OXIDATION OF LIPIDS ON MACKEREL FILLETS DURING COOKING

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ABSTRACT

Mackerel rich in n-3 PUFA is one kind of popular fish in Iceland and China. Lipid changes of mackerel fillets cooked for 6 min and 30 min were studied, in which lipid composition and oxidation were evaluated. Results showed lipid and PL content significantly increased after cooking, while FFA decreased. Not many changes of FAC were detected after cooking, except C22:0 increased 6 folds dramatically, which responded to slight decrease of C22:5n3, and C18:0 increased responding to C18:3n3 reduced. Higher lipid oxidation level was determined with PV and AV after 30 min cooking, which shown marked difference from raw fillets. Furthermore, 12 volatile compounds closely related with lipid oxidation were detected in the three groups. It was found that the content of propanal and (E,Z)-2,6-Nonadienal derived from n-3 PUFA increased dramatically after steaming, especially for (E,Z)-2,6-Nonadienal, which content after cooking was more than 19 fold higher than the raw material. In addition, pure lipid changes during heating were evaluated, in which effect of other components was removed. In pure lipid system, PL content decreased and FFA from different groups had no difference. The change of FAC were similar with FAC in fillets, C18:3n3 and C22:5n3 reduced after heating. But PV was lower after cooking, and AV was detected. It is noteworthy that heating induced darker lipids, which should be studied further to analyse the oxidation products.

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1 INTRODUCTION

Fish is an excellent food source because of its nutrients, especially n-3 polyunsaturated fatty acids (n-3 PUFAs), such as docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA). n-3 PUFAs from fish mainly exist in triacylglycerols (TAG) and phospholipids (PL) (Pacetti, et al., 2015). The bioactivity of n-3 PUFAs has been associated with prevention of heart disease, anti-inflammatory properties, and improving learning ability (Moussa, Shereen, Manal, Mehanni, & Rasha, 2014).

Mackerel, an important fish species widely consumed in Iceland and China, is classified as a high-fat fish and a great source of omega-3 PUFA. (Romotowska, Karlsdottir, Gudjonsdottir, Kristinsson, & Arason, 2016). However, most mackerel are consumed after steaming, boiling, frying and broiling, and the culinary processes undoubtedly alter the content, the composition and the activity of the fish lipids, since PUFAs are susceptible to thermal oxidation with excessive heat. Therefore, several studies have focused on the effects of different cooking procedures on fatty acid composition of the flesh. EPA and DHA levels vary with the fish species and cooking treatment (Larsen, Quek, & Eyres, 2010). Mackerel lipid composition has been analysed to study the effect of deep-fat frying with sunflower oil, which showed omega-3 PUFA decreased significantly from 16.6g/100g to 5.4g/100g of fat (Candela, Astiasaran, & Bello, 1998). In addition, 53 volatile components were found in broiled mackerel as a result of thermal degradation of the lipids, compared with 38 volatile components detected in raw mackerel (Chung, Choi, Cho, & Kim, 2011). Furthermore, there are some compounds from volatile components responsible for flavour, such as alcohols of 8 carbon atoms, 2,6-nonadienal, (Z)-4-heptenal, 2-pentylfuran, 2-ethylfuran, 2,3butanedione and 2,3-pentanedione, produced in cooked fish (Nieva-Echevarría, Manzanos, Goicoechea, & Guillén, 2017) .

In China, attention has been focused on eating food rich in PUFAs. There is limited knowledge about changes of PUFAs in fish during cooking. Most research is related to polyunsaturated fatty acids changes in aquatic products during storage. At the same time, there is no quality, processing and operational standard for fish lipids. Furthermore, there has been confusion related to classification of fish lipids. They can be classified as either animal lipids or aquatic products, so it is difficult for producers and consumers to follow a suitable standard. Another factor to consider is that in China, fish is traditionally cooked for a very long time compared to Europe, in which processed fish lipids (PUFAs especially) may be oxidized. More information about lipid characteristics during cooking will be helpful to build accurate standards for fish lipids and create a more nutritional food through culinary processing.

In this project, oxidation levels and products of mackerel fillets from different cooking time will be compared. At the same time, oxidation of total lipids will be evaluated during cooking, from which, reactions from other constituents will be removed, and more information will be obtained by comparing fish fillets and lipids. This project will be useful to understand the oxidation and changes of lipids in mackerel fillets during steaming. This research also aims to determine optimal steaming time that can be recommended to obtain optimal nutritional qualities in the form of retaining PUFAs.

2 LITERATURE REVIEW

2.1 Fish lipid composition and characteristics

Fat content and fatty acid composition of fish lipids are extremely variable. According to the amount of lipid content in the body, fish are classified into four groups: lean fish, fat <2%, such as cod, haddock, shellfish; low fat fish means 2-4% fat, such as sole, halibut, flounder; medium fat fish with 4-8% fat, includes wild salmon; and high fat fish, whose fat is >8% fat, such as herring, mackerel, sablefish, farmed salmon (Moradi, Bakar, Motalebi, Muhamad, & Man, 2011). The fish lipids are extremely variable, and are often affected by different abiotic and biotic factors such as season, the type and amount of feed available, water temperature, pH, salinity, and reproductive cycle, even within a species (Moradi, Bakar, Motalebi, Muhamad, & Man, 2011). For example, it has been found that the lipid content can vary from 4 to more than 30% in mackerel depending on the season.

