

## THE EFFECT OF SMOKING METHODS ON THE QUALITY OF SMOKED MACKEREL

Dang Thi Thu Huong  
Faculty of Food Technology, Nha Trang University  
02 Nguyen Dinh Chieu, Nha Trang Vietnam  
danghuong2002@yahoo.com

Supervisors:  
Sigurjon Arason  
University of Iceland and Matis, Iceland  
sigurjon@matis.is  
Magnea G. Karlsdottir, Matis, Iceland  
magneag@matis.is  
Guðmundur Stefánsson, Matis, Iceland  
gudmundur.stefansson@matis.is

### ABSTRACT

Atlantic mackerel was smoked with commercial liquid smoked flavourings and wood smoke. After smoking, the mackerels were vacuum packed and stored chilled at  $-1\text{ }^{\circ}\text{C}$  for one week, then increased to  $4\text{-}5\text{ }^{\circ}\text{C}$  for three weeks. The quality changes in sensory attributes (odour, flavour), physical properties (colour, texture), microbial (total plate counts), and chemical properties (total lipid, peroxide value (PV), thiobarbituric acid reactive substance (TBARS), free fatty acids (FFA)) were observed after 0, 1, 2, 3 and 4 weeks of the chilled storage. Smoked mackerel using commercial liquid smoked flavourings was higher in rancid flavour, lightness, redness, and yellowness but had less bitter odour and was softer than the wood smoked mackerel. Further, the number of bacteria was lower in liquid smoke product after smoking but grew fast during chilled storage. The lipid oxidation was higher after the wood smoke process, but was rather stable during chilled storage. In contrast, lipid oxidation in the liquid smoke products increased during chilled storage. The shelf life of liquid smoked mackerel (2.6 % salt content,  $a_w = 0.98$ ) was determined to be three weeks, but wood smoked mackerel (2.6% salt content,  $a_w = 0.98$ ) was at least four weeks.

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## 1 INTRODUCTION

Smoking has been used for the preservation of food for centuries (Alcicek and Atar, 2010; Hattula *et al.*, 2001; Huss *et al.*, 1995; Maga, 1988; Petridis *et al.*, 2012; Martinez *et al.*, 2007; Rora *et al.*, 1998; Simko, 2002). Smoking gives the special colour and flavour to the food (Alcicek and Atar, 2010; Duffes, 1999) and extends its shelf-life via the effects of dehydration, anti-microbial and anti-oxidant of the smoke compounds (Alcicek and Atar, 2010; Goulas and Kontominas, 2005; Hattula *et al.*, 2001; Pagu *et al.*, 2013; Rørvik, 2000; Visciano *et al.*, 2008). Smoking also changes the texture of product (Sigurgisladottir *et al.*, 2001).

This technique has been developed over time, from smoking over the campfire or in a smokehouse, to an industry with large quantities of luxury products. The way to produce smoked food varies among different producers within one country, and the whole world (Huss *et al.*, 1995). In the seafood market, the smoking sector plays an important role. Since the 1990s, the consumption of smoked fish has increased, and smoked salmon is the most consumed product followed by smoked trout and herring (Cardinal *et al.*, 2006).

Smoking has been studied and applied in the world for a long time. However, the average Vietnamese consumer is not familiar with smoked products. In Vietnam there are few smoked fish products. Salmon is the most popular species, but the price of smoked salmon has increased considerably which makes it more difficult for people to purchase it.

The goal of this project is to find out whether one can use a cheaper species instead of salmon, such as smoked mackerel which has a similar flavour, texture, lipid content etc. as salmon. This project also aims to gain better understanding of the influence of different smoking methods on the quality of smoked products would offer prospects for quality improvement of smoked products in Vietnam. The objectives of this project are therefore to determine the quality of smoked mackerel using wood smoke and commercial liquid smoke flavourings, and to determine the quality changes of smoked mackerel during chilled storage.

The tasks of this project are: evaluate sensory attributes of raw material and final product after smoking and chilled storage; evaluate physical properties (colour, texture) of final product after smoking and during chilled storage; evaluate lipid quality (peroxide value (PV), thiobarbituric acid reactive substance (TBARS), free fatty acids (FFA)) of raw material, final product after smoking and during chilled storage; determine water, protein, lipid content and total volatile basic nitrogen (TVB-N) of raw material and final product; determine total plate count (TPC) of raw material and final product after smoking and during chilled storage.

## 2 LITERATURE REVIEW

### 2.1 Fish smoking

Smoking is one of the oldest methods used to process and preserve fish (Bilgin *et al.*, 2008; Hultmann *et al.*, 2004; Simko, 2002; Stołyhwo and Sikorski, 2005; Swastawati *et al.*, 2000). Smoking can inhibit the formation of toxins in products (University of Florida, 2004), reduce the growth of bacteria, due to lower water activity by smoking in combination with salting and drying which creates a physical surface barrier (Rørvik, 2000; Swastawati *et al.*, 2000). The spoilage and pathogenic microflora of smoked products are affected by density of smoke, concentration of active components of the smoke in combination with the salt content, and the time and temperature of smoking (Kolodziejska *et al.*, 2002).

After smoking, the colour and flavour of fish are changed (Visciano *et al.*, 2008). Smoked fish has specific odour, taste and yellow colour (Swastawati *et al.*, 2000). Nowadays, shifting for high sensory quality product is the main purpose of smoking. The smoked products have higher moisture and lower salt content than in the past (Kolodziejska *et al.*, 2002). The smoking process is characteristically a combination of salting, drying, smoking (Alcicek and Atar, 2010; Bhulyan *et al.*, 1986; Kenneth and Hilderbrand, 1992; Sigurgisladottir *et al.*, 2000) followed by vacuum, modified or controlled atmosphere packaging (University of Florida, 2004).

In the past, smoked fish was typically produced with high salt and low moisture content. Nowadays, producers have adjusted the processing condition to produce lower salt products to fulfil consumer demands. The drying step is therefore carried out before and after smoking to remove the moisture from the flesh to increase the water phase salt (WPS) and strengthen the texture of the final product. Drying is affected by heat, humidity, air velocity, and characteristics of material (Kenneth and Hilderbrand, 1992).

Depending on the way smoke gets into products, smoking can be categorized accordingly: the traditional technique – where the smoke is formed directly by burning chips or sawdust from firm wood in the oven (Stołyhwo and Sikorski, 2005; Visciano *et al.*, 2008); or new technique - by using an electric field acts on the ionised smoke particles, which quickens the smoke deposition or by using commercial liquid smoke flavourings (Duffes, 1999; Martinez *et al.*, 2007). Furthermore, smoking can be defined as hot smoking, warm smoking or cold smoking depending on the smoking temperature (Duffes, 1999; University of Florida, 2004; Rørvik, 2000; Stołyhwo and Sikorski, 2005). Cold smoking has only one basic function which is applying smoke to the product while the hot smoking has the function of applying heat and cooks the product (Kenneth and Hilderbrand, 1992). It is therefore not necessary to cook hot smoked fish before consumption because it is a ready-to-eat food.

The quality of smoked fish is affected by raw material (Cardinal *et al.*, 2001; Rora *et al.*, 1998), salting method, brining concentration (Alcicek and Atar, 2010; Goulas and Kontominas, 2005; Sigurgisladottir *et al.*, 2000) condition processing (Duffes, 1999), composition of smoke (Kenneth and Hilderbrand, 1992; Stołyhwo and Sikorski, 2005) smoking method (Cardinal, *et al.*, 2006), smoke agents (Siskos *et al.*, 2007) and storage conditions.



## 2.2 Smoking methods

### 2.2.1 Hot smoking

Hot smoking is known as the traditional smoking method (Arason *et al.*, 2014). The products have high salt and low moisture content. Safe hot smoked fish requires at least 3.5% water phase salt (WPS) and must have achieved an internal product temperature of at least 145 °F (62.8 °C) for at least 30 minutes (Kenneth and Hilderbrand, 1992; University of Florida, 2004). This prevents the production of toxins by *Clostridium botulinum* (Kenneth and Hilderbrand, 1992). Additionally, water activity ( $a_w$ ) of hot smoked fish products must be less than 0.85 to make products stable at room temperature (Arason *et al.*, 2014).

The hot smoking of fish includes five steps: surface drying, smoking, drying, heating/cooking and cooling. Cooling the fish to lower than cooking temperature is carried out immediately in the smoke house. Then, cooling down to less than 38 °F (3.3 °C) as quickly as possible but not in the smokehouse and keeping products at that temperature to reduce the growth of food poisoning bacteria until consumption (Kenneth and Hilderbrand, 1992). Hot smoking has been applied to different fish species such as: tuna, mackerel, halibut and sardine.

### 2.2.2 Cold smoking

Cold smoking is a smoking method where the temperature is maintained below 95 °F (35 °C) and the final salt content in the product must be at least 3.5% WPS (Kenneth and Hilderbrand, 1992; University of Florida, 2004). Arason *et al.*, (2014) suggests that the relative humidity during cold smoking should be remained in the range of 75–85%. Vacuum packed and chilled storage should be followed by cold smoking because product is not completely preserved (Kenneth and Hilderbrand, 1992; Rørvik, 2000).

## 2.3 Smoke agents

### 2.3.1 Wood smoke

Wood smoke is produced by smouldering chips or sawdust of firm wood below the fish in the smokehouse (Visciano *et al.*, 2008). The composition of wood has an effect to the taste of the final product. Wood used as a smoke source is hardwood such as: beech, hickory, oak or fruitwood as apple, pear jackfruit, etc.

### 2.3.2 Commercial liquid smoke flavourings

Smoke flavourings have been used for the preservation and aromatization of meat and fish for over 40 years (Hattula *et al.*, 2001). It is made by distilling dry wood which is then concentrated to a specific concentration (Arason *et al.*, 2014). Concentrated smoke can be used directly on products or dissolved in water or oil (Maga, 1988). Polycyclic aromatic hydrocarbons (PAHs), the largest class of chemical compounds known to be cancer causing agents (Simko, 2002), is not present in liquid smoke flavourings thus, it can be evaluated as safe for health (Alcicek and Atar, 2010). Using liquid smoke has some advantages over traditional smoking techniques such as lowering costs, less

environmental damage and greater availability and variety of application methods (Hattula *et al.*, 2001; Maga, 1988).

## 2.4 Atlantic mackerel (*Scomber scombrus*)

The Atlantic mackerel is classified as a fat fish species that belong to the Scombridae family of fish (Figure 1). Atlantic mackerel is distributed on both sides of the North Atlantic Ocean, including the Baltic Sea. Atlantic mackerel is found in cold and temperate waters. They are typically a surface-living species and swim in schools (NOAA, 2014).



Figure 1: Atlantic mackerel (*Scomber scombrus*).

Mackerel is a valuable pelagic fish and most of the catch is for human consumption (Icelandic Ministry of Fisheries, 2014). The mackerel is a fatty fish, and the fat and water content vary with season. The fat content is about 6-23%, water content is 56-74% and protein content is 18-20 % throughout the year (FAO, 2014). It is considered one of the more healthy fish because it is rich in omega-3 fatty acids and an excellent source of selenium, niacin, and vitamins B6 and B12 (NOAA, 2014).

## 3 MATERIALS AND METHODS

### 3.1 Materials

#### 3.1.1 Atlantic mackerel

Frozen Atlantic mackerel (*Scomber scombrus*) was used in this study. The material was harvested by Purse Seine on 28<sup>th</sup> of July 2013 in the South-East of Iceland. The mackerels were beheaded and gutted on 30<sup>th</sup> of July 2013 and kept at -1.2 °C. The temperature of raw material when landed was -0.9 °C and after packaging was 3.5 °C. Raw material was stored at -18 °C for 6 months before processing. The experiments were carried out at MATIS laboratories in Reykjavik, Iceland.

#### 3.1.2 Smoke agents

- Wood smoke was produced by fireplace wood bought from Husasmidjan in Reykjavik.
- Commercial liquid smoke flavouring (SMOKEZ CLASSIC 5116) was produced by Red Arrow International Company and supplied by the Nokk Company, Iceland.

Concentrated aqueous solution of natural smoke flavours produced by controlled pyrolysis of mixed hardwoods with a foodgrade emulsifier added. The chemical properties of SMOKEZ CLASSIC 5116: pH: 2.5 - 3.5; total acidity (as acetic): 10.5 – 12.0 %; smoke flavour compounds: 13.0 – 20.0 mg/ml; carbonyls: 16.0 – 20.0 %; density (avg): 1.13 kg/litter. The physical properties: clear, brown liquid with characteristic hardwood smoke aroma.

### 3.1.3 Salt

Suprasel salt (25 kg Net) of AkzoNobel A/S company with ISO 9001 certified was used in this study.

### 3.1.4 Packaging material

The vacuum bag (polyamide – PA) was supplied by the PMT Company, Iceland was used in this study.

### 3.1.5 Chemicals

All the chemicals used in this study were of analytical grade and obtained from Sigma Aldrich.

## 3.2 Methods

### 3.2.1 Pre study

Before doing the main experiments, pre-trials were carried out to determine the appropriate brining time and salt concentration to reach final content of 2.6% in the final product. After thawing in air (17 hours), filleting and washing with water at 4 °C, fillets were divided in two groups. Group W was immersed in brine (1:1) containing 100 g/L NaCl at 0-5 °C. After 2, 3 and 4 hours, samples were taken to determine the salt content. Group L was immersed in smoked brining (1:1) containing 5 mL commercial liquid smoke flavourings and 50, 60, 70 g/L NaCl overnight (16 hours) at 0-5 °C. The samples were also taken to determine the salt content. The results from the pre- trials are shown in Table 3 (appendix) and were considered when designing the main experiments.

### 3.2.2 Experimental design

Flowchart of the experimental design is presented in Figure 2. Frozen mackerels were received and thawed in air at 0-5 °C for 17 hours. After filleting and washing with water at 4 °C, the fillets were divided in two groups. Group W was immersed in brine (1:1) containing 100 g/L NaCl at 0-5 °C for three hours, then dried (surface) at room temperature (~20 °C) for two hours and smoked directly with wood smoke in a smoking chamber at 25 °C for 3hours. After smoking, group W was cooled at 0-5 °C for one hour and vacuum packed.

Group L was immersed in smoked brining solution (1:1) containing 5 mL commercial liquid smoke flavourings and 60 g/L NaCl overnight (16 hours) at 0-5 °C. After smoking, group L was dried at 25 °C for two hours in an oven (Memmert –Typ: UFP 500, Germany) with air circulation. Products were cooled at 0-5 °C in one hour and vacuum packed. After vacuum packaging, the final products of two groups were kept at -1 °C for one week. The temperature was then increased 4 °C and stored further for three weeks.

