REPORT NO MA 18-06 | Wenche E. Larssen, Trygg Barnung, Marianne R. Kjøde, Turid S. Fylling, Audny Hellebø, Ingebrigt Bjørkevoll and Margareth Kjerstad

OXIDATION IN FROZEN-STORED, JAPANESE-CUT MACKEREL FILLET

MØREFORSKING

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SUMMARY

The pelagic consumption industry wishes to increase the degree of processing by freezing round mackerel during the catch season and thawing the fish later for further filleting. The fillets are more susceptible to oxidation than the whole fish. This is because the fish meat in a fillet is more exposed to oxygen and light. The focus of this project has therefore been to examine oxidation developments in mackerel fillet produced from frozen raw material, and how the quality is affected by storage time, packaging and use of antioxidant.

Oxidation development has been monitored by chemical and sensory quality parameters. The results show that the use of antioxidant (rosemary) produces a significantly longer shelf life, measured in thiobarbituric acid reactive substances (TBARS) and free fatty acids (FFAs), 12 and 15 months after filleting. Measured yellow colour also increases during the storage time for all the products. Fillets that were vacuum-packed or treated with rosemary were still considered fit for consumption after 18 months, while natural fillets packed in cardboard were evaluated as unfit for consumption.

SAMMENDRAG

Pelagisk konsumindustri ønsker å øke foredlingsgraden ved å fryselagre rund makrell i fangstsesongen for deretter tine og videreforedle makrellen gjennom resten av året. Filetene er mer utsatt for harskning enn rund fisk. Dette skyldes fiskekjøttets eksponering mot oksygen og lys. Blodrester som blir eksponert i fileten vil øke oksidasjonen. Det gjør at makrellfilet får redusert holdbarhet under fryselagring sammenlignet med rundfrosset makrell. Hovedfokus i prosjektet har derfor vært å undersøke hvordan oksidasjonsutviklingen i makrellfilet produsert av fryst råstoff påvirkes av lagringstid, emballering, bruk av antioksidant og alderen på rund frosset makrell.

Holdbarheten har blitt evaluert mht. kjemiske og sensoriske kvalitetsparameter. Resultatene viser at bruk av antioksidant (rosmarin) gir signifikant bedre holdbarhet målt i TBARS og frie fettsyrer, på fryselagret filet, 12 og 15 mnd. etter filetering. En ser også at produsert filet, uavhengig av forbehandling, var mye spaltet og hadde synlig økt gulfarge etter 18 mnd. lagring. Mht. spisekvalitet ble filet som var vakuumpakket eller tilsatt rosmarin fremdeles ansett som egnet for konsum etter 18 mnd. fryselagring mens naturell filet pakket i kartong ble evaluert som uegnet til konsum.

PREFACE

The project 'Mapping of oxidation in mackerel fillet during frozen storage' is a project which aim is to increase the processing of mackerel in Norway. The project is funded by the Norwegian Seafood Research Fund (FHF), and Kristian Prytz and Lars R. Lovund have served as enthusiastic and knowledgeable coordinators. Thanks for valuable input.

The steering committee for the project has comprised Gunnar Domstein and Helge Blålid/Alexander Krokedal Rønnevik (Pelagia AS), Kjetil Peder Sperre (Brødrene Sperre AS), Ole Andre Nilsen (Grøntvedt Pelagic AS) and Tommy Torvanger (Nergård AS). The steering committee has been committed to the project and provided valuable input and recommendations during the project period. Thanks to everyone involved!

The production and storage of the mackerel fillet has taken place at the premises of Pelagia AS in Selje. Thanks to Asbjørn Bøstrand and all of his colleagues for their assistance during the work on the project.

Ålesund, 31 March 2018

Wenche Emblem Larssen

Project manager

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1. INTRODUCTION

Only 2–4% of landed mackerel is processed into fillets in Norway. The rest of the 350,000 tonnes is exported as frozen round mackerel. Together with the industry and the policy instrument system, FHF has recognised the significance and importance of increasing the degree of processing of mackerel landed in Norway. The background for the project is that the pelagic industry wishes to focus more on sustainable production of mackerel fillet in Norway. The project has set the Norwegian pelagic industry the overriding goal of filleting at least 25% of the mackerel landed in the country by 2025. This means that new technological solutions will have to be developed to accommodate the increase in volume. There are many challenges throughout the value chain, from the delivery of mackerel from fishing boats to the realisation of new market opportunities. Pelagic enterprises have cooperated with FHF on establishing a pilot line for filleting mackerel at Pelagia in Selje.

The project aims to increase the degree of processing of round mackerel. It also aims to establish year-round production of mackerel fillet. In order to achieve these aims, the pelagic consumption industry will have to establish new production procedures that will involve buying mackerel during the fishing season and filleting thawed round mackerel the rest of the year. The fat in fish with a high fat content will oxidise over time in frozen storage, which gives the fish an unacceptable rancid odour and taste which make it unfit for consumption. When mackerel are filleted, the fillet is more susceptible to rancidity than round fish (Aubourg et al. 2005), because the fish meat in a fillet is more exposed to oxygen and light. The exposed blood residue in the fillet will increase oxidation (Richards et al. 1998). This means that mackerel fillets have a shorter shelf life in frozen storage than frozen round mackerel.

Chemical characterisation of mackerel (Falch et al. 2006, Remme and Wold 2007) shows that its chemical composition varies during the year, comprising 17–35% fat, 52–61% water and 14–20% protein. The fatty acid composition and development of rancidity (oxidation) during frozen storage have been studied by Aubourg et al. (2005), but the relationship between the raw material and the fillet produced from frozen raw material has not previously been studied. What happens during frozen storage of fillet products with a high lipid content, such as mackerel, requires closer attention. Oxidation can affect the taste, which makes chemical and sensory analysis methods important in the assessment of the raw material and the end product.

The chemical quality parameters used for pelagic raw materials are peroxide value (PV), thiobarbituric acid reactive substances (TBARS) and free fatty acids (FFAs). The oxidation of fat in mackerel fillet during frozen storage follows the normal development of oxidation parameters in marine oils during storage. The PV is the primary (first) oxidation parameter, and it will increase during the initial part of the oxidation process. Several secondary oxidation parameters will become more prominent further on in the oxidation process, and the PV will then decrease. TBARS, which can be compared to the aldehyde value, is a collective term for several secondary oxidation parameters. The TBARS value will start to increase when the PV decreases. When oxidation has reached an advanced stage, the TBARS value will also decrease. The amount of FFAs increases steadily during the storage period (Ackman 2005) (see Appendix 1).

It is important to the appearance and quality of the mackerel fillet that the thawed round mackerel is at the correct temperature (prior to filleting). Trials conducted by Sone et al. (2017) show that filleting produces the best result when the core temperature of the fish is between -2.0 and 1.7°C. This requires a thawing method that uses brine to prevent over-thawing. The effect the salt has on the fish muscle depends on the salt concentration and its solubility effect on the myofibrillar proteins in the muscle fibres. When the fish is salted in advance by immersing it in brine, the fish muscle swells in accordance with the brine concentration. When the fish muscle swells, the filaments of muscle repel each other, which leads the muscle to swell and increase its water-binding capacity (Hellevik 2014). Salt also contains small amounts of minerals and metals, which can serve as catalysts in the process of oxidation of unsaturated fatty acids (Lauritzsen 2004). If the muscle fibre swells, it is probable that these metals have gained easier access to fatty acids, which results in a higher rate of oxidation.

The filleting process affects oxidation. When mackerel is filleted, the fillet is more susceptible to rancidity than round mackerel because the fish meat in a fillet is more exposed to oxygen and light. There is also a risk of blood residue on the surface of the fillet. The blood contains both heme and non-heme iron, which catalyses oxidation (Lauritzsen 2004). The enzyme lipoxygenase, which is particularly prevalent in the gills and skin of fish, also oxidises fatty acids (Lauritzsen 2004). This means that mackerel fillets have a shorter shelf life in frozen storage than frozen round mackerel.

In order to minimise oxidation challenges, it is important to develop processing solutions that make it possible for singly frozen mackerel fillets to have practically the same shelf life as frozen round mackerel. Oxidation can be reduced by reducing the fillet's exposure to oxygen and/or using additives to halt the oxidation process. Japanese-cut fillet (fillet with intact abdomen and peritoneum) is the most popular mackerel fillet in Asia. Customers in the market now demand documentation of the oxidation status of Japanese-cut mackerel fillets.

