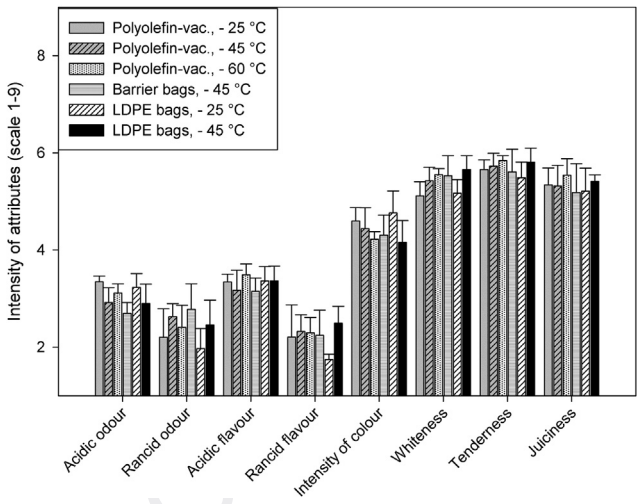

Fig. 2 – Sensory evaluation for cooked salmon samples after 375 days of storage in 3 different packaging materials at 3 different temperatures. The figure shows the results for the most important attributes related to oxidation, colour and texture.

3.6. *Microbiological activity*

Microbiological analyses were performed just after controlled thawing, and after 4, 5 and 6 days of storage at +4 °C. All the microbiological analyses, which were measured at the day of thawing, showed a low microbial activity (CFU<100). The analyses after 4, 5 and 6 days showed a large spread of the microbiological values between the fillets within each category. The results show no significant variation between the test parameters of the storage temperature or packaging technology ($p > 0.05$). A higher microbiological activity (the growth rate) for defrosted salmon stored at 375 days was detected ($p < 0.05$). On average the CFU was higher than 2×10^7 at the 4th day of chilled storage at $+0 \pm 1$ °C, which was quite high and reflected the poor microbial quality of the product. This was related to the increasing of gapes and other possible textural changes, which appeared after 375 days. Such observation leads to the conclusion, that the shelf-life of defrosted fish will be limited by 3 or 4 days.

4. Conclusions

The accumulation of the oxidative products continued in the whole gutted salmon for all investigated storage conditions, even at −60 °C. The application of vacuum packaging inhibited the formation of PV and TBARS at −25 °C, and the effect was similar to the storage at −45 °C for LDPE bags. Decreasing the temperature to −45 °C and −60 °C neutralized this positive influence of the packaging materials.

However, the rancidity of the frozen fish remained at a quite low level for all the samples examined, even after one year of storage. A slight increase in rancid odour and taste was detected by a trained sensory panel, but would probably not be detectable by the consumers.

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Neither the storage temperature nor the packaging materials influenced the colour characteristics and sensor parameters. At the same time, the drip loss, the WHC, and the gaping score of the salmon were lower at −45 °C and −60 °C than at −25 °C.

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