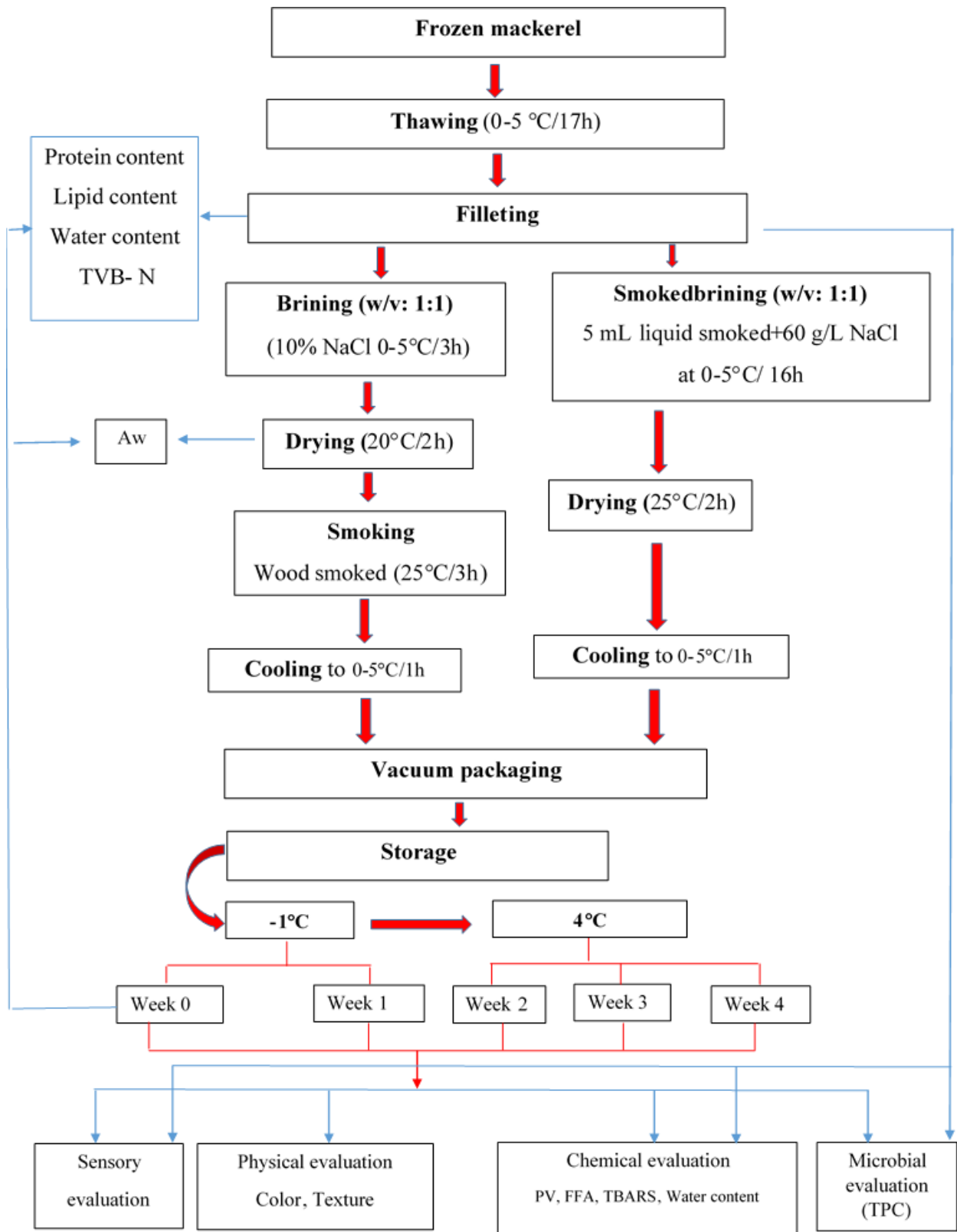


Figure 2: The flowchart of the experiments for processing smoked mackerel.

### 3.2.3 Sampling

Samples were taken on the arrival of the raw material, after smoking and after 1, 2, 3 and 4 weeks of storage for evaluation of sensory, colour, texture, peroxide value (PV), free fatty acid (FFA), thiobarbituric acid reactive substances (TBARS), water content and total plate counts (TPC). Additionally, water content, total volatile basic nitrogen (TVB-N), protein and lipid content were measured on the arrival of the raw material and after smoking. Water activity ( $A_w$ ) was determined after drying and smoking. For analysis, four fillets were taken for each sampling point (chemical, physical, microbial) and six fillets were taken for sensory evaluation. Total of 216 fillets of mackerel were used for 88 analyses in this study.

### 3.2.4 Temperature measurements

The temperature during brining, drying, smoking and storage was recorded with five minutes intervals, by data loggers placed in the brining solutions, on each rack of the oven and smoke house and in the cooling room. Additionally, the temperature in the centre of fish was measured during smoking by a thermometer.

### 3.2.5 Methods of analysis

#### **Sensory evaluation**

*Sensory panels:* 11 panellists of MATIS's sensory panel participated in evaluation of fresh mackerel fillets and smoked mackerel fillets. They had previously been selected by a procedure as describe by Meilgaard *et al.*, (1999). The members of the panel were previously trained specifically in quantitative descriptive analysis (QDA) for odour, flavour and texture attributes of fresh mackerel fillet and odour, flavour attributes of smoked mackerel.

*Evaluation:* Each attribute, as shown in Table 1 for fresh mackerel fillets and in Table 2 for smoked mackerel fillets, was evaluated by every panellist in one whole fillet on a 100 point line scale anchored by the opposites 'none' to 'much'. The panellists evaluated each sample for each sampling day in triplicate while seated in separate booths under normal light in the sensory evaluation laboratory. Panellists used a computerised system for direct recording data. The fresh fish was cooked and the smoked mackerel fillets were cut in to thin pieces, and each portion placed in a small aluminium box.

Table 1: Quantitative descriptive analysis of fresh mackerel fillets.

<b>sensory attribute</b>	<b>short name</b>	<b>scale</b>	<b>definition</b>
<i>ODOUR</i>			
fresh oil	O-oil	none    much	Fresh fishoil odour
metallic	O-metallic	none    much	Metallic odour
sweet	O-sweet	none    much	Sweet odour
mouldy	O-mouldy	none    much	Mouldy odour
butiric acid	O-butiric	none    much	Butiric acid, smelly feet
rancid	O-rancid	none    much	Rancid odour
<i>FLAVOUR</i>			
fresh oil	F-oil	none    much	Fresh fishoil flavour
metallic	F-metallic	none    much	Metallic flavour
sweet	F-sweet	none    much	Sweet flavour
acidic	F-acidic	none    much	Acidic, sour flavour
mouldy	F-mouldy	none    much	Mouldy flavour
bitter	F-bitter	none    much	Bitter flavour
rancid	F-rancid	none    much	Rancid flavour
<i>TEXTURE</i>			
soft	T-soft	firm    soft	Softness in first bite
juicy	T-juicy	dry    juicy	Dry: draws liquid from mouth. Juicy: releases liquid when chewn
tender	T-tender	tough    tender	Tenderness when chewn
mushy	T-mushy	none    much	Mushy, porridge like texture
sticky	T-sticky	none    much	Glues together teeth when biting the fish.

Table 2: Quantitative description analysis of smoked mackerel fillets.

<b>sensory attribute</b>	<b>short name</b>	<b>scale</b>	<b>definition</b>
<i>ODOUR</i>			
butiric acid	O-butiric	none    much	butiric acid, smelly feet
rancid	O-rancid	none    much	rancid odour
spoilage sour	O- sour	none    much	spoilage sour odour
TMA	O-TMA	none    much	TMA odour (trimethylamine)
spoilage	O-spoilage	none    much	other spoilage odour, describe in comment line
<i>FLAVOUR</i>			
bitter	F-bitter	none    much	bitter flavour
rancid	F-rancid	none    much	rancid flavour
spoilage sour	F- sour	none    much	spoilage sour flavour
TMA	F-TMA	none    much	TMA flavour (trimethylamine)
spoilage	F-spoilage	none    much	other spoilage flavour describe in comment line

The data were recorded by FIZZ, Version 2.47B, 1994-2012, Biosystèmes and analysed by NCSS 2000 (NCSS, Utah, USA) using GLM (general linear model) and Duncan's test. Panel performance: Panel check V1.4.0 (Nofima, Tromsø, Norway).

## Physical Analyses

### Colour

The intensity of the flesh colour was measured with a Minolta Chroma Meter CR-400 (Minolta, Osaka, Japan) using the CIE Lab system. The instrument recorded the *L* value, lightness on the scale of 0 to 100 from black to white; *a* value, (+) red or (-) green; *b* value, (+) yellow or (-) blue. The colour was measured above the lateral line at five positions, from the head to the tail of each fillet as shown in Figure 3.

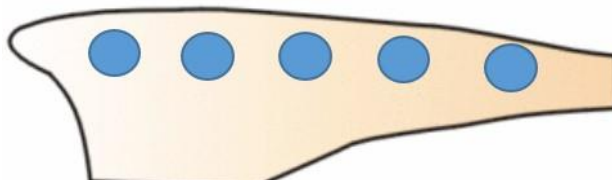


Figure 3: The colour measurement sampling spots on the fillet.

### Texture

Warner-Bratzler shear blade (type HDP/BS) was applied on fillet in each fish. The samples were of equal size, 2.0 cm in diameter and 1.5 cm in thickness above the lateral close to the head as shown in Figure 4. A v-shaped blade with a thickness of 3.20 mm, height of 125 mm and width of 70 mm was assembled to the TA.XT2i Texture Analyses. The maximum peak force in Newton required to shear through the sample was recorded as shear force. This method incorporated compression of fibers beneath the blade, tension in adjoining fibers and shearing of the fibers (Bouton *et al.*, 1975).

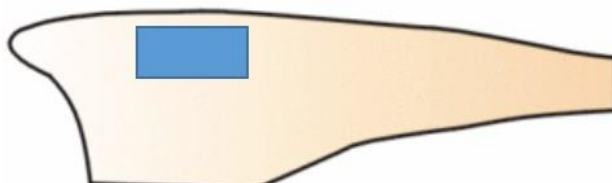


Figure 4: The texture measurement sites on the fillet.

## Chemical Analyses

### Water content

Water content was determined according to ISO 6496:1999. About 5 g of sample was weighed accurately ( $\pm 1$  mg) and placed in an aluminium foil dish which was prepared with a thin layer of sea-sand and a glass rod. The samples were mixed thoroughly with the sand. The glass rod was kept on the dish and then left to dry for  $4 \pm 0.1$  h in the oven at  $103^\circ\text{C}$ . The dish was removed from the oven and allowed to cool to ambient temperature in a desiccator for about 15 minutes. The water content was calculated by the formula as follows:

$$W = \frac{m_1 - (m_3 - m_2)}{m_1} * 100[\%]$$

Where:  $m_1$  was the mass of the test portion (g),  $m_2$  was the mass of the dish, test portion, sand and glass rod (g),  $m_3$  was the mass of the dish, dried test portion, sand and glass rod (g).

#### *Salt content*

Salt content of products was determined according to (AOAC 17<sup>th</sup> ed 2000 no 976.18). Soluble chloride was extracted from the samples with water. Upon addition of nitric acid, the solution was titrated with silver nitrate and the end point was determined potentiometrically.

#### *Protein content*

Protein content of all samples was determined by the Kjeldahl method (ISO 5983-2:2005). A sample of 5 g was digested in sulphuric acid in the presence of copper as a catalyst. Thereafter, the sample was placed in a distillation unit, 2400 Kjeltac Auto Sample System. The acid solution was made alkaline by a sodium hydroxide solution. The ammonia was distilled into boric acid and the acid was simultaneously titrated with diluted  $H_2SO_4$ . The nitrogen content was multiplied by the factor 6.25 to get the ratio of crude protein.

#### *Lipid content*

Total lipids (TL) were extracted from 25 g samples ( $80 \pm 1\%$  water) with methanol/chloroform/0.88 %  $KCl_{(aq)}$  (at 1/1/0.5, v/v/v) according to the Bligh and Dyer (1959) method. The lipid content was determined gravimetrically and the results were expressed as grams lipid per 100 g wet muscle.

#### *Free fatty acids*

Free fatty acids (FFA) were determined according to method from Lowry and Tinsley (1976) with a modification made by Bernardez *et al.*, (2005). About 3 mL of the lower phase resulting from lipid extraction (Bligh and Dyer 1959) was added in a screw cap culture tube. Any solvent present was removed at 55 °C using nitrogen jet. After cooling down, 3 mL of cyclohexane were accurately added by 1 mL of cupric acetate – pyridine reagent and vortex for ~40s. After centrifugation at 2000 rpm for 10 min at 4 °C, the upper layer was read at 710 nm in spectrophotometer. The FFA concentration in the sample was calculated as  $\mu\text{mol}$  oleic acid based on a standard curve spanning a 2-14  $\mu\text{mol}$  range.

#### *Peroxide value (primary oxidation product)*

Lipid hydroperoxides (PV) were determined with a modified version of the ferric thiocyanate method (Shantha and Decker, 1994). Total lipids were extracted from 5.0 g of samples with 10 mL ice-cold chloroform: methanol (1:1) solution, containing 500 ppm BHT to prevent further peroxidation during the extraction process. Sodium chloride (0.5 M) was added (5.0 mL) in to the mixture and homogenized for 30 sec before centrifuging at 5100 rpm for 5 min (TJ-25 Centrifuge, Beckmann Coulter, USA). The chloroform layer was collected (100  $\mu\text{L}$ ) and completed with 900  $\mu\text{L}$  chloroform: methanol solution. A total amount of 5  $\mu\text{L}$  of ammonium thiocyanate (4 M) and



ferrous chloride (80 mM) mixture (1:1) was finally added. The samples were incubated at room temperature for 10 min and read at 500 nm (Tecan Sunrise, Austria). A standard curve was prepared using cumene hydroperoxides. The results were expressed as mmol lipid hydroperoxides per kg of wet muscle.

*Thiobarbituric acid reactive substance (secondary oxidation product)*

A modified method of Lemon (1975) was used for measuring thiobarbituric acid reactive substance (TBARS). A sample (5.0 g) was homogenized with 10.0 mL of trichloroacetic acid (TCA) extraction solution (7.5% TCA, 0.1% propyl gallate and 0.1% EDTA mixture prepared in ultra-pure water) using a homogenizer at maximum speed for 10 seconds (Ultra-Turrax T-25 basic, IKA, Germany). The homogenized samples were then centrifuged at 5100 rpm for 20 min (TJ-25 Centrifuge, Beckmann Coulter, USA). Supernatant (0.1 mL) was collected and mixed with the 0.9 mL thiobarbituric acid (0.02 M) and heated in a water bath at 95°C for 40 min. The samples were cooled down on ice and immediately loaded into 96-wells microplates (NUNC A/S Thermo Fisher Scientific, Roskilde, Denmark) for reading at 530 nm (Tecan Sunrise, Austria). A standard curve was prepared using tetraethoxypropane. The results were expressed as  $\mu\text{mol}$  of malomaldehyde diethylacetal per kg of wet muscle.

*Total volatile basic nitrogen (TVB-N)*

The TVB-N was determined by dissolving 100 g of the fillets mackerel sample extract with 200 mL 7.5% aqueous trichloroacetic acid in a metal beaker and homogenized in Waring blender. Filtering the mixture through a Whatman no 3 filter paper. 25 mL of the filtrate was pipetted into a distillation flask with 6 mL 10% NaOH. Steam distillation was then carried out using the Kjeldahl-type distillator (Struer TVN) and the TVB-N collected in 10 mL 4% boric acid (cont 0.04 mL of methyl red and bromocresol green) indicator which turned green when alkalinized by the TVB-N (Malle and Poumeyro, 1989). The solution was then titrated with 0.0372 N sulphuric acid until there was a complete neutralisation of the base which was indicated by a colour change to pink. The TVB-N content was calculated by the formula:

$$\frac{14 \frac{\text{mg}}{\text{mol}} \times a \times b \times 300}{25 \text{ mL}} \left[ \frac{\text{mgN}}{100\text{g}} \right]$$

Where a: volume of sulphuric acid (mL) b: normality of sulphuric acid (%)

*Water activity*

Water activity was defined as the vapour pressure of water divided by that of pure water at the same temperature, therefore, pure distilled water had a water activity of exactly one. Water activity was measured by Novasina AW – Center (AWC503 RS – C, Axair AG, Swizerland) equipment at standard temperature.