AIMS

The main focus of the project is how the development of oxidation in mackerel fillets produced from frozen raw materials is affected by storage time, packaging, use of antioxidant and the age of frozen round mackerel.

Three aims have been defined on this basis:

1. Document differences in the degree of oxidation in frozen-stored Japanese-cut mackerel fillet, packed in cardboard and vacuum-packed.
2. Document the effect of an approved antioxidant used on frozen fillets produced from frozen round mackerel fillets packed in cardboard.
3. Document the effect of long-term storage of frozen round mackerel on oxidation in Japanese-cut fillets packed in cardboard.

2. MATERIALS AND METHODS

2.1 RAW MATERIAL AND PRODUCTION

The raw material used was round mackerel frozen on 17 September 2015 at Pelagia Selje. The mackerel was caught by Eros (M-29-HØ) on 15 September 2015 on fishing ground 0716. Eros had two hauls of 232 tonnes and 498 tonnes, respectively. The temperature of the mackerel on arrival at Pelagia Selje was -1.2°C. Pelagia Selje produced and froze the catch on 16 and 17 September 2015. The company assessed the freshness and the black belly skin in the abdomen of the fish as good. The parasite evaluation showed that there were more than six parasites in each of the examined mackerel, but that there was an insignificant volume of Calanus finmarchicus in the gastrointestinal system. The mackerel was frozen and stored at Pelagia Selje AS before being sent to Pelagia's main frozen storage facility in Måløy. The batch used in the trial was returned to Pelagia Selje when the trial plan was devised in December, and stored at -30°C.

The first three series of trials were produced from 5 months of frozen round mackerel from 23 to 26 February 2016. The fourth series was produced on 23 May 2016 from the same raw material, which was then 8 months old. Different packaging methods and the use of an antioxidant (rosemary) distinguished the series from each other (see Table 1).

Table 1 Overview of production method, storage and samples for analysis for the different series of trials.

	Raw material	Antioxidant	Glaze	Packaging	Storage time
Series 1	Fillet produced from 5 months of frozen-stored round mackerel		x	Cardboard with plastic wrap	18 months, samples taken every 3 months
Series 2	Fillet produced from 5 months of frozen-stored round mackerel		x	Vacuum-packed	18 months, samples taken every 3 months
Series 3	Fillet produced from 5 months of frozen-stored round mackerel	x	x	Cardboard with plastic wrap	18 months, samples taken every 3 months
Series 4	Fillet produced from 8 months of frozen-stored round mackerel	x	x	Cardboard with plastic wrap	15 months, samples taken every 3 months

The raw material was thawed in three stages in accordance with the procedure developed by Sone et al. (2017). Pursuant to this procedure, the mackerel was thawed in batches in tubs in a three-stage process;

1) 3 tubs (1,000 l) with a water temperature of 11–12°C - for 15 min.
2) 3 tubs (1,000 l) with a water temperature of 4°C - for 30 min.
3) 2 tubs (500 l) with a water temperature of -3°C - for a minimum of 20 min.

To ensure that the water in the tubs had the correct temperature, a mix of fresh water, sea water, refrigerated sea water (RSW), and brine refrigerated to between – 20 and –15°C was used.

Twelve boxes containing 20 kg of mackerel were thawed at a time. The mackerel was removed from the freezer 1–2 hours before being placed in the tub to thaw. The boxes of mackerel were divided up so that the mackerel could be placed individually into the tubs to ensure they thawed evenly.

After thawing, the mackerel was filleted on a Baader 221 filleting machine, which was adjusted to fillet the mackerel in Japanese-cut fillets. After filleting, the fillets were manually trimmed to remove any remaining intestines and blood around the backbone. The filleting was not optimal, so a fairly large number of fillets were rejected. The sorting was based on the criteria described in Table 2.

Table 2 Sorting criteria for rejection of mackerel fillet

Sorting criteria	**Description**
Incorrect cutting	Crooked mackerel, 'banana fish', mean that large parts of the backbone are left in the fillet
	Broken-up fillets because the fish is bent too much to make it fit when being fed into the filleting machine
	Damaged/torn fillet
Blood faults	Blood in front of the remaining backbone that cannot be washed away
	Blood in the tail area with remaining backbone
	Large patches of blood in the muscle
Skin	Skin torn off large areas of the fillet due to the propulsion system in the filleting machine. Small tears/torn off strips of skin were accepted because they were so common
Muscle	Soft muscle – the result of the temperature being too high during cutting
	Clearly gaping fillet

After the fillets were trimmed, they were placed muscle side down on trays covered in plastic and frozen at -30°C. Fillets treated with antioxidant were placed in a box with a mesh bottom and dipped for ten seconds in a 2% solution of rosemary extract (StabilEnhance®OSR 5 from NATUREX) before being placed on a tray covered in a black plastic sheet and frozen at -30°C.

After the fillets had been frozen and stored for two days, they were glazed with water. The water was a mixture of fresh water and ice, and the temperature was equalised overnight in a refrigerated room to a temperature of between 0 and 0.3°C. All ice particles were removed before glazing.

Twelve to fifteen fillets were placed muscle side up in boxes with mesh bottoms before being dipped for 15 seconds in the glaze water. After glazing, 45 fillets from series 1 and 3 were packed in 10 kg cardboard boxes with plastic wrap before being placed in the freezer.

Fillets were frozen before being vacuum-packed. A Multivac Type R50 with Gramvac overfilm 521mm 100my, material CO 95 HFP and Gramvac underfilm 425 mm 230my, material NICE 14 was used for the vacuum packing. Both films were delivered by Wipak Oy. Ten fillets were vacuum-packed per tray. The trays were packed in cardboard boxes containing 40 and 50 fillets, respectively, for further frozen storage. Figure 2 shows an overview of the production process. The last series of samples was produced on 23 May 2016 from eight-month of frozen round mackerel following the same procedure as for the first three series. Series 4 – Japanese-cut mackerel fillet, antioxidant-treated with rosemary (2%), glazed, packed in cardboard boxes with plastic wrap.