Triglycerides (TAGs) are the main lipid in fish flesh, which also includes phospholipids (PLs) and sterols, and minor quantities of glycolypids and sulfolipids, metabolized from lipids glycosylation and sulfation. TAG is the storage lipid in almost all commercial fish species, which is increased with increasing lipid content in the muscles (Drazen, 2007). PLs, important component of cell membrane, are a typical lipid class found in fish flesh but more in fish roe, viscera and brain. Compared with fat fish, lean fish shows a higher proportion of the phospholipids contributes to the total lipids. For this reason, phospholipids comprise almost 90% of total lipids in lean fish (such as cod), with TAG contributing as little as about 1% (Moradi, Bakar, Motalebi, Muhamad, & Man, 2011).

The main groups of fatty acids in fish lipids are classified as saturated fatty acids (SFA), in which palmitic acid (16:0) is often the predominant fatty acid, monounsaturated (MUFA), or polyunsaturated fatty acid (PUFA), in which besides DHA and EPA, linolenic acid is an important PUFA with active roles. The proportion of each fatty acid (FA) group in the total lipid content from tissue is not constant, and differs depending on total lipid content (and hence lipid classes composition) and biological factors (Dunstan, Bailie, Barrett, & Volkman, 1996). The PUFA composition is the most attractive characteristic trait of fish lipids, since n-6/n-3 ratio has been recommended in human diet.

2.2 Lipid oxidation

Hydrolysis and oxidation are the two main pathways for lipids degradation, in which hydrolysis usually occurs before oxidation in the presence of water to produce free fatty acids. On the other hand, several mechanisms for lipid oxidation have been identified, such as autoxidation, photoxidation, and enzymatic or singlet oxygen-mediated oxidation (Lu F. , Nielsen, Baron, & Jacobsen, 2017). Lipid oxidation is a complicated, multi-step process, especially in foods, which includes many interacting components, such as hydroperoxide decomposition catalysts (metal), oxidation related enzymes and natural antioxidant (tocopherol). Besides these, the oxidation variables encompassed include individual fatty acid susceptibility, molecular structure of lipids, physical state of lipids, initiation reactions, presence of oxidized lipids and so on (German, 1997).

Lipid peroxidation often starts with a free radical chain reaction, subsequently yields to primary products, in which oxygen is the most important factor initiating lipid peroxidation in food (Ahn, Wolfe, & Sim, 1993). Theoretically, oxygen molecules cannot interact with lipids directly, but by the production of reactive oxygen species (ROS). Oxygen molecules can be reduced

primarily to ROS, including hydroxyl radical ($^{\cdot}OH$), superoxide anion ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), hydroperoxyl radical ($HO_2^{\cdot}$), lipid peroxyl radical ($LOO^{\cdot}$), alkoxyl radical ($LO^{\cdot}$), iron-oxygen complexes (ferryl- and perferryl radical) and singlet oxygen ($^1O_2$), all of which are highly reactive to participate in lipid peroxidation directly or indirectly. In addition, transition metals (iron and copper) are the most probable catalysts for oxidative processes through nonenzymic mechanisms. Especially free ionic iron plays a critical role in lipid peroxidation. (Min & Ahn, 2005). For enzymic oxidation, some enzymes have been suggested to catalyse lipid oxidation, such as NADPH, lipoxygenase and myeloperoxidase. Except prooxidants, natural antioxidants protect lipids against oxidation. Tocopherol plays a role in pyrroles formation, which prevent krill oil oxidation (Lu, Bruheim, Haugsgjerd, & Jacobsen, 2014).

Besides external factors, the overall rates of oxidation are mainly affected by the own characteristics of lipids, ease of hydrogen abstraction afforded by the abundance of double bonds on fatty acids. Thus, the content of PUFAs often dramatically increases oxidation of oils. The rate of oxidation is increased by the number of methylenic hydrogens, afforded by the number of double bonds in PUFA. The relative oxidation rates for fatty acids with 1,2,3 and 4 double bonds are 1, 50, 100 and 200, respectively. It was further found that the increases in reaction rates for PUFA with more than two double bonds only increased the number of methylene-interrupted sites that do not affect the ease of hydrogen abstraction. The number of double bonds dramatically increases the number of possible hydroperoxide positions, which further probably take part in the breakdown pathways of lipids (German, 1997).

During lipid deterioration, many products are generated from the original lipid, including primary oxidation products and secondary compounds. Hydroperoxides are the major primary products of lipid oxidation, but unstable and essentially odourless, and decompose into a wide range of secondary compounds, such as alkanes, alkenes, aldehydes, ketones, alcohols, esters, acids, and hydrocarbons. Some of these compounds are responsible for food flavour. Asldehydes are considered the most important breakdown products because they possess low threshold values (Carolyn & Denise, 2005).

2.3 Evaluation methods of fish lipid oxidation

Oxidation products are often used to evaluate the lipid oxidation in foods, including hydroperoxides and secondary oxidation products, such as volatile compounds and oligomers and polymers. Many methods have been used to determine both primary and secondary lipid oxidation products, such as spectroscopic to chromatographic techniques and magnetic resonance (Barriuso, Astiasaran, & Ansorena, 2013).