## **Microbial Analysis**

### *Total plate count (TPC)*

According to the method in the laboratory at MATIS ‘The conventional "pour-plate" method was used on Plate Count Agar. 20 g of fish samples was aseptically weighed in stomacher bags and mixed with 180 ml of maximum recovery diluent (MRD) (0.85 % NaCl + 0.1 % peptone). Then homogenized for 2 minutes in a Waring laboratory blender and serially diluted up to  $10^9$  and inoculated in growth media in Petri dishes. For the analysis of total plate count (TPC) 1 mL of 1/10 dilutions was transferred using pipette to Petri plates and melted Iron agar at 45°C poured on the plates and the content mixed to solidify. After solidification the plates were covered with a thin layer of Iron agar then incubated at 22°C for 48 hours. All the microbiological analysis was conducted in duplicate and data expressed as a logarithm of the number of colony-forming units (log cfu/g).

### *Statistical analysis*

All data summaries and statistical analyses were conducted using STATISTICA software (Version 12.0, StatSoft, Inc. 2300 East 14<sup>th</sup> Street Tulsa, OK 74104 USA); SigmaStat software (Version 3.5, Dundas Software Ltd., GmbH, Germany) and MS- Excel 2013. Means between two methods (liquid smoke and wood smoke) were compared using t-test independent. The Tukey HSD test was used to compare the different means between storage times. Pearson correlation was used to test the relation between quality attributes (colour, texture, free fatty acid, peroxide value, thiobarbituric acid reactive substance and total plate counts) and relation with storage time. Significance of difference was defined at  $p < 0.05$ .

## 4 RESULTS

### 4.1 Chemical composition of Atlantic Mackerel

The chemical composition of the raw fillets was measured after thawing. The water content was  $55.85 \pm 1.77\%$ , the crude protein content was  $19.65 \pm 0.35\%$ , and the lipid content was  $21.54 \pm 8.24\%$ . Additionally, total volatile basic nitrogen (TVB-N) was determined to be  $15.7 \pm 0.57$  mg N/100g.

### 4.2 The temperature profiles during brining, smoking, drying and chilled storage

The temperature profiles of smokedbrining of liquid smoke and brining of wood smoke are shown in Figure 5. The smokedbrining and brining temperature were from 3 °C to 4 °C, however, brining temperature was rather stable.

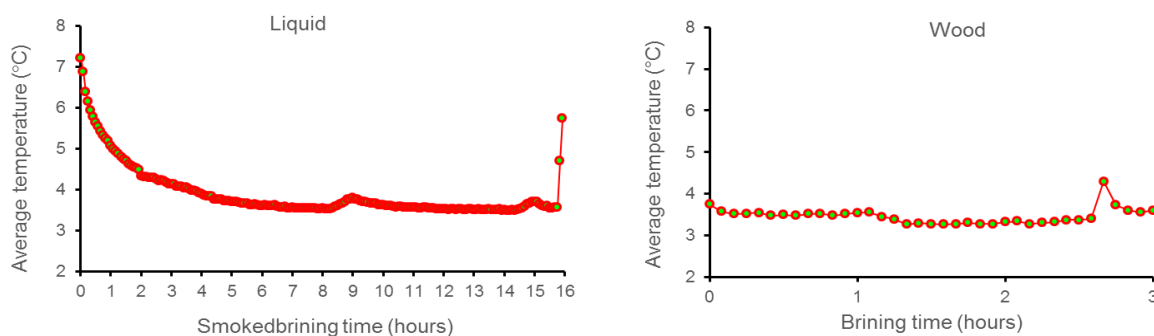


Figure 5: Temperature profile in five minutes interval of smokedbrining and brining.

The temperature profiles during drying of the liquid smoked group and smoking temperature profiles of wood smoked group are shown in Figure 6. The result shows that the drying temperature was rather stable at  $23.9 \pm 0.2$  °C. However, the temperature was not as well distributed in the smokehouse. The temperature was higher on the top and decreased at the bottom. The temperature was  $42.1 \pm 10.65$  °C,  $39.08 \pm 8.04$  °C and  $25.3 \pm 4.99$  °C at the top, middle and bottom respectively.

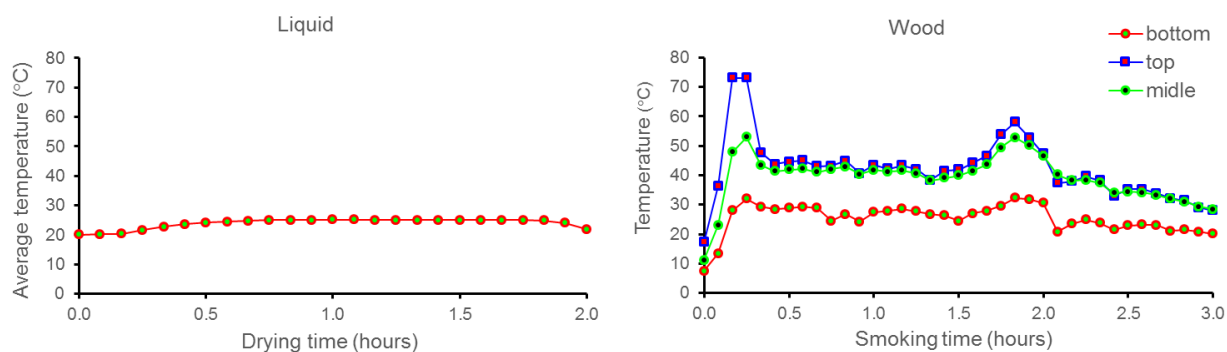


Figure 6: Temperature profile in five minutes interval of drying (in the oven) and smoking (in the smoke house).

After smoking, the final products were kept at  $-1^{\circ}\text{C}$  for one week. Then the temperature was increased up to  $4\text{--}5^{\circ}\text{C}$  for three weeks (Figure 7). In general, the storage temperature was stable, and was on average about  $4.83\pm 0.18^{\circ}\text{C}$ .

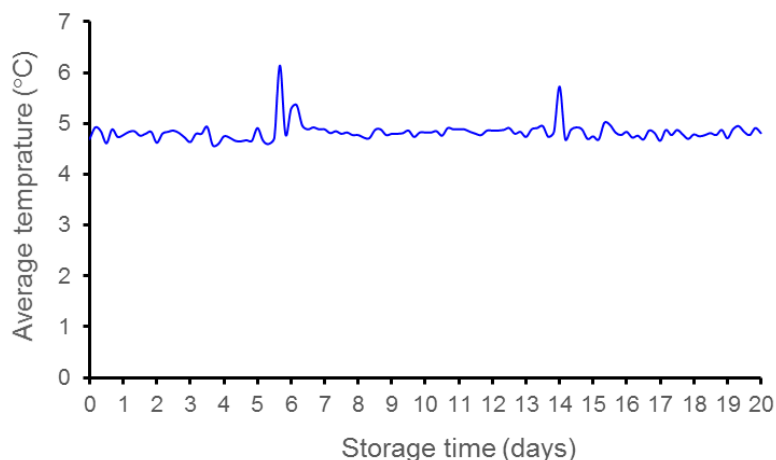


Figure 7: Temperature profile of cooling room during chilled storage.

### 4.3 Chemical composition of final products

After smoking, the salt content of the two sample groups was determined to be 2.6% and water activity was 0.98. In comparison with the raw material, the TVB-N of liquid group was decreased significantly, from initial value of  $15.7\pm 0.57$  mg N/100g to  $9.5\pm 0.99$  mg N/100g. The TVB-N of the wood sample was  $15\pm 0.57$  mg N/100g showing slight decrease compared to the initial raw material. Further, the results indicated that the water content after smoking of the liquid group ( $61.95\pm 5.3\%$ ) was higher than of wood smoke group ( $57.95\pm 0.07\%$ ) and raw material, although the increase was not significant. However, protein ( $15.6\pm 0.14\%$ ) and lipid content ( $20.12\pm 7.9\%$ ) of the liquid group were significantly lower than the raw material. For the wood smoked sample, the water content increased slightly while protein ( $18.95\pm 1.77\%$ ) and lipid content ( $17.29\pm 1.57\%$ ) decreased.

### 4.4 Quality of smoked mackerel using wood smoke and commercial smoke flavourings

#### 4.4.1 Sensory

The odour and flavour of fresh mackerel and smoked mackerel after smoking and during chilled storage are shown in Table 3 and Table 4 in Appendix. The results indicated that after smoking, the liquid smoked product had more rancid flavour but less spoilage odour than the wood smoked group ( $p<0.05$ ). The butyric acid odour, rancid odour, spoilage sour odour, trimethylamine odour, bitter flavour, spoilage sour flavour, trimethylamine flavour and other spoilage flavour were not different after smoking between two methods ( $p<0.05$ ). After one week of chilled storage, significant difference between the liquid and wood groups was observed with regard to spoilage sour odour, rancid and spoilage sour flavour. These parameters were evaluated to be higher for liquid smoke group. However, the wood smoke group was evaluated more bitter than the liquid group ( $p<0.05$ ).

The wood smoked sample had more butyric acid odour and bitter flavour, but less rancid and trimethylamine flavour than the liquid smoked sample after two weeks of chilled storage ( $p < 0.05$ ). For the other attributes, the difference was not significant at that time. Rancid odour was detected at higher level for the liquid group after three weeks of chilled storage, and this trend was the same for spoilage odour sour, other spoilage odour and rancid flavour ( $p < 0.05$ ). After smoking and during chilled storage, trimethylamine odour and other spoilage flavour were evaluated not significant between two groups.

#### 4.4.2 Physical properties

##### Colour

Smoking significantly reduced lightness, redness and yellowness of the fillets compared with the initial raw material.

The lightness of the fillets was measured on a scale from 0 to 100 (from black to white). The results of the final products are shown in Figure 8. After smoking, no significant difference in lightness was observed between the two groups. However, during chilled storage the liquid smoked product was always higher in lightness than the wood smoked product ( $p < 0.05$ ).

For the liquid group, a significant positive correlation between change in lightness and storage time ( $r = 0.26$ ,  $p = 0.007$ ) was observed. The lightness of the liquid group started to increase from week three of storage time ( $p = 0.047$ ). The result also indicated that the wood smoked sample decreased with regard to lightness although the reduction was not significant ( $r = -0.07$ ,  $p = 0.45$ ).

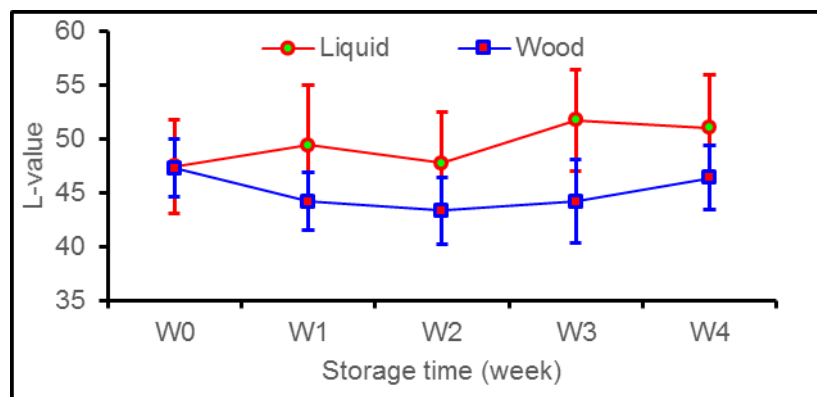


Figure 8: The lightness (L value) of smoked mackerel after smoking and during chilled storage. The fish was liquid and wood smoked.

The a – value (redness) describes the intensity of green colour (negative) and in red colour (positive) of the smoked mackerel. Significant difference in redness was observed between the two groups after smoking, however this difference was not significant during prolonged chilled storage. The redness of the two sample groups was generally rather stable during the storage time (Figure 9).

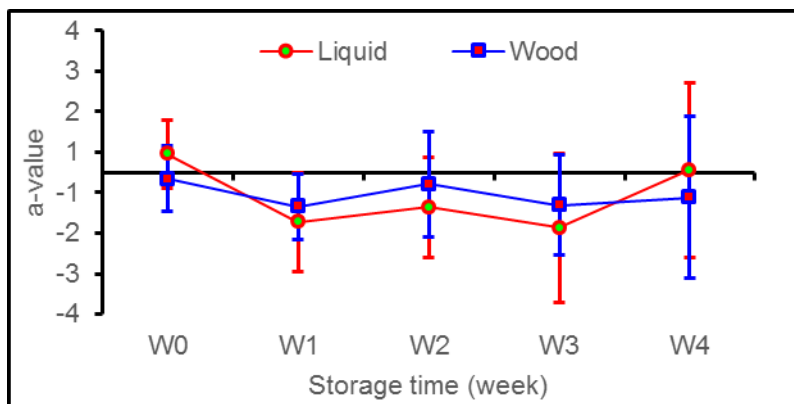


Figure 9: The redness (a-value) of liquid and wood smoked mackerel during chilled storage.

The b value (yellowness) describes intensity of blue (negative) and yellow (positive) colour of the smoked mackerel. The yellowness of the liquid smoked mackerel was significantly higher compared with the wood smoked mackerel after one week ( $p < 0.001$ ) and after three weeks ( $p = 0.003$ ) of storage time (Figure 10). However, no difference was found between the groups at the last week of the chilled storage. With time, the yellowness increased significantly for both the liquid smoked group ( $r = 0.61$ ,  $p < 0.001$ ) and the wood smoke group ( $r = 0.70$ ,  $p < 0.001$ ).

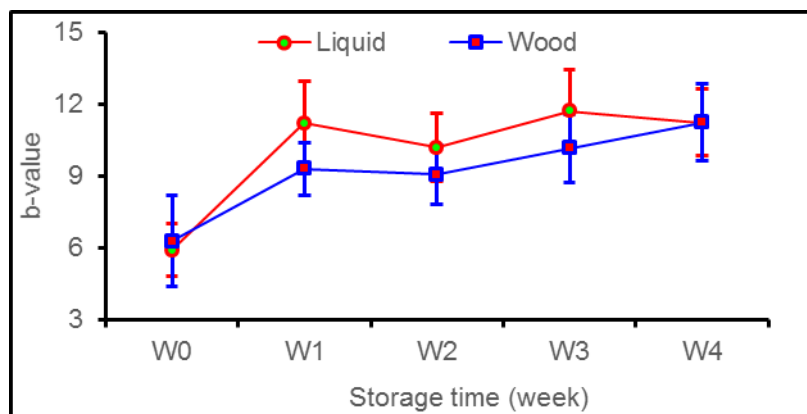


Figure 10: The yellowness (b-value) of liquid and wood smoked mackerel during chilled storage.

### Texture

After smoking, both groups were firmer compared to the raw material. The shear force was 23.07 N and 25.86 N for the liquid smoked sample and the wood smoked sample, respectively, while the shear force of the raw material was 12.84 N.