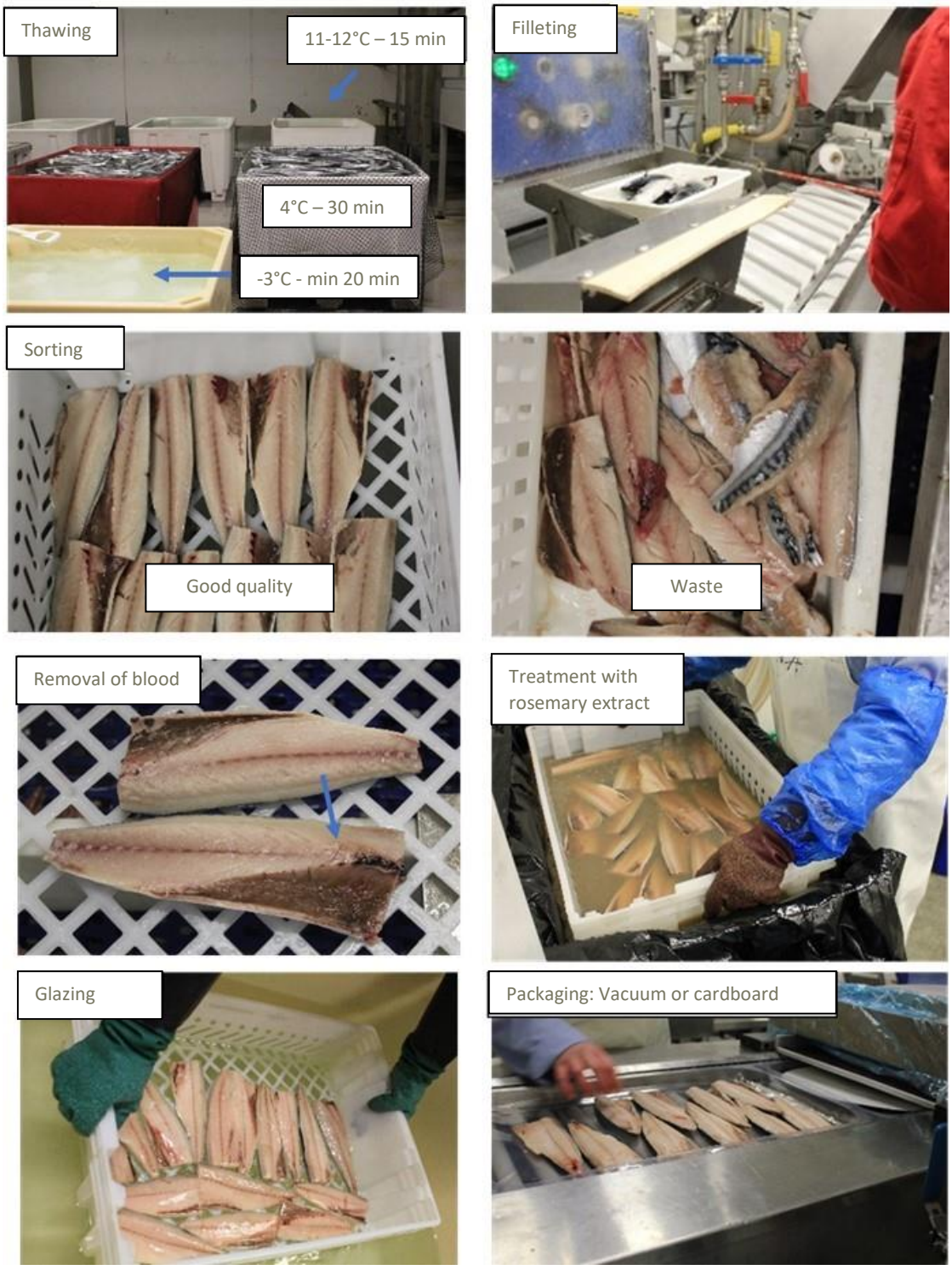

Figure 1 Production process for sample material from frozen round mackerel to frozen fillets.

The temperature profile during the thawing of round mackerel prior to filleting shows that it took around 65 minutes for the frozen product with a core temperature of around -25°C to become ready for filleting at -2.7°C (Figure 2).

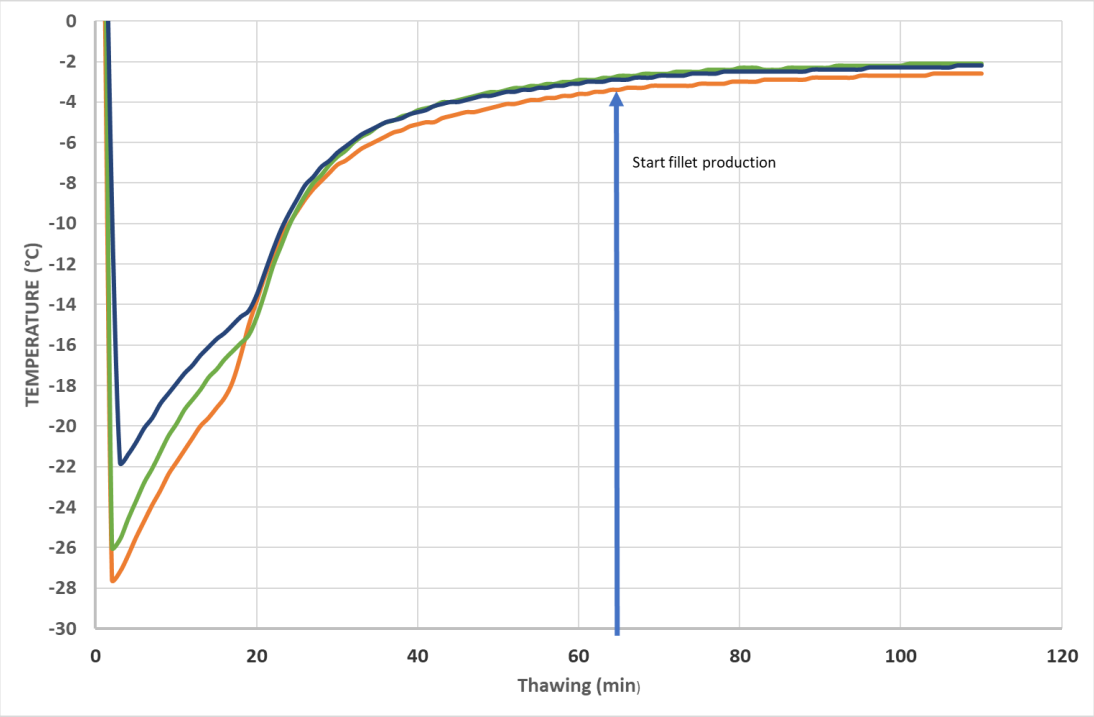

Figure 2 Temperature development during thawing of round mackerel for processing fillets.

2.2 CHEMICAL ANALYSES OF OXIDATION DEVELOPMENT

Shelf life tests were conducted on all four series of fillets. The mackerel fillet was stored at -30 °C at Pelagia Selje and sent frozen to Møreforsking, where it was kept in frozen storage for one week before sampling. Fillets were sampled every three months as described in Table 3. The mackerel was thawed overnight at 4 °C prior to chemical analyses and sensory evaluation. A total of 20 fillets were taken from each series and divided into five composite samples by homogenising four fillets at a time. Two replicates of each sample were analysed.

Table 3 Overview of sampling months and storage times (months) for the different series.

	March 2016	September 2016	December 2016	March 2017	June 2017	August 2017
Round mackerel	0 months					
Series 1		6 months	9 months	12 months	15 months	18 months
Series 2		6 months	9 months	12 months	15 months	18 months
Series 3		6 months	9 months	12 months	15 months	18 months
Series 4		3 months	6 months	9 months	12 months	15 months

The percentage of fat in the raw material was analysed in accordance with Bligh and Dyer (1959).

The PV was determined in accordance with Crowe and White (2001). The TBARS value was determined in accordance with Dulavik et al. (1998). The content of FFAs was determined in accordance with Bernárdez et al. (2005). The pH was measured directly in the minced and homogenised mackerel sample.

2.3. SENSORY EVALUATION OF RAW MATERIAL

Odour

In each series, 20 fillets were evaluated by odour pursuant to a five-point scale from 1 to 5 where 5 is the best score (Table 4). Each fillet was put in a zip-lock plastic bag and placed in a refrigerated room to thaw overnight at 4°C. The 20 fillets from each series were evaluated in random order by a panel of four to five people. The panel smelled the sample by opening the plastic bag and taking a good sniff before closing the bag and passing it to the next judge (Figure 3). The judges took turns at opening the bag and smelling the content first. The panel members smelled the inside of their wrist to 'reset' their sense of smell between the fillets.

Table 4 Evaluation of odour pursuant to a five-point scale from 1 to 5 where 5 is the best score.

Odour	***Scale***
Fresh sea odour	5
Neutral odour (slightly metallic)	4
Slight rancid or strong metallic odour	3
Distinctly rancid or strong metallic odour	2
Rotten odour	1

Figure 3 – Evaluating the odour of the fillets (illustration photo)

Texture

Texture was measured using a TA-XT+ texture analyzer (Stable Micro Systems, Surrey, United Kingdom). Measurements were conducted on the thick part of the fillet by pressing the stamp 30% down into the fillet. The force necessary to do this was registered and presented graphically. A total of 20 fillets from each production series were analysed.

Colour analysis and measurement of gaping

The surface colour of the mackerel fillet was analysed using a computer-controlled imaging technique known as computer vision system (CVS) and described by Girolami et al. (2013). A digital camera (Canon ESO 1300 D) and a 34 mm lens (Canon 34 mm f/5,6) were installed in a black box where all natural light was removed. The box was lit by two fluorescent tubes with a colour temperature of 6500 K (D_{65}, standard light source used in food research), placed at an angle of 45° in relation to the fillet to ensure uniform lighting. As calibration, a photo was taken of the colour palette ColorChecker Passport 1.1.1 (X-Rite Inc.). This was used to make a colour profile. The colour profile was imported into Adobe Photoshop Lightroom CC (Adobe), and all the photos were calibrated in relation to it. A quantitative analysis of the colour was conducted using Photoshop (Photoshop CC 2015, Adobe Systems Inc.) and expressed in CIE L^* (whiteness or clarity), a^* (red/green) and b^* (yellow/blue) coordinates as described by Yam and Papadakis (2004). Twenty fillets from each series were photographed and analysed. The average colour values of the pixels were used to calculate the total colour difference (ΔE) where $L*_0$, $a*_0$ and $b*_0$ are the colour coordinates of the fillets. Figure 4 shows the organisation of Lab values.

$$\Delta E = \sqrt{(L^* - L^*_0)^2 + (a^* - a^*_0)^2 + (b^* - b^*_0)^2}$$

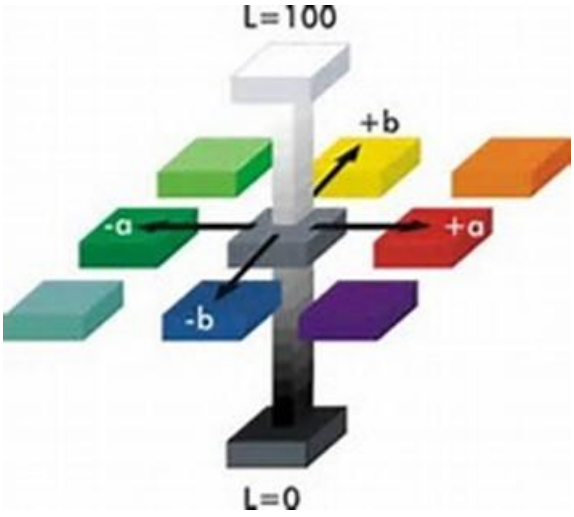

Figure 4 CIE Lab colour system (www.briarpress.org).