2.3.1 Primary oxidation products

Hydroperoxides redox properties are the base of some of the key methods applied in their determination. A number of reagents can be oxidized by hydroperoxides, such as iodide or ferrous ions. Among the different methods, peroxide value (PV) can be measured easily and rapidly without expensive equipment. For example, iodometry has been the most conventional and widespread method mainly due to the simplicity of the experimental procedure, which is as an official method since 1965 offered by AOAC (AOAC, Peroxide value of oils and fats 965.33.12. , 2000). According to this method, PV represents the quantity of active oxygen in 1 kg of lipid by oxidizing the potassium iodide. In addition to the volumetric method, spectroscopic methods are frequently used to determine ferrous or iodide oxidation for peroxide content with specific absorbance wave length (Barriuso, Astiasaran, & Ansorena, 2013).

Actually, chromatographic methods for hydroperoxides determination have been developed instead of volumetric and spectroscopic measurements. A method for measuring the peroxide value has been reported in coloured lipids in which reacted with triphenylphosphine to form a compound measured at 260 nm by Gotoh et al (Gotoh, Miyake, Takei, Sasaki, & Okuda, 2011). In addition to quantitative of hydroperoxides, information about molecular structures can also be presented by liquid chromatography (Saynajoki, Sundberg, Soupas, Lampi, & Piironen, 2003).

2.3.2 Secondary oxidation products

Secondary oxidation products can be generated from primary lipid oxidation products by to further oxidation, from which aldehydes, ketones, epoxides, hydroxy compounds, oligomers and polymers can be detected. Many methods have been developed for analysis of aldehydes and volatiles, which are very frequently used as oxidation marker.

2-thiobarbituric acid reactive substances (TBARS) assay is a traditional and common spectrophotometric measurement for malondialdehyde (MDA), one of the most abundant aldehydes generated during secondary lipid oxidation. The reaction will produce red fluorescent MDA-thiobarbituricacid (MDA-TBA) complex, which is not selective to MDA, since it also reacts with many other aldehydes, carbohydrates, amino acids or nucleic acid. Furthermore, MDA is mainly formed from linolenic acid, and not generated in other oxidized lipids, so it is often a minor secondary oxidation product. Besides spectroscopy, more advanced chromatographic determinations have been developed, from which the formation of the MDA-TBA complex will be detected, the process including purification by chromatography (GC or HPLC) and subsequent detection by MS, UV–Vis or fluorometric detector (Seljeskog, Hervig, & Mansoor, 2006; Mendes R, 2009).

p-anisidine value (AV) are often used to measure other aldehydes than MDA, which are generated during secondary lipid oxidation from other oxidized lipids. AV provides useful information on carbonyl compounds, especially non-volatile α-unsaturated aldehydes (such as 2-alkenals and 2,4-dienals) because it is based on the reactivity of the aldehyde carbonyl bond on the *p*-anisidine amine group, leading to the formation of a Schiff base. The analysis of AV and TBARS could be used together to get overall results for secondary oxidation since they detect different aldehydes from lipids oxidation (Nuchi, et al., 2009).

One kind of special group in secondary oxidation products are volatiles, including aldehydes, ketones, alcohols, short carboxylic acids and hydrocarbons. The volatiles from lipid oxidation contribute to the aroma of food. Measurement of these secondary oxidation products is of great importance, since their formation is closely related to the deterioration of flavour and specific to the oxidative degradation. Analysis of volatiles includes extraction, separation and identification. Dynamic headspace (DHS) and solid-phase microextraction (SPME) are the preferred methods to extract volatile compounds, subsequently GC-MS contributes to identifying them (Barriuso, Astiasaran, & Ansorena, 2013). The method has been applied to applied to determine the volatiles of yellowfin tuna, mackerel, sea bream, even in fish oil emulsions (Iglesias & Medina, 2008; Iglesias J. , et al., 2009; Iglesias, Lois, & Medina, 2007).

2.3.3 Alternative methodologies

Besides methods mentioned above for analysis lipid oxidation, direct spectroscopic analysis has been developed, such as magnetic resonance and infrared spectroscopy (IR). IR is a useful way to study lipid degradation under oxidative conditions, particularly since it is an easy, rapid,

economical and non-destructive technology, which has been used in detection oxidized lipids of horse mackerel, saithe and hoki (Gimenez, Gomez-Guillen, Perez Mateos, Montero, & Marquez Ruiz, 2011; Karlsdottir, Arason, Kristinsson, & Sveinsdottir, 2014). Nuclear magnetic resonance (NMR) relies on the property of certain atoms of absorbing and re-emitting energy in the presence of a strong magnetic field due to the excitation of their atomic nuclei. ^{1}H and ^{13}C NMR have been used in evaluating the oxidative status of the lipid fraction (Dybvik, Falch, & Rustad, 2008; Tyl, Brecker, & Wagner, 2008).