The shear force of liquid and wood smoked mackerel was the same at week 0 (after smoking) and week one of storage time (Figure 11). A marked difference between groups was only found at week two, where the shear force of the wood smoked (40.69 N) was significantly higher ( $p = 0.005$ ) than the shear force of the liquid group (17.35N). The results also showed that the shear force of the liquid smoked mackerel increased slightly ( $r = 0.08$ ,  $p = 0.73$ ) throughout the chilled storage, while in the wood smoked sample, the texture tended to decrease ( $r = -0.17$ ,  $p = 0.47$ ) although the reduction was not significant.

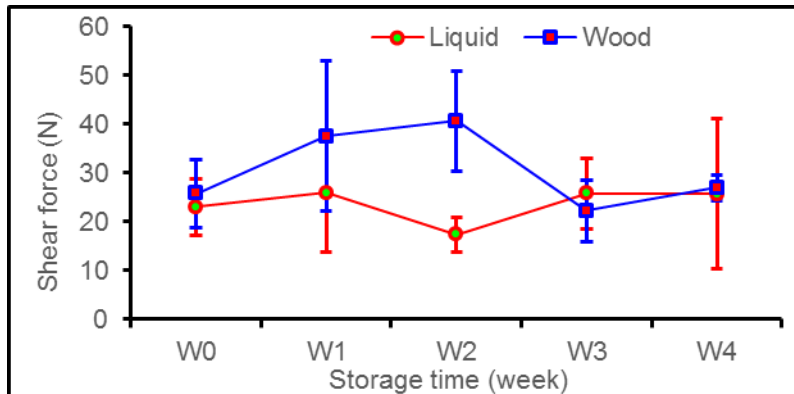


Figure 11: The shear force (N) of liquid and wood smoked mackerel during chilled storage.

#### 4.4.3 Total plate counts

After processing, the initial number of bacteria of raw material was 3.65 (log cfu/g). After smoking TPC of liquid smoked sample was 2.32 (log cfu/g) and 3.33 (log cfu/g) for the wood smoked sample was. However, the difference of bacteria between raw material and product was not significant.

After smoking, the bacteria of liquid smoked mackerel was significantly lower ( $p < 0.04$ ) than wood smoked mackerel (Figure 12). After one week of storage at  $-1\text{ }^{\circ}\text{C}$ , the difference was not significant between the two groups. However, when the temperature was increased up to  $4\text{-}5\text{ }^{\circ}\text{C}$ , the total plate counts (TPC) of the liquid smoked group increased sharply and reached levels of 7.33 (log cfu/g) at week two, and was higher than the wood smoked sample until week four of chilled storage ( $p < 0.05$ ). There was a positive correlation between increasing TPC of liquid smoked mackerel and storage time ( $r = 0.85$ ,  $p = 0.001$ ). The wood smoked mackerel was rather stable in TPC with time.

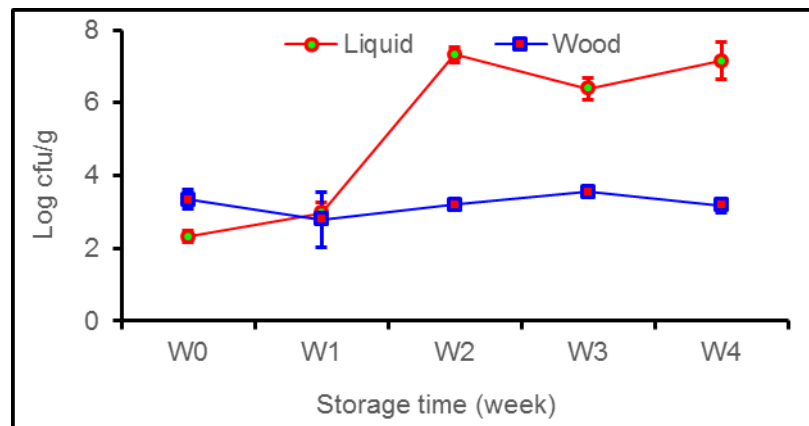


Figure 12: The total plate counts (log cfu/g) of liquid and wood smoked mackerel during chilled storage.

#### 4.4.4 Lipid quality

Lipid content, free fatty acids content (FFA), PV and TBARS are shown in Figure 13a, 13b, 13c and 13d, respectively. The lipid content of the liquid and wood smoked samples after smoking and during storage was rather similar ( $p>0.05$ ).

The lipid content of the liquid smoked mackerel was stable during storage while the lipid content of the wood smoked group, increased ( $r=0.71$ ,  $p=0.02$ ).

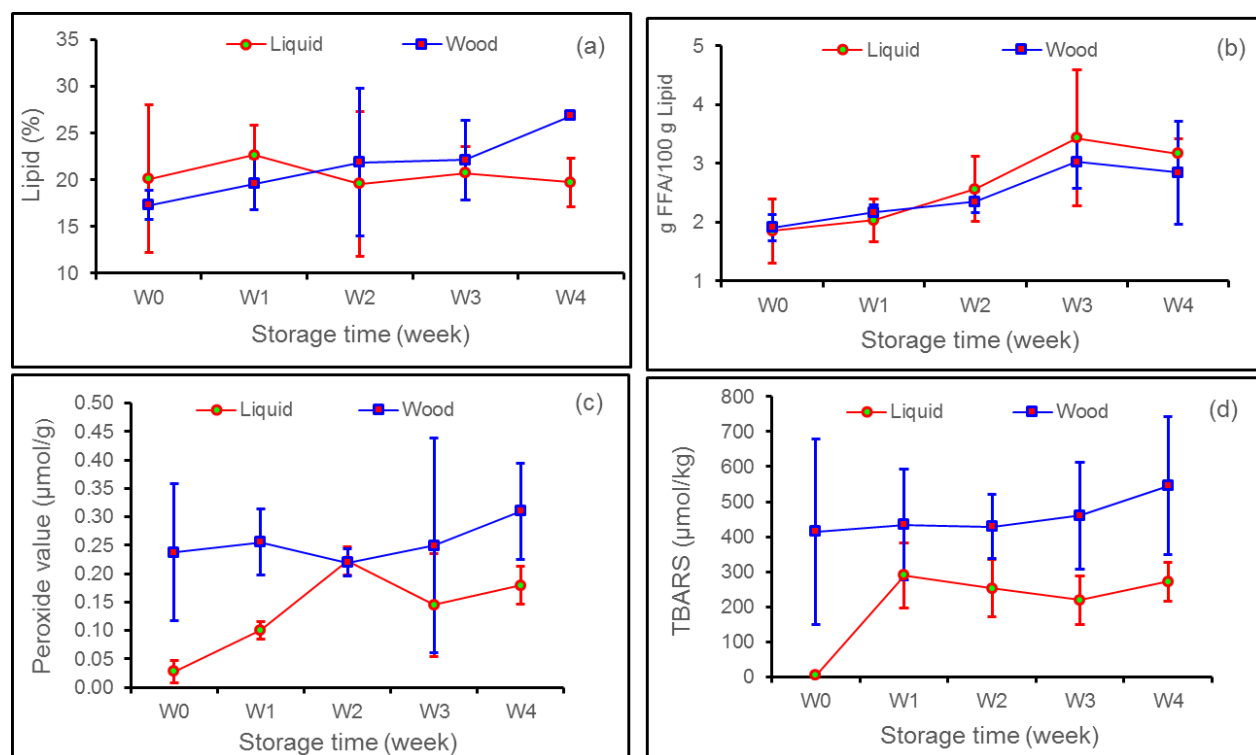


Figure 13: The lipid content (%) (a), free fatty acids (FFA/g lipid) (b), peroxide value ( $\mu\text{mol/g}$ ) (c) and TBARS ( $\mu\text{mol/kg}$ ) (d) of liquid and wood smoked mackerel during chilled storage.

FFA decreased from 2 g FFA/g lipid (raw material) to 1.85 g FFA/g lipid (liquid smoked sample) and 1.91 g FFA/g lipid (wood smoked sample) after smoking although the reduction was not significant.

There was no significant difference in FFA content between the two groups after smoking and during storage time. However, the FFA in liquid smoked sample increased significantly ( $r=0.68$ ,  $p=0.001$ ) from week three of chilled storage. Positive correlation between increased FFA of wood smoked sample and storage time was also indicated from week three ( $r=0.67$ ,  $p=0.001$ ).

Higher primary and secondary lipid oxidation products (PV and TBARS respectively) were observed in the wood smoked samples compared to the liquid smoked samples (Figure 13c and 13d). The PV in wood smoked mackerel was higher after smoking ( $p=0.001$ ), at week one ( $p<0.001$ ) and at the last week of chilled storage ( $p=0.005$ ) than the liquid smoked sample. A positive correlation between increased PV and storage time was observed for liquid smoked



samples ( $r= 0.62$ ,  $p< 0.001$ ). The PV value for liquid smoked sample was highest ( $0.22 \mu\text{mol/g}$ ) at week two, but for the wood smoked sample, the peroxide value was stable with time.

The TBARS of raw material was  $1,370.47 \mu\text{mol/kg}$ . After smoking TBARS decreased strikingly to  $4.25 \mu\text{mol/kg}$  (liquid smoked product) and  $414.62 \mu\text{mol/kg}$  (wood smoked product). The results indicated that the TBARS was lower in the liquid smoked samples, both immediately after smoking and during the storage time, compared to the wood smoked samples ( $p<0.05$ ). Positive correlation between increased TBARS in liquid smoked mackerel and storage time was indicated ( $r=0.54$ ,  $p=0.02$ ), however the wood smoked mackerel was rather stable in TBARS with time.

#### *4.4.5 Correlation between chemical, physical, microbiological and sensory attributes of smoked mackerel*

Some correlation between chemical, physical, microbiological and sensory attributes of the smoked mackerel products was observed. For the liquid group, a positive correlation of FFA between O-TMA ( $r=0.991$ ,  $p=0.008$ ) and F-spoilage ( $r=0.976$ ,  $p=0.02$ ) was observed.

For the liquid smoked sample, PV correlated also positively with TPC ( $r=0.934$ ,  $p=0.02$ ). Similar correlation between TBARS and b value was observed ( $r=0.934$ ,  $p=0.02$ ). Further, similar correlation was found for O-sour ( $r=0.971$ ,  $p=0.0293$ ), O-spoilage ( $r=0.989$ ,  $p=0.01$ ) and F-sour ( $r=0.984$ ,  $p=0.01$ ) with L -value. O-sour correlated positively with O-butyric ( $r=0.956$ ,  $p=0.04$ ), O-spoilage ( $r=0.995$ ,  $p=0.004$ ) and F-sour ( $r=0.979$ ,  $p=0.02$ ). The positive correlation was also indicated between F-spoilage and O-TMA ( $r=0.993$ ,  $p=0.006$ ); O-spoilage and F-sour ( $r=0.99$ ,  $p=0.01$ ).

For the wood group sample, PV correlated positively with TBARS ( $r= 0.924$ ,  $p=0.02$ ). A positive correlation between butyric acid and O-TMA was also found ( $r=0.966$ ,  $p=0.03$ ). Further, similar correlation between butyric acid was observed for F-bitter ( $r=0.967$ ,  $p=0.03$ ).

O-TMA correlated also positively with F-bitter ( $r= 0.965$ ,  $p=0.03$ ) and F-spoilage ( $r= 0.97$ ,  $p= 0.02$ ). A positive correlation of lipid content with b-value ( $r=0.898$ ,  $p= 0.03$ ) and TBARS ( $r=0.924$ ,  $p=0.02$ ) was observed. Further, O butyric correlated positively with F-sour ( $r=0.955$ ,  $p= 0.04$ ) and F-spoilage ( $r=1$ ,  $p<0.001$ ). Similar correlation was found for F-bitter ( $r= 0.974$ ,  $p=0.02$ ) and F- sour ( $0.962$ ,  $p=0.03$ ) with F-spoilage.

## 5 DISCUSSION

### 5.1 Chemical composition of final product after smoking

After smoking, water content of the liquid smoked sample was slightly higher compared with the wood smoked group and the raw material. This increase was probably a consequence of smokebrining for 16 hours. Inversely, both lipid content and protein content were decreased due to the increase in water content. These changes were consistent with Alcicek and Atar (2010) where they found the same result for rainbow trout.

For the wood group sample, the water content increased slightly while protein and lipid content decreased. However, the increased water content in this group can not be explained by getting water from brining because normally, the immersing fillets in brinne solution 10% (1:1) for 3 hours and smoking with the temperature around 39.08°C lead to decrease water content. So, this change maybe due to the raw material diversity.

The total volatile basic nitrogen (TVB-N) is one of the most widely used measurements of seafood quality and is thought to be an important parameter for determining the freshness of fish products. It is a general term which includes trimethylamine (produced by spoilage bacteria), dimethylamine (produced by autolytic enzymes during frozen storage), ammonia (produced by the deamination of amino-acids and nucleotide catabolites) and other volatile basic nitrogenous compounds associated with seafood spoilage (Huss, 1995).

According to Alcicek and Atar (2010), smoking processes influenced the TVB-N level of smoked rainbow trout where the TVB-N increased after smoking and through storage. However, the present study revealed a significant drop in TVB-N of liquid smoked mackerel after the smoking process. The result can be attributed to the preservative effect of liquid smoke flavourings and sodium chloride in prevent bacterial spoilage. This result was also confirmed by microbiology where the total plate counts decreased from raw material to smoked product.

### 5.2 Quality of smoked mackerel using wood smoke and commercial smoke flavourings

Muscle colour is related to the deposition of dietary and pigment (Rosa *et al.*, 1998). In this study there was a significant reduction in lightness, redness and yellowness from raw to smoked product which was consistent with Rosa *et al.*, (1998), where they found the same result for Atlantic salmon. Cardinal *et al.*, (2001) also reported similar result for Atlantic salmon with lightness, and redness, however the yellowness was increased regardless of the raw material.

According to Bugueno *et al.*, (2003), colour of smoked salmon produced through smoked brining was stable during 25 days storage at 2 °C. However, in the present study, the lightness and yellowness of the liquid smoked mackerel increased significantly while redness was stable with time. For the wood smoked group, only yellowness correlated positively with time while lightness and redness were stable during the chilled storage.

The force required to shear the smoked mackerel was significantly higher than for the raw material. This result was similar with result observed by Sigurgisladottir *et al.*, (2000) in smoked Atlantic salmon. The liquid smoked mackerel, in present study became stiffer with time which was in

accordance to the finding of Siskos *et al.*, (2007) where increased storage time, liquid smoked fillets of trout became more firmer. Further, same trend was also seen for liquid smoked salmon (Bugueno *et al.*, 2003) where the products became tougher during storage time.

Smoking agents and methods affected the growth of microbial counts. After smoking, TPC was reduced. However, TPC rose sharply when the temperature storage increased from  $-1^{\circ}\text{C}$  to  $4^{\circ}\text{C}$  in the liquid group. These findings were similar to previous results (Siskos *et al.*, 2007), where the microbial flora in smoked trout fillets after the liquid smoked processing was lower than raw material and remained stable for 14 days of storage at  $4 \pm 1^{\circ}\text{C}$ . Antonia da Silva *et al.*, (2008) found the same results in smoked blue catfish, where smoking sharply reduced all microbial population counts. Bilgin *et al.*, (2008) reported smoking reduced TPC in cold traditional smoke gilthead seabream. However TPC increased during storage time at  $4^{\circ}\text{C}$ .