The area to undergo colour analysis was marked on each individual photo. The tail area and the abdomen with the black belly skin were not included in the analyses (Figure 5).

Figure 5 The area for colour analysis of the mackerel fillet is indicated with a dotted line.

The mackerel fillets were also analysed in terms of the prevalence of gaping and cracking. Each fillet was assessed by the prevalence and extent of gaping and cracking in the fillet before it was graded on a scale from 1 to 5. The scale used for the evaluation of gaping is shown in Table 5. The higher the prevalence of gaping, the higher the score. The highest value (5) requires both gaping and cracking.

Table 5 Scale for evaluating gaping in mackerel fillet.

Grading 1	**Grading 2**	**Grading 3**	**Grading 4**	**Grading 5**
No gaping areas in the fillet	<5 gaping areas in the fillet	<10 gaping areas in the fillet	>10 gaping areas in the fillet	>10 gaping areas and cracking in the fillet
				

Elasticity

Elasticity was assessed on the basis of the criteria set out in Table 6. The fillet was folded in two and released (Figure 6). The score on the scale from 1 to 5 was based on how quickly the fillet unfolded. The highest value (5) denotes best elasticity.

Table 6 Evaluation of elasticity pursuant to a five-point scale from 1 to 5.

Elasticity	***Scale***
Unfolds quickly	5
Unfolds	4
Unfolds slowly	3
Attempts to unfold	2
Remains folded	1

Figure 6 Folding of the fillet to examine the elasticity of the muscle.

2.4 SENSORY QUALITY OF RAW MATERIAL AFTER HEAT TREATMENT

A sensory quality test was conducted on heat-treated fillets from the four series (Table 1). A panel of six people (Figure 7) evaluated the odour, colour, texture and flavour of the fillets by means of difference testing (ISO:4120 2007) and simple quality assessment (ISO:13299 2003) using an open evaluation form. All the samples were tested in duplicate. To ensure that the samples given to the panel were as uniform as possible, they were served two pieces of mackerel fillet weighing around 20 grams taken from the middle section of the mackerel fillets. A total of four to six pieces with skin were cut from each fillet (Figure 8). The pieces were heat-treated in a steam oven at 200°C for two minutes.

Figure 7 Sensory quality evaluation of the heat-treated raw material.

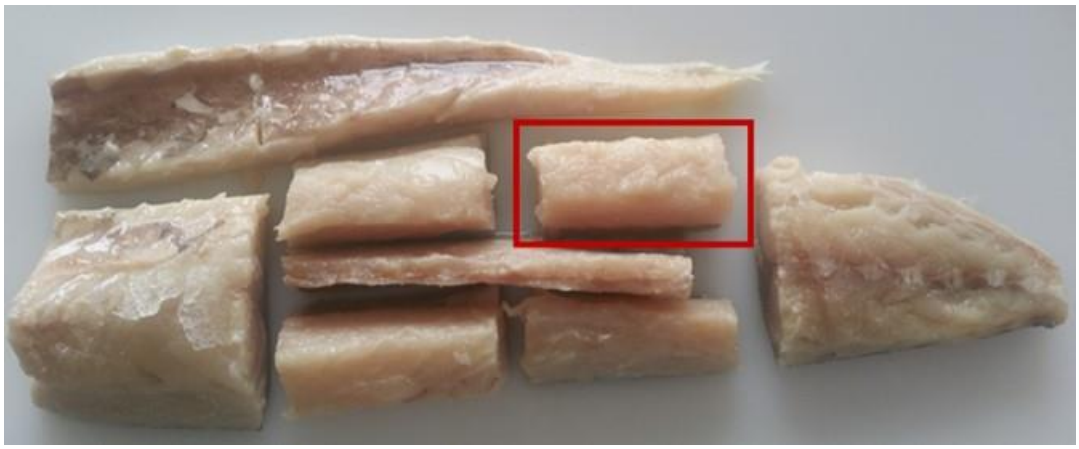

Figure 8 Selection of fillet pieces for sensory analysis (red box). Up to 4 to 6 pieces were taken from each fillet.

2.5 STATISTICAL ANALYSIS

Significant differences were studied by means of a one-way variance analysis using Stata (Stata Corp). Bonferroni was used as a post-hoc test. Significant differences in the sensory analyses based on difference tests are retrieved from a binomial table (SensoriskStudiegruppe 2015).

3. RESULTS

3.1 CHEMICAL ANALYSES OF OXIDATION PARAMETERS

The raw material had a fat content of 27.4±2.7 g/100g. The fat content of mackerel fillet varies during the season. According to the Institute of Marine Research (IMR) seafood database, it is in the range between 2.8 and 37 g/100g with an average of around 25 g/100 g (www.sjomatdata.hi.no).

TBARS

Irrespective of the pre-treatment of the four series, the TBARS value rose steadily throughout the storage period (Figure 9). The exception is series 2, which, after 18 months, decreased in value from 104 nmol/g to 66 nmol/g. After 12 and 15 months, the TBARS values were significantly higher in fillets that had not been treated with antioxidant. The difference is no longer significant after 18 months, although the average value of the fillet treated with antioxidant is markedly lower. There is no significant difference between the fillets produced from five-month and eight-month old raw material. That means that, after 12 months, mackerel fillet produced from round mackerel that had been frozen-stored for 5 months (series 3) has the same TBARS value as the mackerel fillet produced from round mackerel frozen-stored for 8 months (series 4). In general, there are great individual differences between the fillets, which is shown by the high standard deviations. This particularly applies to fillets packed in cardboard (series 1, 3 and 4).

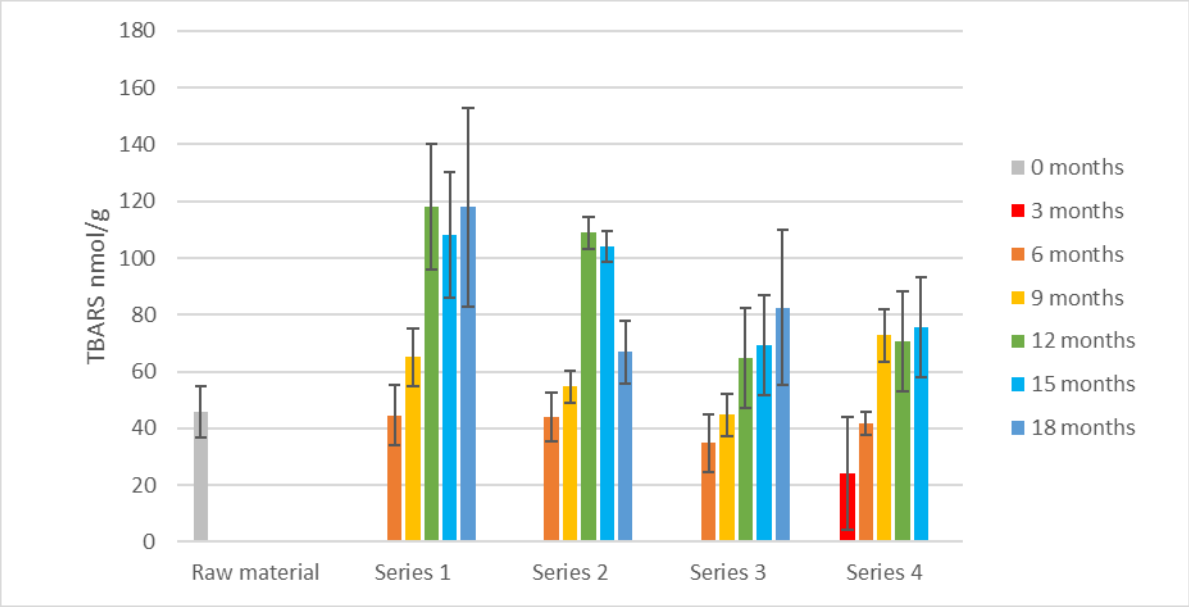

Figure 9 TBARS value of mackerel fillets with different pre-treatment and packaging over a period of 18 months in frozen storage. N = 5.