2.4 Effects of cooking methods on the fish lipid characteristics

Generally, cooking methods can modify lipid composition and content. Cooking methods are divided into three categories: dry-heat, moist-heat, and combination-heat method. Dry-heat methods cook the foods with hot air or fat (pan-frying, deep-frying, grilling, broiling, roasting, baking); moist-heat cooking methods cook the food with a liquid, usually water, stock, or steam (poaching, boiling, steaming); and combination cooking methods use a combination of dry- and moist-heat methods (braising, stewing) (Moradi, Bakar, Motalebi, Muhamad, & Man, 2011).

Frying. Frying often changes the fat content of the fish. The mechanisms for the changes probably due to the absorption of culinary fat into the fish, moisture loss of the food, leaching of fat soluble molecules out of the food, and oxidation reactions. Shallow- and deep-frying induce different alternation of fish PUFA. It has been found DHA and EPA levels in silver catfish were significantly reduced during frying in canola and hydrogenated vegetable oil due to the oil absorption during frying (Weber J. , Bochi, Ribeiro, Victorio, & Emanuelli, 2008). A comparison of cooking methods including of broiling, baking and frying, found cooking treatments had little effect on omega-3 fatty acid content, but had higher omega-6 and MUFA content as a result of cooking oil (Neff, Bhavsar, Braekevelt, & Arts, 2014).

Baking and grilling. Similar with other cooking, oven baking caused fillets to loose water with a consequent increase in protein, fat, and ash content, which has been found in silver catfish. However, dehydration was lower than during frying (Weber J. , Bochi, Ribeiro, Victorio, & Emanuelli, 2008). Grilling also resulted in an increase in total lipids and PUFAs by decreasing water content in fish (Nichols, Mooney, & Elliott, 2001), and grilling made fish lose more water than oven baking but lower than frying.

Cooking without oil. Effects of cooking without oil on FA change have been studied. FA contents were increased without great differences between individual FAs in lean pike in microwave baking methods because shorter cooking time decreased the loss of fluidified lipids outside the flesh (Agren & Hanninen, 1993). Boiling affected the silver catfish fillets fatty acid content, in which C14:1n-5, C20:0, C22:0, and C22:1n-9 were not detected in raw fillets, but were found at low levels after these heating treatments (Nichols, Mooney, & Elliott, 2001). Changes provoked by boiling, steaming and sous-vide cooking in the lipid and volatile profile of European sea bass were studied with ^{1}H NMR, but none of hydrolytic or oxidation processes were detected. However, steaming and sous-vide cooking modified the volatile profile of sea bass meat (Nieva-Echevarría, Manzanos, Goicoechea, & Guillén, 2017).

3 METHODS OF STUDY

3.1 Experimental design

Fatty fish, mackerel, was used as experimental material. In order to investigate the lipid oxidation of mackerel by cooking, mackerel fillets were divided to three groups, raw fillets,

fillets cooked 6 min, and fillets cooked 30 min, respectively (shown in Fig.1). Lipids were extracted in all the groups of fillets, and were evaluated oxidation level with chemical analysis (PV, AV, TBARS, et al). Due to complicated components and reactions in fish fillets, pure lipids heated were also compared, from which other affection were removed.

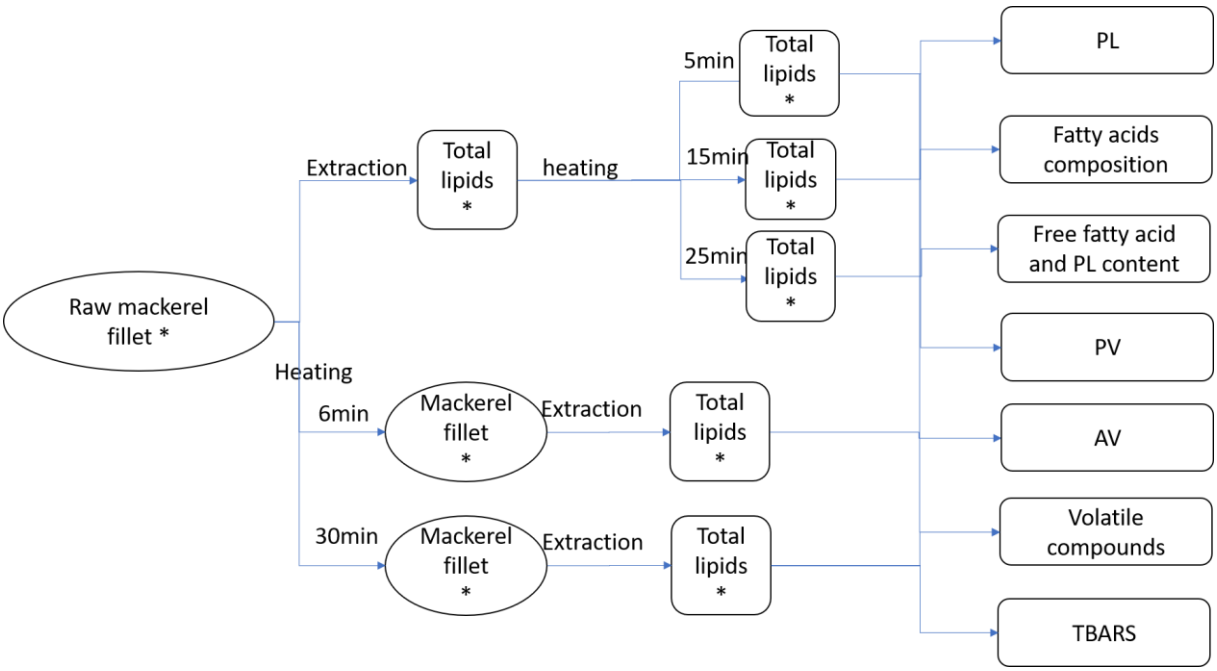


Figure 1. Technical roadmap in the project (All the processes marked with stars mean samples for analyses)

3.2 Samples and cooking method

Frozen whole mackerel was obtained from HB Grandi. Fishes were filleted by hand. Mackerel fillets were cooked by steaming at 100 ℃ for 6 min and 30 min separately.