According to Alcicek and Atar (2010), the lipid content of the samples were not significantly influenced by different smoking and brining processing conditions during storage. In present study, lipid content decreased from raw material to smoked product, similar result was found on smoked salmon by Espea *et al.*, (2002). However there was no significant difference in lipid content of liquid and wood smoked mackerel in present study. This study also confirmed the result from Espea *et al.*, (2002) on smoked salmon, where no correlation between lipid content of raw material and TBARS was observed, and oxidation was more progressive at the higher smoking temperature.

The lipid oxidation increased with time. These results were in contrast with Siskos *et al.*, (2007) where they reported that storage at  $4 \pm 1^{\circ}\text{C}$  had little effect on lipid oxidation of smoked trout fillet.

Antonia da Silva *et al.*, (2008) reported that smoking increased the TBARS value of smoked blue catfish, and reduced PV significantly in the sample soaked in 5% sorbic acid/30 min. However in present study, TBARS decreased surprisingly and PV increased during the storage time.

In the study of Gómez-Estaca *et al.*, (2007) on cold smoked dolphin fish treated high pressurized, TBARS level was stable during chilled storage and little higher at the end of the storage. In comparison with this study, TBARS increased after one week then stable at the end of storage. The results from this study contrast with Bugueno *et al.*, (2003) where no changes in TBARS value of smokebrined salmon under vacuum until 25 days of smokebrined.

## 6 CONCLUSIONS

The results in present study indicated that, generally, smoked mackerel using commercial liquid smoke flavourings tended to be higher in lightness, redness, and yellowness but softer than traditional smoking. Although, the number of bacteria was lower after smoking in liquid smoke product, the higher water content led to growth of bacteria during storage and the shelf life of this product at 4-5°C is three weeks.

The lipid oxidation was represented by free fatty acid, peroxide value and thiobarbituric acid reactive substance. These attributes was higher after wood smoke processing and rather stable during chilled storage. In contrast, liquid smoke processing led less oxidation of lipid in the product but it increased during storage time. This along with the microbial growth limited the shelf life of the liquid smoke product.

The options of smoking traditionally or by liquid smoke technique is highly dependent on the material, point of view of processor and the processing conditions. However, liquid smoke technique could be better to control the temperature than traditional – smoking house.

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## APPENDICES

### Appendix 1: Pre-trial result

Table 3: The salt content (%) of mackerel fillets in different time (wood smoked) and different salt concentration solution of smoked brining (liquid smoked). Red number in the table express the salt concentration was chose for this study.

Parameters of brinning	Liquid smoke	Wood smoke
100 g/L NaCl / 2 hours		2.2
<b>100 g/L NaCl / 3 hours</b>		<b>2.6</b>
100 g/L NaCl / 4 hours		2.4
50 g/L NaCl / 16 hours	1.7	
<b>60 g/L NaCl / 16 hours</b>	<b>2.6</b>	
70 g/L NaCl / 16 hours	2.9	

### Appendix 2: Sensory result

Table 4: The odour, flavour and texture of fresh mackerel fillet.

Sensory attribute	Score	Sensory attribute	Score	Sensory attribute	Score
O-oil	36	F-oil	35	T-soft	45
O-metallic	29	F-metallic	36	T-juicy	50
O-sweet	39	F-sweet	37	T-tender	58
O-mouldy	2	F-acidic	15	T-mushy	31
O-butyric	1	F-mouldy	2	T-sticky	29
O-rancid	9	F-bitter	12		
		F-rancid	9		

Table 5: The odour and flavour changes of liquid and wood smoked mackerel, after smoking and chilled storage.

Group	O-butyric	O-rancid	O-sour	O-TMA	O-spoilage	F-bitter	F-rancid	F-sour	F-TMA	F-spoilage
<b>Day 0</b>										
L	4	7	3	1	1	11	17	1	1	1
W	4	3	5	1	2	13	6	4	0	1
p-value	0.890	0.258	0.350	0.664	0.002 **	0.616	0.024 *	0.111	0.664	0.359
<b>Day 7</b>										
L	3	6	4	1	4	7	14	5	1	1
W	5	2	1	1	0	12	4	3	0	2
p-value	0.058 ms	0.122	0.003 **	0.572	0.026 *	0.037 *	0.004 **	0.042 *	0.418	0.784
<b>Day 14</b>										
L	3	3	3	2	1	11	8	3	4	3
W	6	2	2	1	1	15	3	4	2	3
p-value	0.027 *	0.590	0.101	0.330	0.428	0.036 *	0.000 ***	0.329	0.041 *	0.922
<b>Day 21</b>										
L	13	12	9	3	10	13	16	11	1	4
W	10	6	2	1	2	20	7	10	1	7
p-value	0.218	0.002 **	0.002 **	0.114	0.000 ***	0.081 ms	0.019 *	0.647	0.427	0.411
ms (marginal significance, p = 0.05-0.10); * (p < 0.05); ** (p < 0.01); *** (p < 0.001)										

### Appendix 3: Summary results from data analysis

#### Pearson correlation between chemical, physical, microbiological and sensory attributes of smoked mackerel

##### *Liquid smoked mackerel*

Cell Contents:

Value of the first row: Correlation Coefficient

Value of the second row: P Value

Value of the third row: Number of Samples

The pair(s) of variables with positive correlation coefficients and P values below 0.050 tend to increase together. For the pairs with negative correlation coefficients and P values below 0.050, one variable tends to decrease while the other increases. For pairs with P values greater than 0.050, there is no significant relationship between the two variables.

	L-value	a-value	b-value	Shear force	TPC	FFA	PV	TBARS
L-value	1.000	-0.387	0.752	0.715	0.431	0.827	0.276	0.510
	4.965E-025	0.519	0.142	0.175	0.468	0.0844	0.653	0.381
	5	5	5	5	5	5	5	5
L-value		-0.387	0.752	0.715	0.431	0.827	0.276	0.510
		0.519	0.142	0.175	0.468	0.0844	0.653	0.381
		5	5	5	5	5	5	5

a-value	-0.737	-0.0607	-0.241	-0.316	-0.391	-0.655
	0.155	0.923	0.697	0.605	0.515	0.231
	5	5	5	5	5	5
b-value		0.294	0.611	0.687	0.683	0.934
		0.632	0.274	0.200	0.204	0.0201
		5	5	5	5	5
Shear force			-0.290	0.228	-0.411	0.107
			0.636	0.713	0.492	0.863
			5	5	5	5
TPC				0.823	0.934	0.569
				0.0867	0.0203	0.317
				5	5	5
FFA					0.633	0.465
					0.252	0.430
					5	5
PV						0.741
						0.152
						5

	<b>O-butyric</b>	<b>O-rancid</b>	<b>O- sour</b>	<b>O-TMA</b>	<b>O-spoilage</b>	<b>F-bitter</b>	<b>F-rancid</b>	<b>F- sour</b>
L-value	0.863	0.849	0.971	0.696	0.989	0.283	0.336	0.984
	0.137	0.151	0.0293	0.304	0.0110	0.717	0.664	0.0165
	4	4	4	4	4	4	4	4
a-value	-0.410	-0.272	-0.637	-0.575	-0.689	0.138	0.337	-0.781
	0.590	0.728	0.363	0.425	0.311	0.862	0.663	0.219
	4	4	4	4	4	4	4	4
b-value	0.410	0.262	0.634	0.585	0.686	-0.127	-0.352	0.779
	0.590	0.738	0.366	0.415	0.314	0.873	0.648	0.221
	4	4	4	4	4	4	4	4
Shear force	0.467	0.804	0.564	-0.0196	0.602	-0.133	0.787	0.528
	0.533	0.196	0.436	0.980	0.398	0.867	0.213	0.472
	4	4	4	4	4	4	4	4

	<b>O-butyric</b>	<b>O-rancid</b>	<b>O- sour</b>	<b>O-TMA</b>	<b>O-spoilage</b>	<b>F-bitter</b>	<b>F-rancid</b>	<b>F-</b>
<b>sour</b>								
TPC	0.388	-0.0640	0.391	0.827	0.364	0.486	-0.638	0.455
	0.612	0.936	0.609	0.173	0.636	0.514	0.362	0.545
	4	4	4	4	4	4	4	4
FFA	0.872	0.575	0.876	0.991	0.850	0.665	-0.0538	0.876
	0.128	0.425	0.124	0.00855	0.150	0.335	0.946	0.124
	4	4	4	4	4	4	4	4
PV	0.100	-0.314	0.183	0.611	0.185	0.139	-0.848	0.311
	0.900	0.686	0.817	0.389	0.815	0.861	0.152	0.689
	4	4	4	4	4	4	4	4

TBARS	0.0460 0.954 4	-0.0955 0.905 4	0.302 0.698 4	0.328 0.672 4	0.367 0.633 4	-0.402 0.598 4	-0.600 0.400 4	0.490 0.510 4
O-butyric		0.889 0.111 4	0.956 0.0436 4	0.839 0.161 4	0.925 0.0752 4	0.722 0.278 4	0.442 0.558 4	0.882 0.118 4
O-rancid			0.874 0.126 4	0.504 0.496 4	0.864 0.136 4	0.466 0.534 4	0.761 0.239 4	0.783 0.217 4
O- sour				0.814 0.186 4	0.995 0.00462 4	0.505 0.495 4	0.351 0.649 4	0.979 0.0212 4
O-TMA					0.779 0.221 4	0.727 0.273 4	-0.107 0.893 4	0.807 0.193 4
O-spoilage						0.420 0.580 4	0.335 0.665 4	0.990 0.0105 4
F-bitter							0.242 0.758 4	0.366 0.634 4
F-rancid								0.200 0.800 4

	<b>F-TMA</b>	<b>F-spoilage</b>	<b>Lipid</b>
L-value	-0.305 0.695 4	0.631 0.369 4	0.167 0.789 5
a-value	-0.250 0.750 4	-0.587 0.413 4	-0.544 0.343 5
b-value	0.270 0.730 4	0.600 0.400 4	0.287 0.640 5
Shear force	-0.878 0.122 4	-0.123 0.877 4	0.536 0.352 5
TPC	0.789	0.888	-0.573

	0.211 4	0.112 4	0.312 5
FFA	0.229 0.771 4	0.976 0.0240 4	-0.336 0.581 5
PV	0.893 0.107 4	0.696 0.304 4	-0.385 0.523 5
TBARS	0.446 0.554 4	0.376 0.624 4	0.290 0.636 5
O-butiric	-0.257 0.743 4	0.769 0.231 4	-0.0616 0.938 4
O-rancid	-0.664 0.336 4	0.400 0.600 4	0.270 0.730 4
O- sour	-0.244 0.756 4	0.750 0.250 4	0.181 0.819 4
O-TMA	0.309 0.691 4	0.993 0.00696 4	-0.327 0.673 4
O-spoilage	-0.257 0.743 4	0.715 0.285 4	0.268 0.732 4
F-bitter	0.0717 0.928 4	0.697 0.303 4	-0.721 0.279 4
F-rancid	-0.949 0.0511 4	-0.222 0.778 4	0.260 0.740 4
F- sour	-0.138 0.862 4	0.757 0.243 4	0.275 0.725 4
F-TMA		0.418 0.582 4	-0.527 0.473 4
F-spoilage			-0.369 0.631 <b>4</b>

**Wood smoked mackerel**

	<b>a-value</b>	<b>b-value</b>	<b>Shear force</b>	<b>TPC</b>	<b>FFA</b>	<b>PV</b>	<b>TBARS</b>	<b>O-butyric</b>
L-value	0.395 0.511 5	-0.361 0.551 5	-0.586 0.299 5	0.165 0.791 5	0.107 0.864 5	0.437 0.462 5	0.251 0.684 5	-0.513 0.487 4
a-value		-0.700 0.188 5	0.111 0.859 5	0.265 0.667 5	0.432 0.467 5	-0.443 0.455 5	-0.371 0.539 5	-0.553 0.447 4
b-value			-0.0424 0.946 5	-0.0766 0.903 5	0.146 0.815 5	0.651 0.234 5	0.801 0.103 5	0.772 0.228 4
Shear force				-0.703 0.185 5	0.535 0.353 5	-0.388 0.518 5	-0.347 0.568 5	-0.436 0.564 4
TPC					-0.401 0.504 5	-0.166 0.789 5	0.0332 0.958 5	0.587 0.413 4
FFA						0.243 0.694 5	0.344 0.570 5	-0.621 0.379 4
PV							0.924 0.0247 5	0.204 0.796 4
TBARS								0.949 0.0514 4
	<b>O-rancid</b>	<b>O- sour</b>	<b>O-TMA</b>	<b>O-spoilage</b>	<b>F-bitter</b>	<b>F-rancid</b>	<b>F- sour</b>	<b>F-TMA</b>
L-value	-0.0427 0.957 4	0.915 0.0850 4	-0.584 0.416 4	0.757 0.243 4	-0.359 0.641 4	0.612 0.388 4	-0.252 0.748 4	-0.623 0.377 4
a-value	-0.475 0.525 4	0.640 0.360 4	-0.374 0.626 4	0.520 0.480 4	-0.336 0.664 4	-0.123 0.877 4	-0.374 0.626 4	0.353 0.647 4
b-value	0.442 0.558 4	-0.813 0.187 4	0.741 0.259 4	-0.589 0.411 4	0.602 0.398 4	-0.204 0.796 4	0.551 0.449 4	0.268 0.732 4
Shear force	-0.814 0.186 4	-0.668 0.332 4	-0.306 0.694 4	-0.810 0.190 4	-0.546 0.454 4	-0.987 0.0134 4	-0.655 0.345 4	0.598 0.402 4
TPC	0.717 0.283 4	0.570 0.430 4	0.631 0.369 4	0.802 0.198 4	0.771 0.229 4	0.634 0.366 4	0.784 0.216 4	0.0789 0.921 4

FFA	-0.891 0.109 4	-0.191 0.809 4	-0.415 0.585 4	-0.359 0.641 4	-0.588 0.412 4	-0.857 0.143 4	-0.700 0.300 4	0.695 0.305 4
PV	0.429 0.571 4	-0.147 0.853 4	-0.0525 0.948 4	-0.146 0.854 4	0.0466 0.953 4	0.497 0.503 4	0.163 0.837 4	-0.840 0.160 4
TBARS	0.813 0.187 4	-0.453 0.547 4	0.859 0.141 4	-0.169 0.831 4	0.840 0.160 4	0.277 0.723 4	0.843 0.157 4	-0.0236 0.976 4
O-butyric	0.870 0.130 4	-0.263 0.737 4	0.966 0.0339 4	0.0585 0.941 4	0.967 0.0329 4	0.338 0.662 4	0.955 0.0446 4	0.125 0.875 4
O-rancid		0.150 0.850 4	0.757 0.243 4	0.424 0.576 4	0.887 0.113 4	0.757 0.243 4	0.946 0.0538 4	-0.309 0.691 4
O- sour			-0.272 0.728 4	0.946 0.0543 4	-0.0456 0.954 4	0.670 0.330 4	0.0334 0.967 4	-0.382 0.618 4
O-TMA				0.0551 0.945 4	0.965 0.0353 4	0.179 0.821 4	0.919 0.0815 4	0.365 0.635 4
O-spoilage					0.281 0.719 4	0.770 0.230 4	0.350 0.650 4	-0.295 0.705 4
F-bitter						0.428 0.572 4	0.988 0.0116 4	0.165 0.835 4
F-rancid							0.553 0.447 4	-0.720 0.280 4
F- sour								0.0152 0.985 4