Peroxide value

Peroxide value (PV) is an indicator of primary oxidation which increases in the initial phases of an oxidation process and then decreases (Olsen et al. 2005). The results (Figure 10) show that all the series reach their highest PV 6–9 months after filleting and that series 1, which was not treated with antioxidant and not vacuum-packed, has significantly higher peroxide levels than the three other series when they had been stored for 9 months after filleting. The PV in all the series decreases when they are stored longer (12, 15 and 18 months). There is only a significant decrease between 12 months and 18 months for vacuum-packed fillets (series 2).

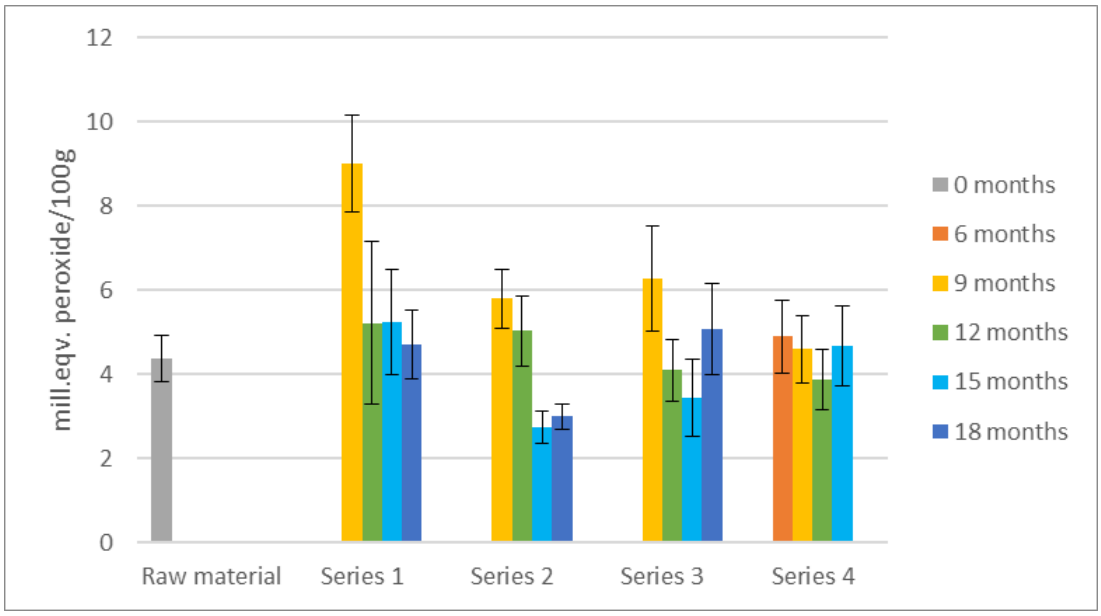

Figure 10 Peroxide values (PV) of mackerel fillets with different pre-treatment and packaging over a period of 18 months in frozen storage. N = 5.

Free fatty acids

There is a steady increase in the level of free fatty acids (FFAs) in the fat phase of the mackerel fillets during the storage trial (Figure 11). The percentage of FFAs is significantly higher already after 6 months, compared with chemical analysis of round mackerel when it is landed. No significant differences were registered in FFAs between the different series through the frozen storage period, with the exception of vacuum-packed fillets, where, after 12 months' storage, series 2 showed a significantly higher percentage of FFAs than series 3 and 4, which were treated with antioxidant.

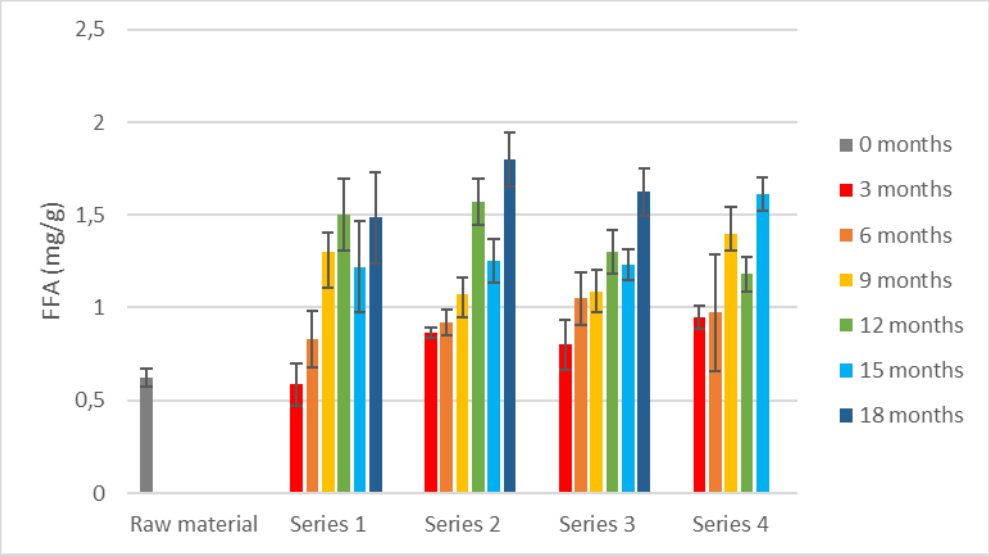

Figure 11 Free fatty acids (FFAs) in mackerel fillets with different pre-treatment and packaging over a period of 18 months in frozen storage. N = 5.

3.2 SENSORY EVALUATION OF RAW MATERIAL

Odour

The odour of the thawed fillets was evaluated pursuant to a 5-point scale where 5 is the best score. The results (Figure 12) show that there is a steady decrease in odour preference. Series 3 has a stronger average odour than series 1 and 2 from 6 months' storage and onwards, but the difference is not significant. Nor is there a significant difference between series 3 and 4. Irrespective of the pre-treatment and storage period, none of the fillets studied were deemed to be unfit for consumption.

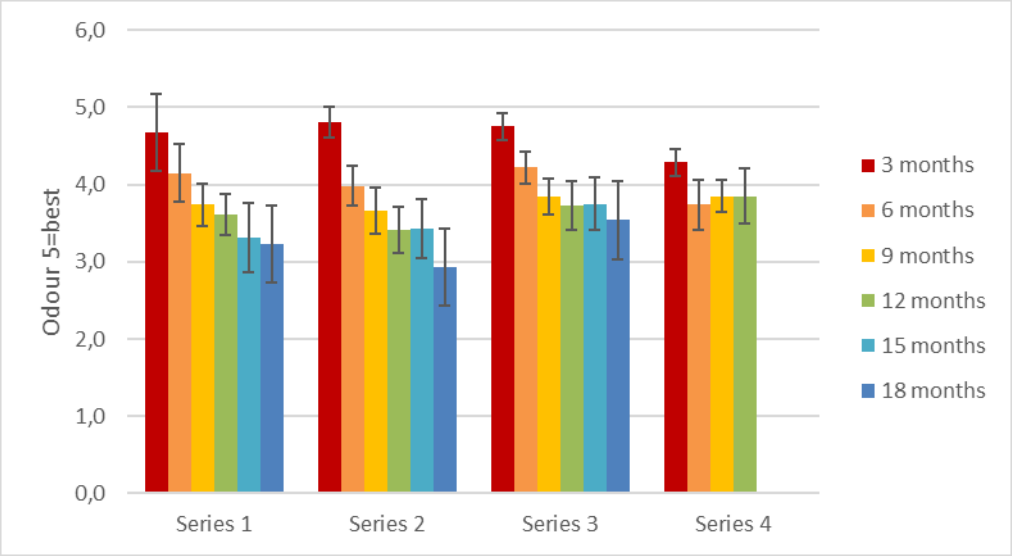

Figure 12 Evaluation of odour development in mackerel fillets with different pre-treatment and packaging over a period of 18 months in frozen storage. The odour was evaluated using a scale from 1 to 5 where 5 is best. N = 20.