Total lipids from raw mackerel fillets were cooked in 100 ℃ water bath for 5 min, 15 min, 25 min, separately.

3.3 Water and lipid content

Water content was determined by the difference in weight of the homogenised fillet samples before and after drying for 4h at 102 to 104℃ (ISO, 1993). Results were calculated as grams of water/100g fillet. Total lipids were extracted from fillet samples with methanol/chloroform/0.88% KCl (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 expressed as gram lipid/100g wet fillet.

3.4 Total lipids extraction and composition

3.4.1 Extraction total lipids

Total lipids were extracted from mackerel with a solution of chloroform and methanol according to Bligh & Dyer (1959) with adaptations. 10g of ground sample, and 15g water were added into 250/500ml centrifuge bottle with 25ml of chloroform and 50ml of methanol and homogenized for 2 minutes. Then another 25ml of chloroform was added and continued mixing for 1 minute. Subsequently 25mL 0.88% KCl was added and mixed for 1 minute. The centrifuge bottle was centrifuged for 20 minutes at 2500 rpm at 4°C. After that, the lower chloroform phase containing the fat were transferred with pipettes. The chloroform phase was then filtrated

on a glass microfibre under suction. The suction flask content was then poured into a 50mL volumetric flask. Every trace of the upper phase (aqueous phase) was removed and the 50mL volumetric flask filled with chloroform to the mark.

3.4.2 Fatty acid profile

The fatty acid composition of the total lipids was determined by gas chromatography of fatty acid methyl esters (FAMEs). The methylation of fatty acids was carried out according to AOCS. The total lipids were vaporised at 55°C under nitrogen to a constant weight. 75mg of extracted lipids were dissolved in 1.5ml 0.5N NaOH in methanol and incubated for 7 minutes at 100°C. After cooling to room temperature, 2ml of BCl_3/MeOH (12% boron trichloride) were added and the samples incubated again for 30 minutes at 100°C. After subsequent cooling, 1ml of internal standard (1mg/ml of 23:0 methyl ester in isooctane) and 5ml of concentrated NaCl solution were added. After phase separation, the isooctane layer was transferred into a glass tube containing 1mm bed of anhydrous Na_2SO_4. This was repeated again with 1ml of clean isooctane. The combined isooctane layers, containing the FAMEs, were then transferred to GC vials. The FAMEs were separated on a Varian 3900 GC equipped with a fused silica capillary column (HP-88, 100m*0.25mm*0.20µm film, Agilent Technologies), split injector and flame ionisation detector fitted with Galaxie Chiromatography Data System (Version 1.9.3.2 software). The oven ramp was programmed as followed: 100°C for 4 minutes, then increased to 240°C at 3°C/min and held at this temperature for 15 minutes. The injector and detector temperature were 225 and 285°C, respectively. Helium was used as the carrier gas and the column flow rate was 0.8ml/min; with a split ratio of 200:1. This programme was based on the AOAC 996.06 (AOAC, Officail method Ce 996.06. Fat (Total, Saturated and Unsaturated) in Food, Hydrolytic Extraction Gas Chromatographic Method., 2001). Results were expressed as percentage of total lipids. This analysis was performed on four replicates (n=4) per sample.

3.4.3 Free fatty acids (FFA) content

Free fatty acid content was determined on the total lipids according to Lowry and Tinsley (Lowry & Tinsley, 1976), with modifications from Bernardez et al (Bernardez, Pastoriza, Sampedro, Herrera, & Cabo, 2005). The FFA concentration was calculated as µM quantities of oleic acid based on a standard curve spanning a 2-22µM range. Results were expressed as grams FFA/100 g of total lipids.

3.4.4 Phospholipid (PL) content

The phospholipid content was determined on the total lipid extracts and measured using a colorimetric method (Stewart, 1980). This method estimates the formation of a complex between phospholipids and ammonium ferrothiocyanate, by evaluation of absorbance of the resultant solution at 488nm (UV-1800 spectrophotometer, Shimadzu, Kyoto, Japan). A standard curve was prepared with phophatidylcholine in chloroform (5-50 mL^{-1}), and results were expressed as a percentage of the total lipid content (g PL/g TL)*100.

3.5 Chemical measurements of lipid oxidation

3.5.1 Determination of peroxide value (PV)

Lipid hydroperoxides (PV) were determined with a modified version of the ferric thiocyanate method. Total lipids or mackerel fillets were collected (500µL) and matched with 500µL chloroform: methanol solution. A total amount of 5µL of ammonium thiocyanate (4M) and ferrous chloride (80mM) mixture (1:1) was finally added. The samples were then brought to room temperature for 10 minutes and read at 500nm on a spectrometer (Tecan Sunrise, Austria).