	<b>F-spoilage</b>	<b>Lipid</b>	<b>Lipid</b>
L*value	-0.499 0.501 4	-0.0770 0.902 5	-0.0770 0.902 5
a*value	-0.531 0.469 4	-0.343 0.572 5	-0.343 0.572 5
b*value	0.757 0.243	0.898 0.0387	0.898 0.0387



	4	5	5
Shear force	-0.448	-0.126	-0.126
	0.552	0.840	0.840
	4	5	5
TPC	0.608	0.0756	0.0756
	0.392	0.904	0.904
	4	5	5
FFA	-0.619	0.427	0.427
	0.381	0.474	0.474
	4	5	5
PV	0.187	0.724	0.724
	0.813	0.167	0.167
	4	5	5
TBARS	0.940	0.924	0.924
	0.0600	0.0248	0.0248
	4	5	5
O-butiric	1.000	0.804	0.804
	0.000363	0.196	0.196
	4	4	4
O-rancid	0.874	0.407	0.407
	0.126	0.593	0.593
	4	4	4
O- sour	-0.241	-0.649	-0.649
	0.759	0.351	0.351
	4	4	4
O-TMA	0.969	0.884	0.884
	0.0309	0.116	0.116
	4	4	4
O-spoilage	0.0820	-0.380	-0.380
	0.918	0.620	0.620
	4	4	4
F-bitter	0.974	0.730	0.730
	0.0264	0.270	0.270
	4	4	4
F-rancid	0.348	-0.284	-0.284
	0.652	0.716	0.716
	4	4	4
F- sour	0.962	0.635	0.635
	0.0383	0.365	0.365
	4	4	4
F-TMA	0.130	0.620	0.620
	0.870	0.380	0.380

	4	4	4
F-spoilage		0.799 0.201 4	0.799 0.201 4
Lipid			1.000 4.965E-025 5

**T-test, Tukey HSD test and linear regression**

Df: degree of freedom; t-value: statistic from T-test; Std.Dev: Standard deviation; Valid N; Number of samples; M: Mean  
 W0: Week 0 (after smoking); W1, W2, W3, W4: Week 1,2,3,4 of chilled storage; Group 1: liquid smoked mackerel (L); Group 2:  
 wood smoked mackerel (W).

Differences significant ( $p < 0.05$ ) are mark red colour

**Colour**

Table 6: T-test for L-value (lightness) between the liquid smoked group and wood smoked group.

Group 1 vs. Group 2	T-test for Independent Samples (DATA-FINAL 25214) Note: Variables were treated as independent samples										
	Mean Group 1	Mean Group 2	t-value	df	p	Valid N Group 1	Valid N Group 2	Std.Dev. Group 1	Std.Dev. Group 2	F-ratio Variances	p Variances
L- L-value (W0) vs. W-L-value(W0)	47.43900	47.29650	0.124973	38	0.901203	20	20	4.328244	2.696179	2.577068	0.045446
L- L-value(W1) vs. W -L-value(W1)	49.44050	44.20250	3.803381	38	0.000504	20	20	5.541842	2.687254	4.252955	0.002758
L- L-value(W2) vs. W -L-value(W2)	47.72900	43.33500	3.444848	38	0.001408	20	20	4.772217	3.124961	2.332120	0.072560
L- L-value(W3) vs. W -L-value(W3)	51.75400	44.18200	5.535282	38	0.000002	20	20	4.712152	3.901472	1.458753	0.418051
L- L-value(W4) vs. W -L-value(W4)	51.04500	46.40300	3.618902	38	0.000859	20	20	4.897180	2.987389	2.687253	0.036999

Table 7: Tukey HSD test for L-value (lightness) of the liquid smoked group between weeks of chilled storage.

Time (colour change)	Tukey HSD test; Variable: Liquid - L-value (DATA-FINAL 25214) Marked differences are significant at $p < .05000$				
	{1} (M=47.439)	{2} (M=49.440)	{3} (M=47.729)	{4} (M=51.754)	{5} (M=51.045)
W0 {1}		0.691529	0.999747	0.047194	0.140602
W1 {2}	0.691529		0.799816	0.562942	0.834889
W2 {3}	0.999747	0.799816		0.075644	0.206152
W3 {4}	0.047194	0.562942	0.075644		0.990654
W4 {5}	0.140602	0.834889	0.206152	0.990654	

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Table 8: Tukey HSD test for L-value (lightness) of the wood smoked group between weeks of chilled storage.

Time (colour change)	Tukey HSD test; Variable: Wood - L-value (DATA-FINAL 25214) Marked differences are significant at $p < .05000$				
	{1} (M=47.297)	{2} (M=44.202)	{3} (M=43.335)	{4} (M=44.182)	{5} (M=46.403)
W0 {1}		0.018527	0.001156	0.017433	0.893190
W1 {2}	0.018527		0.903036	1.000000	0.175469
W2 {3}	0.001156	0.903036		0.910421	0.019987
W3 {4}	0.017433	1.000000	0.910421		0.168207
W4 {5}	0.893190	0.175469	0.019987	0.168207	

Table 9: Linear Regression for L-value (lightness) of the liquid smoked group with storage time.

N=100	Regression Summary for Dependent Variable: Liquid - L-value (DATA-FINAL 25214) R= .26693520 R <sup>2</sup> = .07125440 Adjusted R <sup>2</sup> = .06177741 F(1,98)=7.5187 p<.00726 Std.Error of estimate: 4.9128					
	b*	Std.Err. (of b*)	b	Std.Err. (of b)	t(98)	p-value
Time (colour change)	0.266935	0.097350	0.9525	0.34739	2.74202	0.007260

Table 10: Linear Regression for L-value (lightness) of the wood smoked group with storage time.

N=100	Regression Summary for Dependent Variable: Wood - L-value (DATA-FINAL 25214) R= .07553145 R <sup>2</sup> = .00570500 Adjusted R <sup>2</sup> = ----F(1,98)=.56230 p<.45513 Std.Error of estimate: 3.4089					
	b*	Std.Err. (of b*)	b	Std.Err. (of b)	t(98)	p-value
Time (colour change)	-0.075531	0.100727	-0.18075	0.24104	-0.749865	0.455132

Table 11: T-test for a-value (redness) between the liquid smoked group and the wood smoked group.

Group 1 vs. Group 2	T-test for Independent Samples (DATA-FINAL 25214) Note: Variables were treated as independent samples										
	Mean Group 1	Mean Group 2	t-value	df	p	Valid N Group 1	Valid N Group 2	Std.Dev. Group 1	Std.Dev. Group 2	F-ratio Variances	p Variances
L-a-value (W0) vs. W-a-value(W0)	0.464000	-0.161000	2.398025	38	0.021501	20	20	0.839589	0.808494	1.078400	0.871043
L-a-value(W1) vs. W-a-value(W1)	-1.22700	-0.846000	-1.15568	38	0.255026	20	20	1.230084	0.812782	2.290452	0.078683
L-a-value(W2) vs. W-a-value(W2)	-0.864000	-0.291500	-1.41928	38	0.163970	20	20	1.246487	1.304027	1.094454	0.846093
L-a-value(W3) vs. W-a-value(W3)	-1.37250	-0.804000	-1.14209	38	0.260561	20	20	1.851611	1.235737	2.245159	0.085963
L-a-value(W4) vs. W-a-value(W4)	0.055000	-0.616000	1.022325	38	0.313092	20	20	2.162003	1.985339	1.185887	0.713990

Table 12: Tukey HSD test for a-value (redness) of the liquid smoked group between weeks of chilled storage.

Time (colour change)	Tukey HSD test; Variable: Liquid - a-value (DATA-FINAL 25214) Marked differences are significant at p < .05000				
	{1} (M=.46400)	{2} (M=-1.227)	{3} (M=-.8640)	{4} (M=-1.372)	{5} (M=.05500)
W0 {1}		0.006973	0.057734	0.002698	0.917726
W1 {2}	0.006973		0.945347	0.998294	0.072927
W2 {3}	0.057734	0.945347		0.834505	0.332131
W3 {4}	0.002698	0.998294	0.834505		0.033843
W4 {5}	0.917726	0.072927	0.332131	0.033843	

Table 13: Tukey HSD test for a-value (redness) of the wood smoked group between weeks of chilled storage.

Time (colour change)	Tukey HSD test; Variable: Wood -a-value (DATA-FINAL 25214) Marked differences are significant at p < .05000				
	{1} (M=-.1610)	{2} (M=-.8460)	{3} (M=-.2915)	{4} (M=-.8040)	{5} (M=-.6160)
W0 {1}		0.461706	0.997830	0.525968	0.803777
W1 {2}	0.461706		0.663257	0.999978	0.980692
W2 {3}	0.997830	0.663257		0.725609	0.933632
W3 {4}	0.525968	0.999978	0.725609		0.990992
W4 {5}	0.803777	0.980692	0.933632	0.990992	

Table 14: Linear Regression for a-value (redness) of the liquid smoked group with storage time.

N=100	Regression Summary for Dependent Variable: Liquid - a-value (DATA-FINAL 25214) R= .08172057 R <sup>2</sup> = .00667825 Adjusted R <sup>2</sup> = ----F(1,98)=.65887 p<.41893 Std.Error of estimate: 1.6787					
	b*	Std.Err. (of b*)	b	Std.Err. (of b)	t(98)	p-value
Time (colour change)	-0.081721	0.100677	-0.096350	0.11870	-0.811707	0.418927

Table 15: Linear Regression for a-value (redness) of the wood smoked group with storage time.

N=100	Regression Summary for Dependent Variable: Wood -a-value (DATA-FINAL 25214) R= .09451746 R <sup>2</sup> = .00893355 Adjusted R <sup>2</sup> = ----F(1,98)=.88338 p<.34959 Std.Error of estimate: 1.3061					
	b*	Std.Err. (of b*)	b	Std.Err. (of b)	t(98)	p-value
Time (colour change)	-0.094517	0.100563	-0.086800	0.092352	-0.939883	0.349589

Table 16: T-test for b-value (yellowness) between the liquid smoked group and wood smoked group.

Group 1 vs. Group 2	T-test for Independent Samples (DATA-FINAL 25214) Note: Variables were treated as independent samples										
	Mean Group 1	Mean Group 2	t-value	df	p	Valid N Group 1	Valid N Group 2	Std.Dev. Group 1	Std.Dev. Group 2	F-ratio Variances	p Variances
L-b-value (W0) vs. W-b-value(W0)	5.915500	6.289500	-0.768597	38	0.446883	20	20	1.074163	1.892560	3.104267	0.017474
L-b-value(W1) vs. W-b-value(W1)	11.19850	9.295500	4.061303	38	0.000235	20	20	1.779122	1.107185	2.582092	0.045019
L-b-value(W2) vs. W-b-value(W2)	10.19250	9.054000	2.708988	38	0.010065	20	20	1.414712	1.237372	1.307181	0.565061
L-b-value(W3) vs. W-b-value(W3)	11.72100	10.16450	3.116139	38	0.003481	20	20	1.731023	1.411910	1.503114	0.382314
L-b-value(W4) vs. W-b-value(W4)	11.23150	11.22900	0.005273	38	0.995820	20	20	1.393040	1.598255	1.316331	0.555005

Table 17: Tukey HSD test for b-value (yellowness) of the liquid smoked group between weeks of chilled storage.

Time (colour change)	Tukey HSD test; Variable: Liquid - b-value (DATA-FINAL 25214) Marked differences are significant at p < .05000				
	{1} (M=5.9155)	{2} (M=11.198)	{3} (M=10.193)	{4} (M=11.721)	{5} (M=11.231)
W0 {1}		0.000117	0.000117	0.000117	0.000117
W1 {2}	0.000117		0.220258	0.805577	0.999995
W2 {3}	0.000117	0.220258		0.014812	0.192629
W3 {4}	0.000117	0.805577	0.014812		0.840161
W4 {5}	0.000117	0.999995	0.192629	0.840161	

Table 18: Tukey HSD test for b-value (yellowness) of the liquid smoked group between weeks of chilled storage.

Time (colour change)	Tukey HSD test; Variable: Wood - b-value (DATA-FINAL 25214) Marked differences are significant at $p < .05000$				
	{1} (M=6.2895)	{2} (M=9.2955)	{3} (M=9.0540)	{4} (M=10.165)	{5} (M=11.229)
W0 {1}		0.000117	0.000118	0.000117	0.000117
W1 {2}	0.000117		0.985481	0.344832	0.000799
W2 {3}	0.000118	0.985481		0.129916	0.000206
W3 {4}	0.000117	0.344832	0.129916		0.160140
W4 {5}	0.000117	0.000799	0.000206	0.160140	

Table 19: Linear Regression for b-value (yellowness) of the liquid smoked group with storage time.

N=100	Regression Summary for Dependent Variable: Liquid - b-value (DATA-FINAL 25214) R= .6111371 R <sup>2</sup> = .37345997 Adjusted R <sup>2</sup> = .36706670 F(1,98)=58.415 $p < .00000$ Std.Error of estimate: 2.0640					
	b*	Std.Err. (of b*)	b	Std.Err. (of b)	t(98)	p-value
Time (colour change)	0.611114	0.079958	1.115	0.14595	7.64294	0.000000

Table 20: Linear Regression for b-value (yellowness) of the wood smoked group with storage time.

N=100	Regression Summary for Dependent Variable: Wood - b-value (DATA-FINAL 25214) R= .69536645 R <sup>2</sup> = .48353450 Adjusted R <sup>2</sup> = .47826444 F(1,98)=91.751 $p < .00000$ Std.Error of estimate: 1.5869					
	b*	Std.Err. (of b*)	b	Std.Err. (of b)	t(98)	p-value
Time (colour change)	0.695366	0.072595	1.075	0.11221	9.57869	0.000000

**Texture**

Table 21: T-test for shear force between the liquid smoked group and wood smoked group.

Group 1 vs. Group 2	T-test for Independent Samples (DATA-FINAL 25214) Note: Variables were treated as independent samples										
	Mean Group 1	Mean Group 2	t-value	df	p	Valid N Group 1	Valid N Group 2	Std.Dev. Group 1	Std.Dev. Group 2	F-ratio Variances	p Variances
Shear force (L0) v.s Shear force (W0)	23.06589	25.85887	-0.616281	6	0.560348	4	4	5.806214	6.960135	1.436975	0.772924
Shear force (L1) vs. Shear force (W1)	25.98776	37.51773	-1.17337	6	0.285113	4	4	12.17531	15.42695	1.605460	0.706808
Shear force (L2) vs. Shear force (W2)	17.35389	40.69048	-4.30917	6	0.005042	4	4	3.460323	10.26350	8.797470	0.107261
Shear force (L3) vs. Shear force (W3)	25.82202	22.22126	0.745286	6	0.484260	4	4	7.227972	6.412925	1.270342	0.848747
Shear force (L4) vs. Shear force (W4)	25.74837	26.95385	-0.154344	6	0.882399	4	4	15.41204	2.544154	36.69725	0.014552

Table 22: Tukey HSD test for shear force of the liquid smoked group between weeks of chilled storage.