Elasticity

Despite there being major individual differences between fillets in each series, a steady decrease in elasticity was observed during the period in frozen storage (Figure 13). For series 1 and 2, it is not until after 18 months that the difference becomes significant compared with the three-month sample. There are no significant differences in elasticity in fillets treated with antioxidant (series 3 and 4) from 3 to 15 and 18 months' storage.

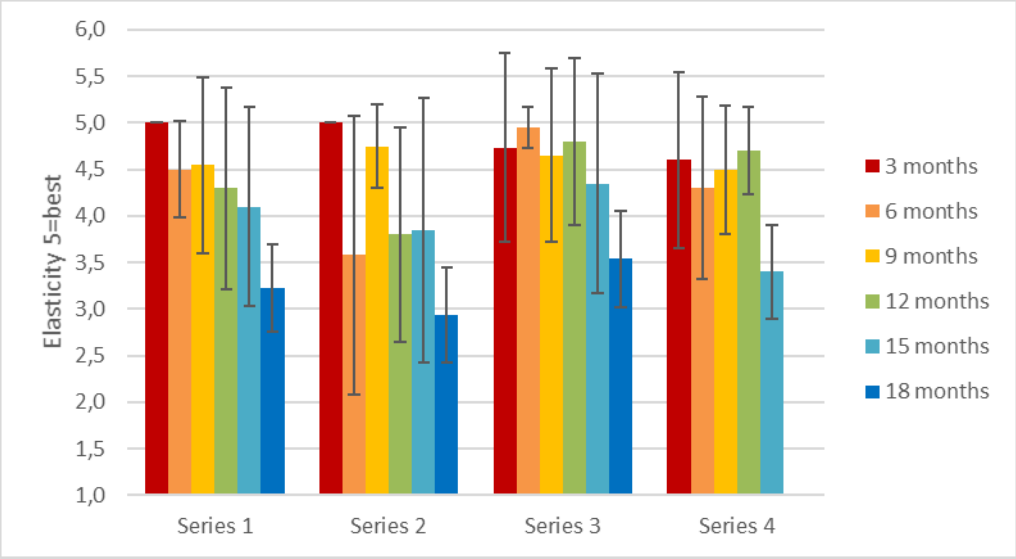

Figure 13 Change in elasticity in mackerel fillets with different pre-treatment and packaging over a period of 18 months in frozen storage. N = 20.

Texture

Figure 14 shows that the resistance in the texture of the fillet was highest in measurements performed at 9 and 12 months after filleting. There are no significant differences between the series, but series 3 has the highest average resistance after 9 months' storage.

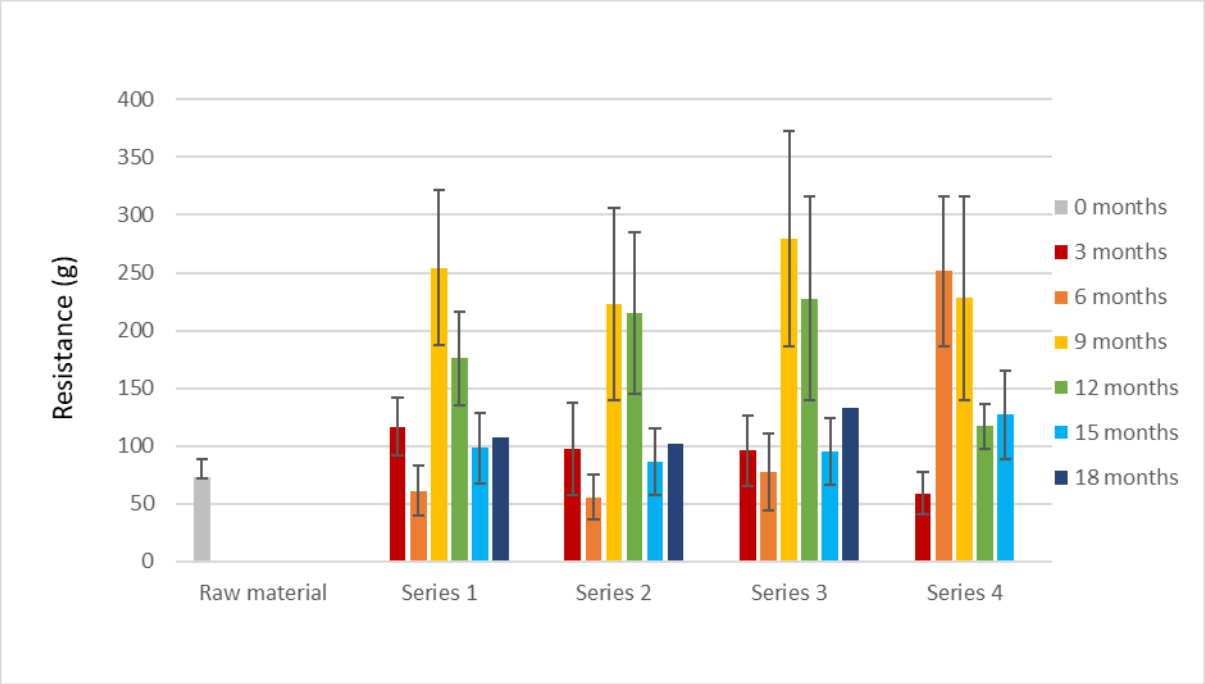

Figure 14 Change in texture in mackerel fillets with different pre-treatment and packaging over a period of 18 months in frozen storage. N = 20.

Early in the storage period, the fillet was observed to have maintained its shape better and had a convex shape that meant that the whole fillet was not in contact with the surface below it. This shape became less pronounced as the storage period progressed, so that the fillet came to lay flatter on the surface (Figure 15).

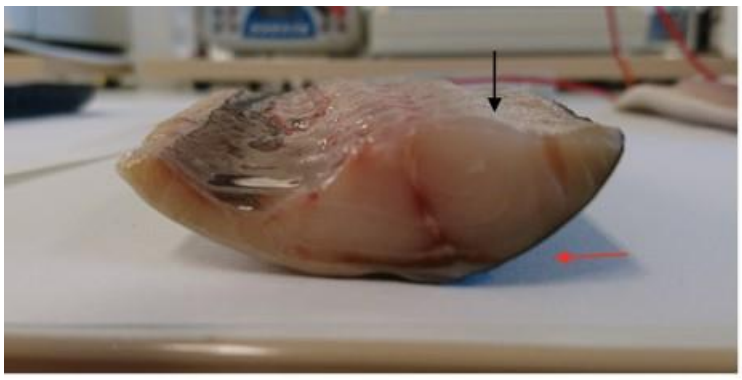

Figure 15 Illustration photo of the convex shape of the mackerel fillet while its texture was being analysed. The probe was inserted into the muscle in the area indicated by the black arrow. The area on the underside of the fillet that was not in contact with the surface during texture analysis is indicated by a red arrow.

Colour

The colour of the fillets steadily became more yellow during the storage period, and there are no significant differences between the series. Figure 16 shows that there was a significant increase in yellow colour from 3 to 9 months and again from 9 to 15 and 18 months for series 1, 2 and 3. For series 1, this means that the intensity of the yellow colour increased from 13.4 in the initial samples to 16,9 after 9 months. It continued to increase to 20.6 after 18 months. For series 4, which was filleted 3 months later, the same colour development is observed as for series 1, 2 and 3 if the criterion applied is the age of the raw material and not the time since filleting.

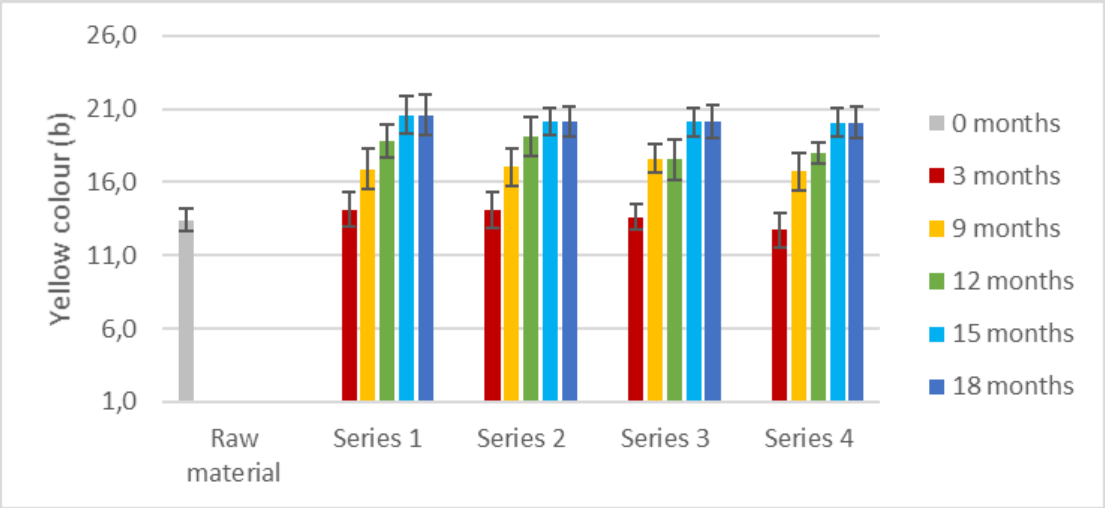

Figure 16 Development of yellow colour in mackerel fillets with different pre-treatment and packaging over a period of 18 months in frozen storage. N = 20.