A standard curve was prepared using cumene hydroperoxides. The results were expressed as mmol lipid hydroperoxides/g of lipid.

3.5.2 Thioarbituric reactive substances (TBARS)

A modified method of Lemon (Lemon, 1975) was used for measuring TBARS. A minced fillet sample (5.0g) was homogenized with 5.0ml of trichloroacetic acid (TCA) extraction solution (7.5% TCA, 0.1% propyl gallate and 0.1% ethylenediamine tetraacetic acid mixture prepared in ultrapure water) using a homogenizer at maximum speed for 10 seconds (Ultra-Turrax T-10 basic, IKA, Germany). The homogenized samples were added with 5.0ml TCA extraction solution and centrifuged at 9400 x g for 15 minutes (Model Z323K, Hermle laboratories, Germany). 0.5ml supernatant was collected and mixed with the same volume (0.5ml) of thiobarbituric acid (0.02M) and heated in a water bath at 95°C for 40 minutes. 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 530nm (POLARstar OPTIMA, BMG Labtech, Offenburg, Germany). A standard curve was prepared using tetraethoxypropane. The results were expressed as µmol of malomaldehyde diethylacetal per kg of samples.

3.5.3 p-anisidine value (AV)

The total amount of lipids needed is approximately 0.5g (m). The lipids were dissolved with isooctane and transfered into 25ml volumetric flask. The glass tube was cleaned several times with isooctane. Finally, 25ml volumetric flask was filled the up with isooctane. The absorbance of this solution (A1) was measured in glass cuvette at 350nm. 5ml of the same solution was transferred in to glass tube with cap and 1ml of reagent solution added into the glass tube and shaken well. Blank was prepared from 5ml of clean isooctane and 1ml of reagent. After 10 minutes, the tube was shaken again and the absorbance read (A2) at 350nm and also the absorbance of the blank (At)

$$Anisidne\ value = \frac{25\ x\ (1.2(A2 - At) - A1)}{m}$$

3.5.4 Method setting for HS-SPME-GC-MS

A HS-SPME-GC-MS method was set at first. 1-penten-3-ol and 1-octen-3-ol mixed with in ultrapure water or of ultrapure water saturated in NaCl were used as external standards (range 0.125 to 2 ppm) to test fibres. The volatiles were desorbed in the GC injection port for 10 minutes at 250 ℃ PDMS/DVB fibre, at 270 ℃ DVB/Carboxen/PDMS fibre.

3.5.5 Volatile compounds analysis with HS-SPME-GC-MS

For raw and cooking fish samples, 3g of minced fillet were homogenized for 2 minutes with a volume of 8ml of ultrapure water saturated in NaCl. The mixture was centrifuged (20 minutes, 5000 rpm) and 1ml supernatant was analysed.

All the SPME experiments were performed using a manual injection device. The PDMS/DVB fibre was exposed to the headspace of a 20ml vial containing supernatant of minced fish fillet heated for 6 minutes or 30 minutes.

GC-MS analysis was carried out on a Thermo Finnigan ThermoQuest gas chromatograph equipped with a split injector and coupled with a Trace quadrupole mass detector. Compounds were separated on a 30m*0.32mm*1µm film thickness, fused silica DB-1701 capillary column. The GC oven temperature programme was: 35℃ for 3 minutes, followed by an increase of

3℃/minute to 70℃; then an increase of 10℃/minute to 200℃ and finally an increase of 20℃/minute to a final temperature of 260℃, held for 5minutes. Helium, with a constant flow of 1.5ml/minute, was used as the carrier gas. Transfer line temperature was maintained at 265℃. The quadrupole mass spectrometer was operated in the electron impact mode and the source temperature was set at 200℃. Acquisition was performed in the 35-200amu mass range with a scan rate 0.220s/scan. All the analyses were performed setting ionisation energy at 70eV, filament emission current at 150µA and the electron multiplier voltage at 500V. The identification of the volatile compounds was achieved by comparing their mass spectra with those stored in the National Institute of Standards and Technology (NIST) US Government library. Pure standards were also injected to confirm MS identifications. In order to evaluate relative quantitative differences in the aromatic profiles of the samples investigated, GC peak areas of the compounds identified were calculated as total ion current (Iglesias J. , et al., 2009).

4 RESULTS

4.1 Changes of chemical composition of mackerel fillets after cooking

As shown in Figure 2 below, the water content of raw mackerel fillets was 53.35±0.26%, and steaming decreased water content in fillets, in which the lowest water content was 48.06±0.34% after 30 minutes cooking. The total lipids in the three groups range from 29.03±2.80% to 32.95±3.15%, and water losses increased total lipids content in steaming group, in which lipids in fillets cooked 30 minutes was significantly different than raw fillets. These results are similar with most results of lipids changes in fish during different cooking methods, such as frying, baking, grilling, boiling and steaming (Moradi, Bakar, Motalebi, Muhamad, & Man, 2011; Nieva-Echevarría, Manzanos, Goicoechea, & Guillén, 2017).