Time(texture change)	Tukey HSD test; Variable: L-shear force (DATA-FINAL 25214)				
	{1} (M=23.066)	{2} (M=25.988)	{3} (M=17.354)	{4} (M=25.822)	{5} (M=25.748)
W0 {1}		0.992846	0.919983	0.994289	0.994851
W1 {2}	0.992846		0.728576	1.000000	1.000000
W2 {3}	0.919983	0.728576		0.741831	0.747661
W3 {4}	0.994289	1.000000	0.741831		1.000000
W4 {5}	0.994851	1.000000	0.747661	1.000000	

Table 23: Tukey HSD test for shear force of the wood smoked group between weeks of chilled storage.

Time(texture change)	Tukey HSD test; Variable: W-shear force (DATA-FINAL 25214) Marked differences are significant at p < .05000				
	{1} (M=25.859)	{2} (M=37.518)	{3} (M=40.690)	{4} (M=22.221)	{5} (M=26.954)
W0 {1}		0.430794	0.219015	0.980478	0.999824
W1 {2}	0.430794		0.988252	0.196046	0.523072
W2 {3}	0.219015	0.988252		0.086848	0.281343
W3 {4}	0.980478	0.196046	0.086848		0.950075
W4 {5}	0.999824	0.523072	0.281343	0.950075	



Table 24: Linear Regression for shear force of the liquid smoked group with storage time.

N=20	Regression Summary for Dependent Variable: L-shear force (DATA-FINAL 25214) R= .08048215 R <sup>2</sup> = .00647738 Adjusted R <sup>2</sup> = ----F(1,18)=.11735 p<.73589 Std.Error of estimate: 9.5989					
	b*	Std.Err. (of b*)	b	Std.Err. (of b)	t(18)	p-value
Time(texture change)	0.080482	0.234938	0.5199	1.5177	0.342568	0.735891

Table 25: Linear Regression for shear force of the wood smoked group with storage time.

N=20	Regression Summary for Dependent Variable: W-shear force (DATA-FINAL 25214) R= .17133567 R <sup>2</sup> = .02935591 Adjusted R <sup>2</sup> = ----F(1,18)=.54439 p<.47013 Std.Error of estimate: 11.235					
	b*	Std.Err. (of b*)	b	Std.Err. (of b)	t(18)	p-value
Time(texture change)	-0.171336	0.232217	-1.3107	1.7764	-0.737826	0.470131

### Total plate counts (TPC)

Table 26: T-test for TPC between the liquid smoked group and wood smoked group.

Group 1 vs. Group 2	T-test for Independent Samples (DATA-FINAL 25214) Note: Variables were treated as independent samples										
	Mean Group 1	Mean Group 2	t-value	df	p	Valid N Group 1	Valid N Group 2	Std.Dev. Group 1	Std.Dev. Group 2	F-ratio Variances	p Variances
L-TPC (W0) vs. W-TPC (W0)	2.317742	3.332321	-4.65140	2	0.043244	2	2	0.160686	0.263317	2.685360	0.697624
L-TPC (W1) vs. W-TPC (W1)	2.969760	2.788746	0.316466	2	0.781626	2	2	0.291797	0.754445	6.684884	0.469889
L-TPC (W2) vs. W-TPC (W2)	7.326606	3.203270	26.95886	2	0.001373	2	2	0.212860	0.038437	30.66889	0.227461
L-TPC (W3) vs. W-TPC (W3)	6.377937	3.540313	12.76452	2	0.006082	2	2	0.285454	0.131741	4.694904	0.550535
L-TPC (W4) vs. W-TPC (W4)	7.145240	3.171211	10.38671	2	0.009142	2	2	0.508990	0.183591	7.686237	0.440761

Table 27: Tukey HSD test for TPC of the liquid smoked group between weeks of chilled storage.

Time (TPC change)	Tukey HSD test; Variable: L-TPC (DATA-FINAL 25214) Marked differences are significant at p < .05000				
	{1} (M=2.3177)	{2} (M=2.9698)	{3} (M=7.3266)	{4} (M=6.3779)	{5} (M=7.1452)
W0 {1}		0.354992	0.000311	0.000466	0.000325
W1 {2}	0.354992		0.000400	0.000815	0.000436
W2 {3}	0.000311	0.000400		0.134218	0.973319
W3 {4}	0.000466	0.000815	0.134218		0.243994
W4 {5}	0.000325	0.000436	0.973319	0.243994	

Table 28: Tukey HSD test for TPC of the wood smoked group between weeks of chilled storage.

Time (TPC change)	Tukey HSD test; Variable: W-TPC (DATA-FINAL 25214) Marked differences are significant at $p < .05000$				
	{1} (M=3.3323)	{2} (M=2.7887)	{3} (M=3.2033)	{4} (M=3.5403)	{5} (M=3.1712)
W0 {1}		0.621665	0.995870	0.975826	0.990439
W1 {2}	0.621665		0.794313	0.372108	0.833431
W2 {3}	0.995870	0.794313		0.883310	0.999983
W3 {4}	0.975826	0.372108	0.883310		0.848858
W4 {5}	0.990439	0.833431	0.999983	0.848858	

Table 29: Linear Regression for TPC of the liquid smoked group with storage time.

N=10	Regression Summary for Dependent Variable: L-TPC (DATA-FINAL 25214) R= .87870260 R <sup>2</sup> = .77211827 Adjusted R <sup>2</sup> = .74363305 F(1,8)=27.106 p<.00082 Std.Error of estimate: 1.1502					
	b*	Std.Err. (of b*)	b	Std.Err. (of b)	t(8)	p-value
Time(texture change)	0.878703	0.168776	2.530	0.48603	5.20634	0.000816

Table 30:

Linear Regression for TPC of the wood smoked group with storage time.

N=10	Regression Summary for Dependent Variable: W-TPC (DATA-FINAL 25214) R= .19094672 R <sup>2</sup> = .03646065 Adjusted R <sup>2</sup> = -----F(1,8)=.30272 p<.59720 Std.Error of estimate: .39526					
	b*	Std.Err. (of b*)	b	Std.Err. (of b)	t(8)	p-value
Time(texture change)	0.190947	0.347048	0.09190	0.16703	0.550202	0.597203

## Lipid

Table 31: T-test for lipid between the liquid smoked group and wood smoked group.

Group 1 vs. Group 2	T-test for Independent Samples (DATA-FINAL 25214) Note: Variables were treated as independent samples										
	Mean Group 1	Mean Group	t-value	df	p	Valid N Group 1	Valid N Group 2	Std.Dev. Group 1	Std.Dev. Group 2	F-ratio Variances	p Variances
L-lipid (W0) vs. W-lipid (W0)	20.11966	17.29014	0.496837	2	0.668543	2	2	7.899593	1.569575	25.33058	0.249728
L-lipid (W1) vs. W-lipid (W1)	19.73519	19.59101	0.064936	2	0.954132	2	2	3.138219	0.103190	924.9011	0.041851
L-lipid (W2) vs. W-lipid (W2)	21.36678	21.89645	-0.086135	2	0.939206	2	2	7.773795	3.898038	3.977169	0.591793
L-lipid (W3) vs. W-lipid (W3)	20.01029	22.15707	-0.736593	2	0.538054	2	2	2.861981	2.966048	1.074046	0.977267
L-lipid (W4) vs. W-lipid (W4)	23.43764	26.86451	-0.594121	2	0.612684	2	2	2.594966	7.733373	8.881261	0.412209

Table 32: Tukey HSD test for lipid of the liquid smoked group between weeks of chilled storage.

Time (lipid change)	Tukey HSD test; Variable: L-lipid (DATA-FINAL 25214) Marked differences are significant at $p < .05000$				
	{1} (M=20.120)	{2} (M=19.735)	{3} (M=21.367)	{4} (M=20.010)	{5} (M=23.438)
W0 {1}		0.999992	0.999179	1.000000	0.967182
W1 {2}	0.999992		0.997634	0.999998	0.952404
W2 {3}	0.999179	0.997634		0.998860	0.994096
W3 {4}	1.000000	0.999998	0.998860		0.963316
W4 {5}	0.967182	0.952404	0.994096	0.963316	

Table 33: Tukey HSD test for lipid of the wood smoked group between weeks of chilled storage.

Time (lipid change)	Tukey HSD test; Variable: W-lipid (DATA-FINAL 25214) Marked differences are significant at $p < .05000$				
	{1} (M=17.290)	{2} (M=19.591)	{3} (M=21.896)	{4} (M=22.157)	{5} (M=26.865)
W0 {1}		0.976679	0.797153	0.767279	0.278923
W1 {2}	0.976679		0.976504	0.965852	0.483074
W2 {3}	0.797153	0.976504		0.999995	0.755420
W3 {4}	0.767279	0.965852	0.999995		0.785699
W4 {5}	0.278923	0.483074	0.755420	0.785699	

Table 34: Linear Regression for lipid of the liquid smoked group with storage time.

N=10	Regression Summary for Dependent Variable: L-lipid (DATA-FINAL 25214) R= .23957872 R <sup>2</sup> = .05739796 Adjusted R <sup>2</sup> = ----F(1,8)=.48714 p<.50498 Std.Error of estimate: 4.4282					
	b*	Std.Err. (of b*)	b	Std.Err. (of b)	t(8)	p-value
Time (lipid change)	0.239579	0.343257	0.6911	0.9902	0.697958	0.504981

Table 35: Linear Regression for lipid of the wood smoked group with storage time.

N=10	Regression Summary for Dependent Variable: W-lipid (DATA-FINAL 25214) R= .70881735 R <sup>2</sup> = .50242204 Adjusted R <sup>2</sup> = .44022480 F(1,8)=8.0779 p<.02174 Std.Error of estimate: 3.4168					
	b*	Std.Err. (of b*)	b	Std.Err. (of b)	t(8)	p-value
Time (lipid change)	0.708817	0.249394	2.171	0.76402	2.84216	0.021740

**FFA**

Table 36: T-test for FFA between the liquid smoked group and wood smoked group.

Group 1 vs. Group 2	T-test for Independent Samples (DATA-FINAL 25214) Note: Variables were treated as independent samples										
	Mean Group 1	Mean Group	t-value	df	p	Valid N Group 1	Valid N Group 2	Std.Dev. Group 1	Std.Dev. Group 2	F-ratio Variances	p Variances
L-FFA-(W0) vs. W-FFA-(W0)	1.854003	1.911442	-0.195975	6	0.851098	4	4	0.544272	0.217678	6.251760	0.166486
L-FFA-(W1) vs. W-FFA-(W1)	2.035065	2.172720	-0.721955	6	0.497481	4	4	0.362990	0.116868	9.647137	0.094929
L-FFA-(W2) vs. W-FFA-(W2)	2.569062	2.349325	0.751801	6	0.480611	4	4	0.555042	0.183418	9.157267	0.101731
L-FFA-(W3) vs. W-FFA-(W3)	3.432989	3.028546	0.653492	6	0.537662	4	4	1.155342	0.444200	6.764941	0.150708
L-FFA-(W4) vs. W-FFA-(W4)	3.168051	2.847330	0.703008	6	0.508396	4	4	0.249824	0.877556	12.33907	0.068104

Table 37: Tukey HSD test for FFA of the liquid smoked group between weeks of chilled storage.

Time (FFA change)	Tukey HSD test; Variable: L- FFA (DATA-FINAL 25214) Marked differences are significant at p < .05000				
	{1} (M=1.8540)	{2} (M=2.0351)	{3} (M=2.5691)	{4} (M=3.4330)	{5} (M=3.1681)
W0 {1}		0.994521	0.549561	0.026862	0.078167
W1 {2}	0.994521		0.775051	0.056109	0.154642
W2 {3}	0.549561	0.775051		0.373121	0.697111
W3 {4}	0.026862	0.056109	0.373121		0.977058
W4 {5}	0.078167	0.154642	0.697111	0.977058	

Table 38: Tukey HSD test for FFA of the wood smoked group between weeks of chilled storage.

Time (FFA change)	Tukey HSD test; Variable: W-FFA (DATA-FINAL 25214) Marked differences are significant at p < .05000				
	{1} (M=1.9114)	{2} (M=2.1727)	{3} (M=2.3493)	{4} (M=3.0285)	{5} (M=2.8473)
W0 {1}		0.926107	0.670021	0.026400	0.074489
W1 {2}	0.926107		0.981360	0.115229	0.282315
W2 {3}	0.670021	0.981360		0.276505	0.561320
W3 {4}	0.026400	0.115229	0.276505		0.979517
W4 {5}	0.074489	0.282315	0.561320	0.979517	

Table 39: Linear Regression for FFA of the liquid smoked group with storage time.

Regression Summary for Dependent Variable: L- FFA (DATA-FINAL 25214) R= .68125754 R <sup>2</sup> = .46411184 Adjusted R <sup>2</sup> = .43434027 F(1,18)=15.589 p<.00094 Std.Error of estimate: .64490						
N=20	b*	Std.Err. (of b*)	b	Std.Err. (of b)	t(18)	p-value
Time (FFA change)	0.681258	0.172544	0.4026	0.10197	3.94830	0.000942

Table 40: Linear Regression for FFA of the wood smoked group with storage time.

Regression Summary for Dependent Variable: W-FFA (DATA-FINAL 25214) R= .66835271 R <sup>2</sup> = .44669535 Adjusted R <sup>2</sup> = .41595620 F(1,18)=14.532 p<.00128 Std.Error of estimate: .45253						
N=20		Std.Err. (of b*)	b	Std.Err. (of b)	t(18)	p-value
Time (FFA change)	0.668353	0.175326	0.2728	0.071552	3.81206	0.001277

**PV**

Table 41: T-test for PV between the liquid smoked group and wood smoked group.

T-test for Independent Samples (DATA-FINAL 25214) Note: Variables were treated as independent samples											
Group 1 vs. Group 2	Mean Group 1	Mean Group	t-value	df	p	Valid N Group 1	Valid N Group 2	Std.Dev. Group 1	Std.Dev. Group 2	F-ratio Variances	p Variances
L-PV- (W0) vs.W - PV (W0)	0.027649	0.237414	-4.19075	10	0.001856	6	6	0.020320	0.120912	35.40818	0.001319
L-PV- (W1) vs.W - PV (W1)	0.100516	0.255728	-6.33594	10	0.000085	6	6	0.015513	0.057966	13.96248	0.011660
L-PV- (W2) vs.W - PV (W2)	0.222252	0.219715	0.179937	10	0.860796	6	6	0.024372	0.024466	1.007764	0.993435
L-PV- (W3) vs.W - PV (W3)	0.145058	0.249426	-1.21997	10	0.250466	6	6	0.090867	0.188827	4.318357	0.134270
L-PV- (W4) vs.W - PV (W4)	0.178893	0.309781	-3.52229	10	0.005517	6	6	0.033421	0.084665	6.417630	0.062312

Table 42: Tukey HSD test for PV of the liquid smoked group between weeks of chilled storage.

Tukey HSD test; Variable: L-PV (DATA-FINAL 25214) Marked differences are significant at p < .05000					
Time (PV change)	{1} (M=.02765)	{2} (M=.10052)	{3} (M=.22225)	{4} (M=.14506)	{5} (M=.17889)
W0 {1}		0.076279	0.000130	0.001591	0.000184
W1 {2}	0.076279		0.001090	0.467258	0.049181
W2 {3}	0.000130	0.001090		0.054124	0.493522
W3 {4}	0.001591	0.467258	0.054124		0.710423
W4 {5}	0.000184	0.049181	0.493522	0.710423	

Table 43: Tukey HSD test for PV of the wood smoked group between weeks of chilled storage.