As illustrated in Figure 16, there is a visible difference between a newly filleted fillet from five-month old raw material (fillet A in Figure 17), with a b-value of 12.4 compared with a fillet that has been in frozen storage for 9 and 18 months respectively (fillets B and C in Figure 17), with b-values of 16.6 and 20.5.

Figure 17 Colour difference between a newly filleted mackerel fillet (A) and fillets that has been frozen-stored for 9 (B) and 18 (C) months.

Gaping

The results (Figure 18) show that the degree of gaping is the same irrespective of the type of pre-treatment and storage time. There is generally a high degree of gaping in the fillets, which is probably a result of its treatment during processing, freezing and thawing.

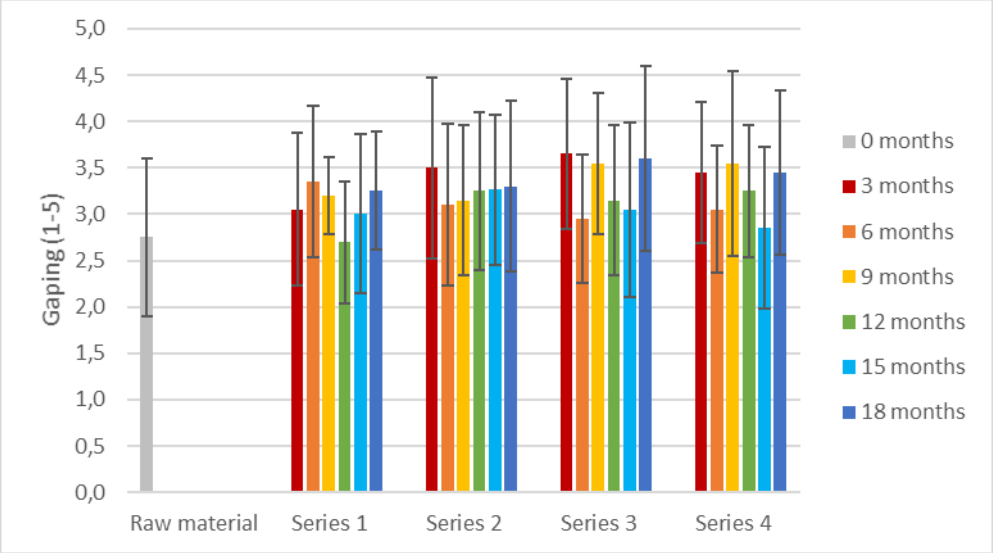

Figure 18 Development of gaping in mackerel fillets with different pre-treatment and packaging over a period of 18 months in frozen storage. Gaping evaluated on a scale from 1 to 5 where 5 is the highest degree of gaping. N = 20.

3.3 SENSORY QUALITY OF HEAT-TREATED FILLETS

Heat-treated mackerel fillets were tested every three months for differences between series and relevance for consumption. Table 7 shows that there were minor sensory differences between the different pre-treatment methods. However, there was a sensory difference between three-months frozen-stored mackerel fillet and newly-filleted fillets from raw material stored for 8 months (series 4). A sensory difference was observed between fillets packed in cardboard versus vacuum-packed after 6 months in frozen storage. After 18 months, fillets not treated with antioxidant were deemed to be of lower sensory quality than fillets treated with antioxidant. The fillets packed in cardboard (series 1) and frozen-stored for 18 months was not deemed fit for consumption in that 50% of the tested samples were described as unacceptable.

Tabell 7 Significant differences between frozen-stored mackerel fillets. All of the series were tested by comparison to series 1.

	Series 1 vs series 2	Series 1 vs series 3	Series 1 vs series 4
3 months	-	-	p = 0.1
6 months	p = 0.05	-	-
9 months	-	-	-
12 months	-	-	-
15 months	-	-	-
18 months	-	p = 0.05	-

4. DISCUSSION

Japanese-cut fillets (fillets with intact abdomen and peritoneum) are the most sought-after type of mackerel fillet in Asia, and the market demands documentation of the oxidation status of the end product. The results of the project have generated new knowledge about how oxidation in mackerel fillets produced from frozen raw material is affected by the age of the raw material, storage time, packaging and use of antioxidant on frozen round mackerel.

The results show that fillets produced from eight-month of frozen-stored round mackerel have the same shelf life as fillets produced from five-month of frozen-stored mackerel, and the shelf life of the products is good irrespective of pre-treatment, packaging and the use of antioxidant. It is only after one year in frozen storage that the fillets show signs of deteriorating quality.

In general, there are only minor differences between fillets packed in cardboard and those that are vacuum-packed. Previous trials with herring have shown that vacuum-packed products are considerably less prone to oxidation than products packed in cardboard (Bjørkevoll et al. 2002). The fact that this difference is not as pronounced in this trial may be due to the fillets being glazed after filleting and that glazing negates some of the advantages previously seen from vacuum-packing.

TBARS is a measurement of the rancidity of the fish muscle. The high fat content in mackerel makes products made from this raw material susceptible to rancidity. The results of the TBARS measurements in the trials show that fillets treated with the antioxidant rosemary have significantly lower oxidation than fillets packed in cardboard and vacuum-packed fillets in frozen storage for 12 and 15 months. Plant extracts are often used as natural antioxidants in fatty products, and have replaced synthetic food preservatives in recent years (Shah et al. 2014). The use of rosemary extract is widespread in the fish oil industry, and it is one of the most effective natural antioxidants on the market (abingredients.com).

The natural occurrence of antioxidants in the fish muscle can increase its shelf life. The breakdown of the natural antioxidant tocopherol (vitamin E) has been proven to lead to increased oxidation over time, when there is no longer a sufficient quantity of it to protect membrane lipids and prevent propagation (Erickson 1993). Trials on herring show a decrease in the antioxidant vitamin E during frozen storage (Kjerstad et al. 2014). In order to describe the consumption of vitamin E during storage, further studies analysing the vitamin E content in fish muscle should be considered. Another factor that contributes to major individual differences is that the fillets packed in cardboard boxes are subject to different degrees of air exposure depending on their position in the box. This hypothesis is supported by the fact that the individual differences were smaller when fillets were vacuum-packed on trays of ten.

The vacuum-packed fillet which had been in frozen storage for 18 months was the only series in which the TBARS value decreased. According to Ackman (2005), the TBARS value will increase initially before decreasing when the oxidation process is fairly advanced. As the last sample was taken at 18 months, it is not possible to determine whether the value will continue to decrease or whether it is a coincidence that precisely this value was so low.

Peroxide is used to measure primary oxidation, as it increases at the early stages of the oxidation process and then decreases (Ackman 2005, Olsen et al. 2005). The PV for all the different packaging and treatment methods were found to be highest from 6 to 9 months before decreasing. Only the vacuum-packed fillets (series 2) showed a significant decrease between 12 and 18 months. This result supports the TBARS results.

There was a significantly higher percentage of FFAs in the mackerel fillets in all the series compared with the raw material already after six months' storage. After 12 months' storage, vacuum-packed fillets have a significantly higher percentage of FFAs than fillets treated with antioxidant (series 3 and 4). The difference then levels out.

The oxidation measurements show major differences between fillets in the same series, which is reflected in the high standard deviations. Oxidation takes place differently in the different parts of the fillet. Studies on both herring and mackerel show differences between the skin, dark muscle and light muscle (Ke et al. 1977, Undeland et al. 1998). Studies of frozen-stored round mackerel show that the skin and the dark muscle right under the skin oxidises more quickly (Ke et al. 1977). Studies of frozen-stored herring fillet show the same result. The muscle right under the skin is more susceptible to oxidation, but this evens out over time (Undeland et al. 1998). The same is probably the case for mackerel fillet.