Figure 2. Water content of mackerel fillet affected by different cooking

Phospholipids in Atlantic mackerel affected by season range from 0.7% up to 4% in lipids, and have indicated that majority of the lipids were present as triglycerides (Romotowska, Karlsdottir, Gudjonsdottir, Kristinsson, & Arason, 2016), and therefore, the initial phospholipids in raw material was 2.26±0.21%. But after cooking, the phospholipids content

significantly increased to 2.71±0.07% and 3.07±0.19%（*P<0.05*）, which may be explained by increased extractability of phospholipids resulting from the protein denaturation during cooking, similar with extended frozen storage (Romotowska, Karlsdottir, Gudjonsdottir, Kristinsson, & Arason, 2016). At the same time, in the pure lipids system without protein existence, phospholipids tended to decreased, which was shown in Figure 3.

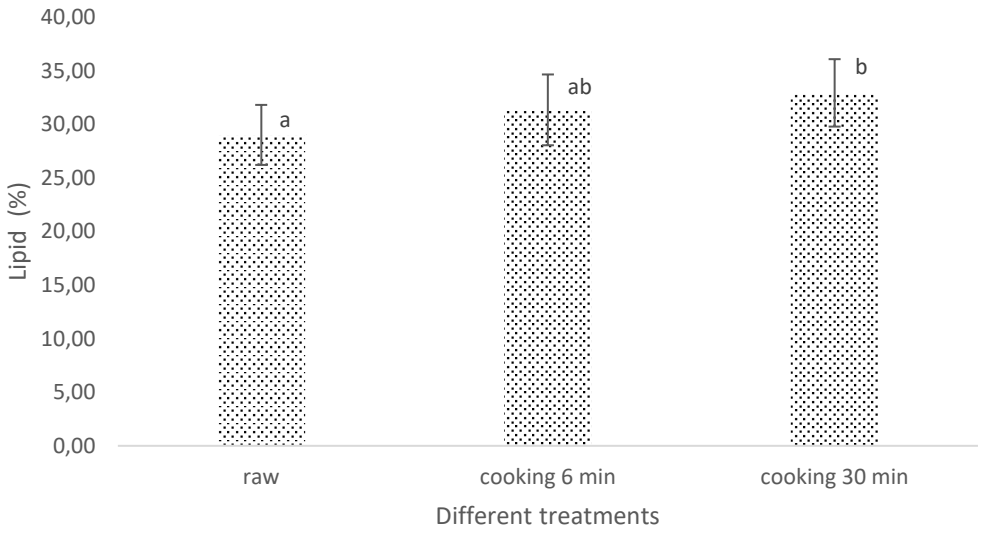


Figure 3. Lipid content of mackerel fillet affected by different cooking (lowercase letters different indicates that the difference is significant (P<0.05))

FFA produced by lipid hydrolysis due to enzymatic activity, was summarized in Figure 4. It was shown that FFA content was higher in raw fillets, which was 0.79±0.14g per 100g lipid. Cooking significantly decreased FFA content, in which FFA was 0.45±0.07g per 100g lipid （*P<0.05*）after cooking 6 minutes, similar with cooking 30 minutes. This is in agreement with the observation of Weber *et al*. who stated that decreased FFA content probably because of loss of volatile FFA and deactivation of hydrolysis enzyme occurred during heating (Weber J. , Bochi, Ribeiro, Victorio, & Emanuelli, 2008). PL content is illustrated in Figure 5.

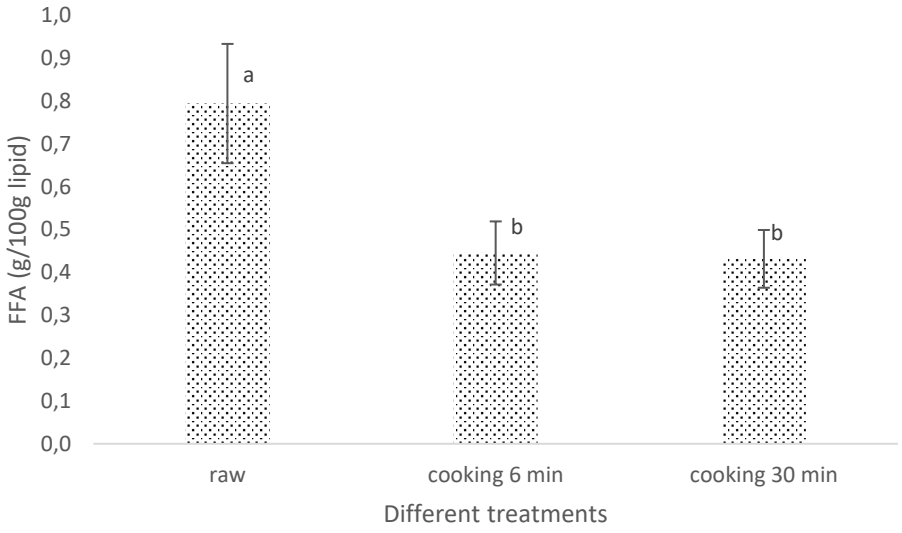


Figure 4. FFA content of mackerel fillet affected by different cooking (lowercase letters completely different indicates that the difference is significant (P<0.05))