Time (PV change)	Tukey HSD test; Variable: W-PV (DATA-FINAL 25214) Marked differences are significant at $p < .05000$				
	{1} (M=.23741)	{2} (M=.25573)	{3} (M=.21971)	{4} (M=.24943)	{5} (M=.30978)
W0 {1}		0.998490	0.998679	0.999726	0.788825
W1 {2}	0.998490		0.979298	0.999979	0.913918
W2 {3}	0.998679	0.979298		0.989920	0.628658
W3 {4}	0.999726	0.999979	0.989920		0.877192
W4 {5}	0.788825	0.913918	0.628658	0.877192	

Table 44: Linear Regression for PV of the liquid smoked group with storage time.

N=30	Regression Summary for Dependent Variable: L-PV (DATA-FINAL 25214) R= .6212374 R <sup>2</sup> = .38593136 Adjusted R <sup>2</sup> = .36400033 F(1,28)=17.598 $p < .00025$ Std.Error of estimate: .06408					
	b*	Std.Err. (of b*)	b	Std.Err. (of b)	t(28)	p-value
Time (PV change)	0.621234	0.148091	0.03470	0.008273	4.19494	0.000249

Table 45: Linear Regression for PV of the wood smoked group with storage time.

N=30	Regression Summary for Dependent Variable: W-PV (DATA-FINAL 25214) R= .18539511 R <sup>2</sup> = .03437135 Adjusted R <sup>2</sup> = ----- F(1,28)=.99665 $p < .32667$ Std.Error of estimate: .10741					
	b*	Std.Err. (of b*)	b	Std.Err. (of b)	t(28)	p-value
Time (PV change)	0.185395	0.185706	0.01384	0.013866	0.998326	0.326671

**TBARS**

Table 46: T-test for TBARS between the liquid smoked group and wood smoked group.

Group 1 vs. Group 2	T-test for Independent Samples (DATA-FINAL 25214) Note: Variables were treated as independent samples										
	Mean Group 1	Mean Group	t-value	df	p	Valid N Group 1	Valid N Group 2	Std.Dev. Group 1	Std.Dev. Group 2	F-ratio Variances	p Variances
L-TBARS- (W0) vs.W - TBARS (W0)	4.253327	414.6242	-3.78968	10	0.003545	6	6	8.365006	265.1148	1004.466	0.000000
L-TBARS- (W1) vs.W - TBARS (W1)	290.6856	434.9088	-1.92477	10	0.083150	6	6	93.07137	158.1920	2.888930	0.269140
L-TBARS- (W2) vs.W - TBARS (W2)	253.3249	429.5334	-3.50585	10	0.005671	6	6	81.29211	92.45939	1.293616	0.784452
L-TBARS- (W3) vs.W - TBARS (W3)	219.3508	460.1900	-3.51958	10	0.005542	6	6	69.65618	152.4554	4.790341	0.110608
L-TBARS- (W4) vs.W - TBARS (W4)	271.7845	545.8666	-3.29338	10	0.008104	6	6	56.04936	195.9948	12.22779	0.015711

Table 47: Tukey HSD test for TBARS of the liquid smoked group between weeks of chilled storage.

Time (TBARS change)	Tukey HSD test; Variable: L-TBARS (DATA-FINAL 25214) Marked differences are significant at $p < .05000$				
	{1} (M=4.2533)	{2} (M=290.69)	{3} (M=253.32)	{4} (M=219.35)	{5} (M=271.78)
W0 {1}		0.000130	0.000145	0.000226	0.000139
W1 {2}	0.000130		0.875587	0.391180	0.988651
W2 {3}	0.000145	0.875587		0.908252	0.989617
W3 {4}	0.000226	0.391180	0.908252		0.676140
W4 {5}	0.000139	0.988651	0.989617	0.676140	

Table 48: Tukey HSD test for TBARS of the wood smoked group between weeks of chilled storage.

Time (TBARS change)	Tukey HSD test; Variable: W- TBARS (DATA-FINAL 25214) Marked differences are significant at $p < .05000$				
	{1} (M=414.62)	{2} (M=434.91)	{3} (M=429.53)	{4} (M=460.19)	{5} (M=545.87)
W0 {1}		0.999693	0.999910	0.992226	0.723300
W1 {2}	0.999693		0.999998	0.999255	0.826669
W2 {3}	0.999910	0.999998		0.998371	0.801114
W3 {4}	0.992226	0.999255	0.998371		0.923397
W4 {5}	0.723300	0.826669	0.801114	0.923397	

Table 49: Linear Regression for TBARS of the liquid smoked group with storage time.

N=30	Regression Summary for Dependent Variable: L-TBARS (DATA-FINAL 25214) R= .53890364 R <sup>2</sup> = .29041714 Adjusted R <sup>2</sup> = .26507489 F(1,28)=11.460 p<.00212 Std.Error of estimate: 106.11					
	b*	Std.Err. (of b*)	b	Std.Err. (of b)	t(28)	p-value
Time (TBARS change)	0.538904	0.159192	46.37	13.699	3.38523	0.002122

Table 50: Linear Regression for TBARS of the wood smoked group with storage time.

N=30	Regression Summary for Dependent Variable: W- TBARS (DATA-FINAL 25214) R= .23584558 R <sup>2</sup> = .05562314 Adjusted R <sup>2</sup> = .02189539 F(1,28)=1.6492 p<.20960 Std.Error of estimate: 173.57					
	b*	Std.Err. (of b*)	b	Std.Err. (of b)	t(28)	p-value
Time (TBARS change)	0.235846	0.183651	28.78	22.408	1.28420	0.209595

**Appendix 5: Pictures of smoked mackerel**

L0, 1, 2, 3, 4: Liquid smoked mackerel after smoking and 1,2,3,4 weeks of chilled storage.  
W0, 1, 2, 3, 4: Wood smoked mackerel after smoking and 1,2,3,4 weeks of chilled storage.  
a, b: Replicate

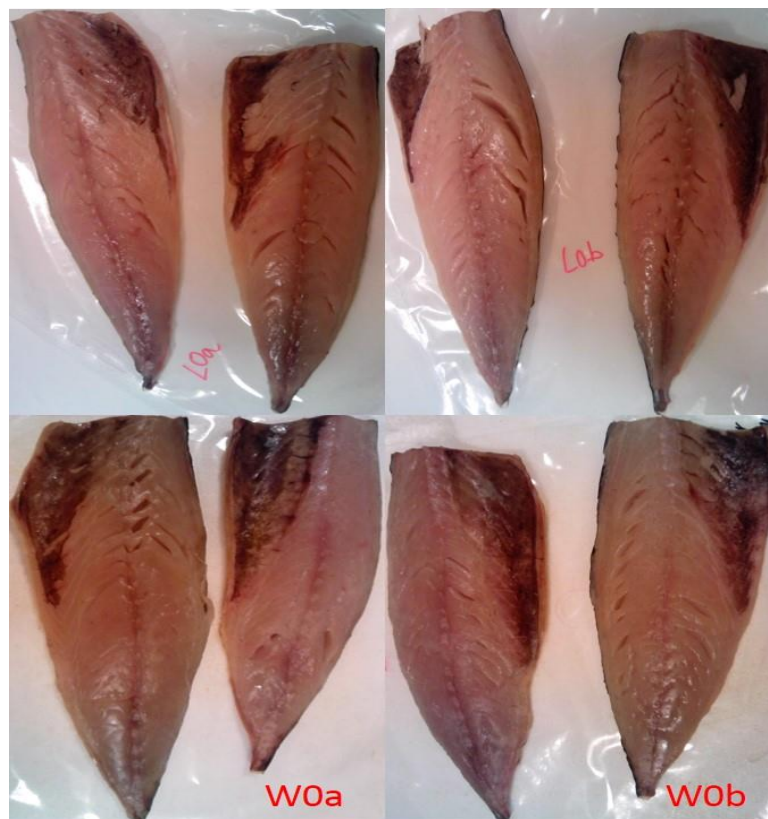


Figure 14: Mackerel fillets after smoking.



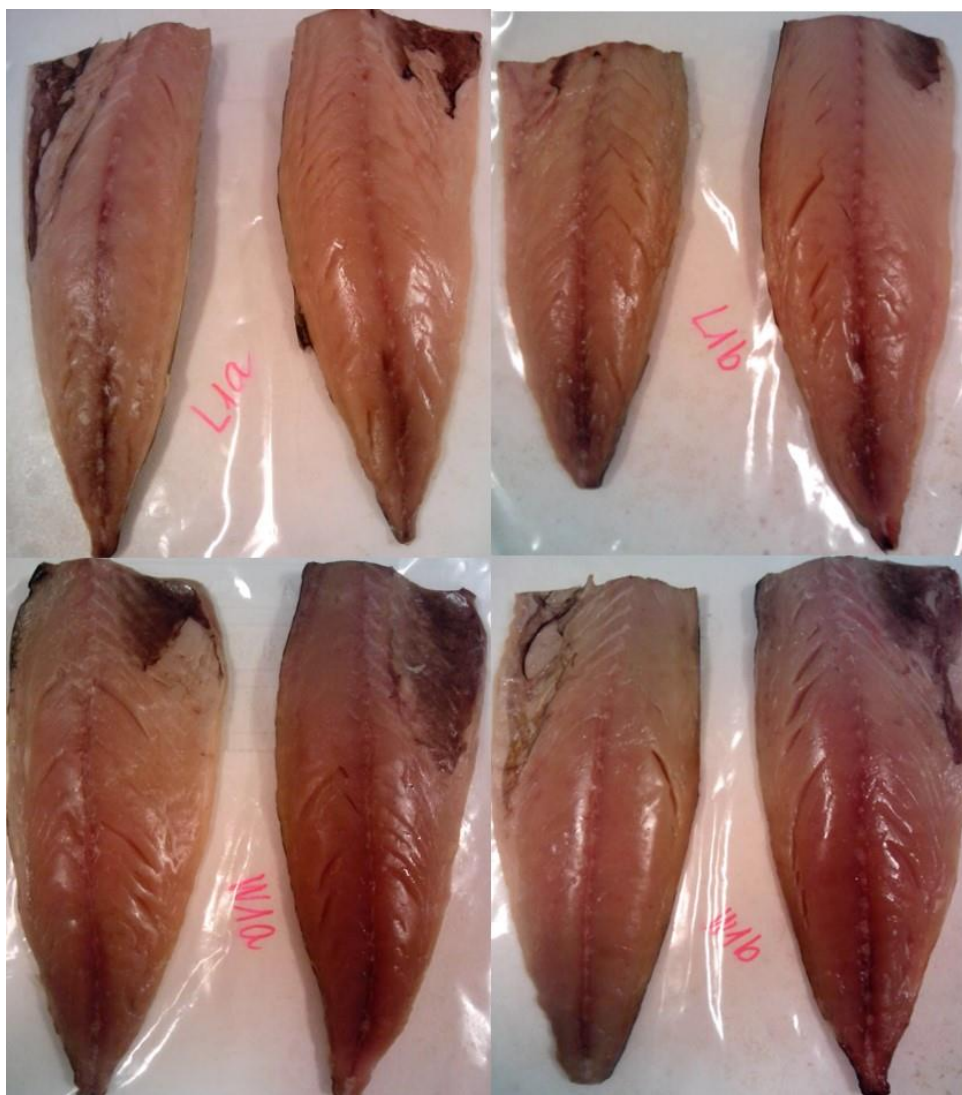


Figure 15: Smoked mackerel fillets after one week of storage time.

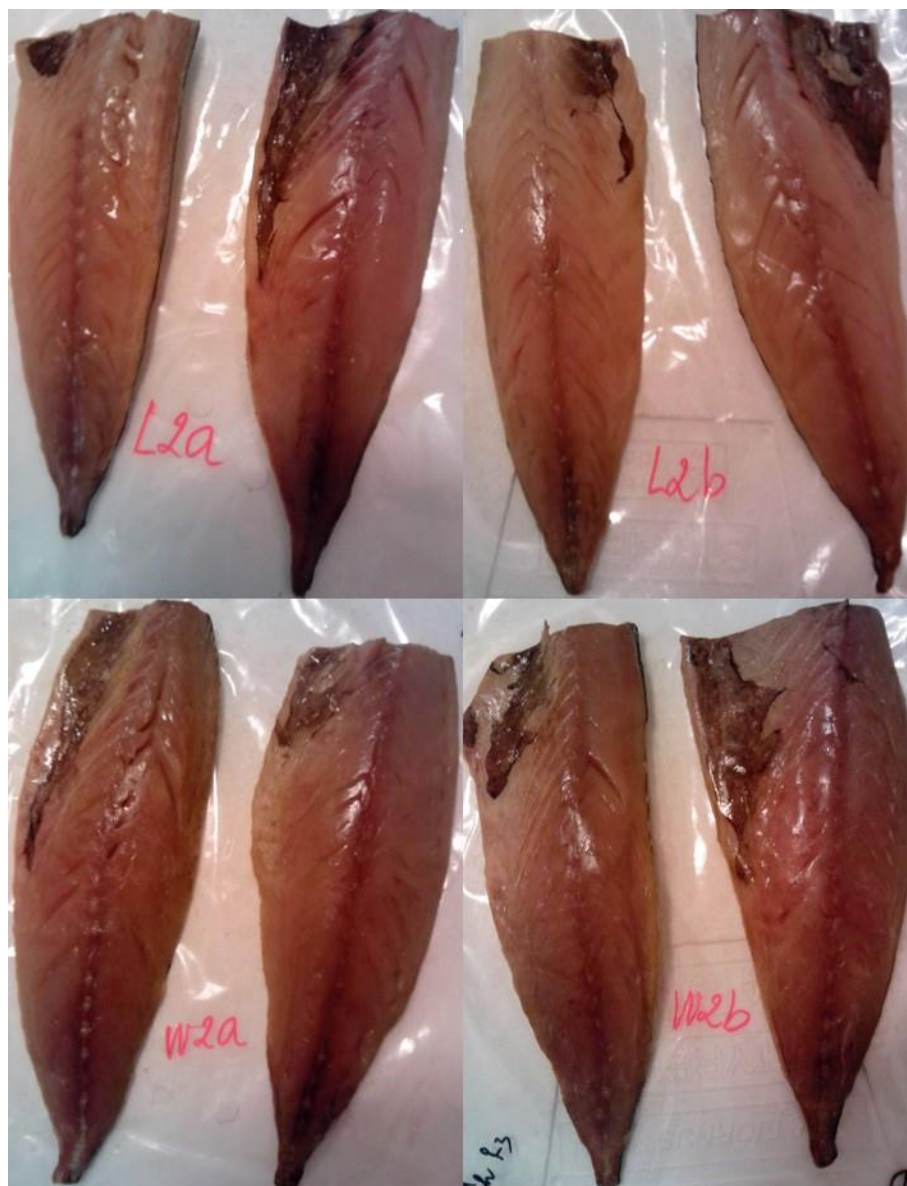


Figure 16: Smoked mackerel fillets after two weeks of storage time.



Figure 17: Smoked mackerel fillets after three weeks of storage time.



Figure 18: Smoked mackerel fillets after four weeks of storage time.