A sensory evaluation of odour and elasticity was conducted on all the samples (N = 20) included in the storage trial. Mackerel has a very strong distinctive odour and, with the exception of a few individual fillets, the expert panel only detected a slight degree of rancidity even after 18 months' frozen storage. Mackerel fillet that had been pre-treated with rosemary had the best odour on average, but, unlike the chemical results, these differences are not significant.

Previous trials on herring products have shown that a TBARS value of 50–80 correlates with reduced sensory quality (Bjørkevoll et al. 2002, Kjerstad et al. 2014). This is not in line with our trials on mackerel where, despite values approaching 120, we have not disqualified the raw material for consumption.

Heat-treated mackerel fillet will have a stronger smell and taste than raw fillet, and we assume that rancidity will only be detected once the fillet is heat-treated. Despite this, it was not until the 18-month mark that the panel deemed traditionally frozen and packed fillet as unfit for consumption.

Measurements of the yellow colour of the fillets show a steady increase during storage, from a b-value of 13.4 when the first samples were taken to just over 20 after 18 months' storage. The same development is described by Sone et al. (2017), where the yellow colour (b-value) increased from 12 to just over 14 after 12 months' storage. There are no significant differences between the different packaging and processing methods in our study. Sone et al. (2017) reported that the b-value (yellow colour) of fillet treated with antioxidant was 2% higher than in untreated fillet after four months' storage. This difference was not observed in this trial.

The results show that the elasticity of the fillet decreases during storage and the fillets thus, on average, become less flexible. This is a natural development during frozen storage and also correlates with the softer texture of the fillets after 12–18 months' frozen storage. Instrumental

texture analysis of the firmness of the fish fillets showed greater variation between the different frozen storage periods than between the different types of treatment. The firmest fillets were measured at 9 and 12 months' frozen storage, where resistance was as much as three times higher than in samples taken before and after. Before and after these two sampling rounds, the resistance (firmness) was about the same (approx. 100 grams resistance at 30% compression). The texture was expected to become softer after a long period of frozen storage as found in the study by Aubourg et al. (2013). We have not been able to find a good explanation for why, in our measurements, the firmness increases after 9 and 12 months' storage and then decreases, but one possible explanation is that, early in the trial, the mackerel fillets took on a convex shape (v-shape) so that the outer edges of the fillet were not in contact with the surface below (Figure 15). This may have led to movement in the fillet when the probe pressed into the fillet during the texture analysis. This may in turn have led to lower texture/resistance values than if the fillet had lain flat against the surface throughout the analysis. Another source of error in relation to the fillets bending upwards is that the height of the fillet is registered as higher than was actually the case, and this gives incorrect measurements since the texture analyzer is programmed to compress 30% of the registered initial height of the fillet. This error was not detected until after several rounds of samples had been analysed, and it was then deemed best to continue using the same texture analysis method for all the samples to ensure that the basis for comparison was as similar as possible.

Further on in the storage period, the fillets became softer and the whole skin side lay against the surface during the analyses, which is assumed to have resulted in more resistance/higher texture during analysis, since there was no air/space between the fillet and the surface as in earlier sample rounds (Figure 15). This may explain why the texture value increased in sampling at 9 and 12 months. The fact that the values decreased after 12 months may be due to the fillets becoming softer, which was registered during the elasticity analyses and when the fillets were handled after they had thawed. One of the reasons for the reduction in firmness and flexibility during frozen storage may also be due to the breakdown and changes in functionality in proteins, which is also reported to increase at higher frozen storage temperatures (Saeed and Howell 2002).

Although we see, as in Sone et al. (2017), that the structure of the fillet gradually becomes firmer during frozen storage of up to 12 months, studies involving texture analysis must ensure that the muscle measured is in full contact with the surface below it during the analyses. This can be done by analysing a cube of muscle of a given size rather than using the whole fillet and/or using the shear force method to analyse texture (Romotowska et al. 2016).

The production method was still under development during this trial, and thawing and packing involved many manual operations. The production process has been significantly improved since this trial started through optimisation and rationalisation as part of the development of the Pelagic pilot line at Selje over the past year. It is therefore reasonable to assume that this development work will also affect and prolong the shelf life of the product. There was a high prevalence of gaping in all the fillets in the trial. No differences were observed with respect to gaping between the series, and nor were differences observed in relation to storage times. The

optimisation that has taken place in the Pelagic pilot line ensures more gentle handling of the fillets, which can also be assumed to lead to less gaping in the product.

The fillets in this trial were stored at -30°C. The temperature during frozen storage affects oxidation and Saeed and Howell (2002) found major differences in the oxidation process between storage at temperatures of -20 and -30°C. It is therefore reasonable to assume that if the storage trial had been conducted at a higher temperature, we would have seen a faster deterioration in quality.

The results of the project contribute to reduce the uncertainty about Norwegian mackerel fillet products and their properties in relation to the Asian market. Knowledge of oxidation processes linked to the packaging and processes selected have provided important information about how the products should be processed and packed to achieve the quality the market demands. The project results will have utility value in that it will be easier to steer production towards optimal oxidation of the end product. Such factors will be vital for meeting important market requirements when Norwegian mackerel fillet is launched in the market.

5. CONCLUSION

The results of the oxidation study on frozen-stored mackerel fillet shows they have a good shelf life. According to the pelagic industry, it is important that the fillets have a shelf life of at least one year in order to meet customer requirements. The results show that the fillet series, irrespective of pre-treatment, had a shelf life of at least 15 months and thus meet the market requirement by a good margin.

- Fillets produced from raw material that had been in frozen storage for eight months have as long a shelf life as fillets produced from five-month old raw material.
- Using an antioxidant (rosemary) significantly improved shelf life measures in TBARS and FFAs in fillets stored frozen for 12 and 15 months after filleting.
- The fillet, irrespective of pre-treatment, had significant gaping and became visibly yellower in colour after 18 months' storage.
- After 18 months in frozen storage, the untreated mackerel fillet packed in cardboard boxes lined with plastic, was assessed as unfit for consumption by the sensory panel.

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7. APPENDIX

Appendix 1

Oxidation of marine oils pursuant to Ackman (2005).

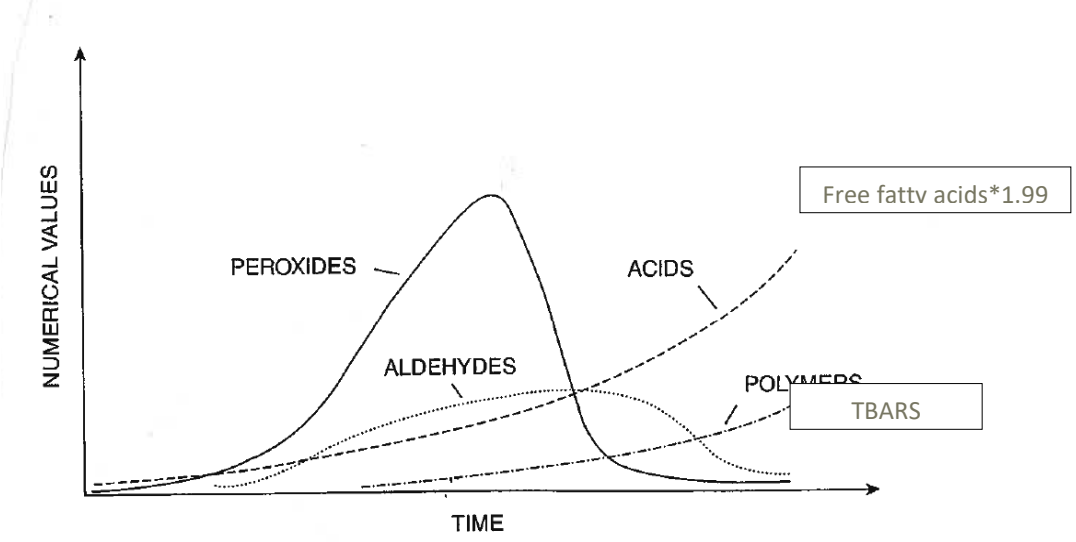

Figure 7: The figure shows the pattern for the development of peroxides and their degradation products in the oxidation of marine oils. Acids = FFA*1.99. (Ackman 2005).

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