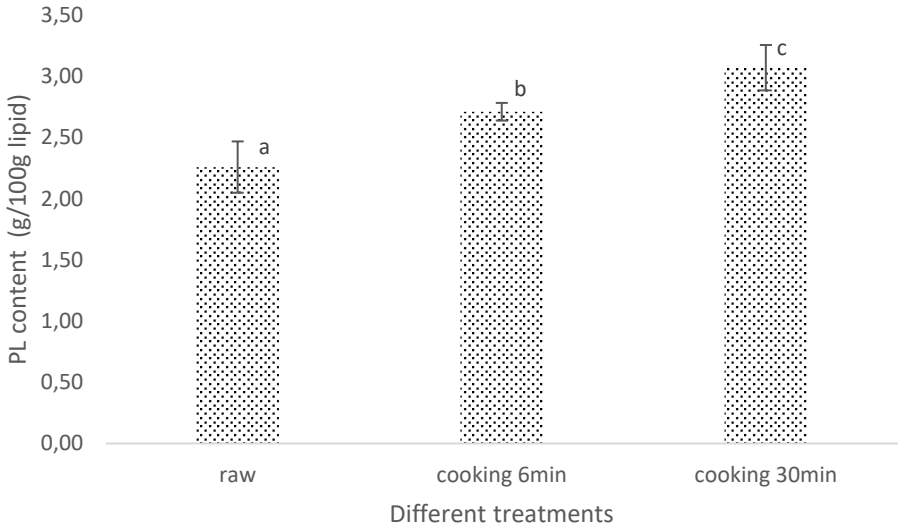


Figure 5. Phospholipid content of mackerel fillet affected by different cooking (lowercase letters completely different indicates that the difference is significant (P<0.05))

4.2 Fatty acid profile of mackerel fillets

The lipid composition of raw fillets and cooked fillets was investigated. Among SFAs, palmitic acid (16:0) was the predominant fatty acid observed, followed by eicosanoic acid (20:0) and stearic acid (18:0). The major fatty acid among MUFAs was palmitoleic acid (C16:1n7), followed by oleic acid (C18:1n7 and C18:1n9). Amongst the PUFAs, the predominating fatty acids were EPA, followed by C22:5n3 and least DHA was found. These results were different with Romotowska's result (Romotowska, Karlsdottir, Gudjonsdottir, Kristinsson, & Arason, 2016), in which DHA was predominant, probably from different source of mackerel. But not

many changes were found, even after cooking 30 minutes, shown in Table 1. For SFA, it was found C22:0 increased 6-fold, dramatically, which responded to slight decrease of C22:5n3. Besides C22:0, EPA showed a slight increase, and C18:0 increased responding to C18:3n3 reduced. The least change was in agreement with the results from baking black pomfret, and boiling and steaming European sea bass (Moradi, Bakar, Motalebi, Muhamad, & Man, 2011; Nieva-Echevarría, Manzanos, Goicoechea, & Guillén, 2017). On the contrary, Chung *et al*. found PUFAs and saturated fatty acids were at a low level in broiled mackerel, possibly as a result of thermal degradation of the lipids (Chung, Choi, Cho, & Kim, 2011). It is possible that steaming could not induce fatty acid degradation as much as broiling, since the broiling temperature was 250℃ for 20 minutes.

Table 1. Fatty acid composition of mackerel fillets

Fatty acid	Content in total fatty acids (%)		
	Raw fillets	Cooking 6min	Cooking 30min
C11:0	0.15±0.00	0.14±0.02	0.15±0.00
C14:0	6.90±0.02	6.65±0.58	6.51±0.17
C15:0	0.41±0.00	0.41±0.01	0.39±0.01
C16:0	11.89±0.01	11.84±0.28	11.77±0.26
C17:0	0.25±0.01	0.24±0.00	0.24±0.00
C18:0	8.50±0.05	8.74±1.51	8.70±0.33
C20:0	10.43±0.16	10.02±0.48	10.04±0.48
C21:0	0.37±0.00	0.38±0.00	0.38±0.01
C22:0	0.09±0.00	0.38±0.43	0.56±0.37
C24:0	1.23±0.05	1.23±0.01	1.25±0.06
SFA total	40.24±0.06	40.02±1.16	40.00±0.30
C14:1	0.23±0.01	0.22±0.01	0.21±0.01
C16:1n7	3.76±0.09	3.66±0.30	3.80±0.09
C17:1	0.55±0.00	0.55±0.06	0.57±0.02
C18:1n9	1.64±0.05	1.67±0.16	1.72±0.11
C18:1n7	1.72±0.03	1.68±0.08	1.66±0.04
C20:1n11	0.18±0.02	0.18±0.00	0.17±0.01
MUFA total	8.08±0.19	7.97±0.29	8.14±0.19
C16:2n4	0.29±0.00	0.29±0.01	0.28±0.00
C16:3n4	0.09±0.00	0.08±0.01	0.08±0.00
C18:2n6	0.27±0.01	0.27±0.02	0.28±0.02
C18:3n6	1.55±0.08	1.50±0.09	1.48±0.03
C18:3n3	5.24±0.03	5.13±0.28	4.91±0.25
C20:2	0.27±0.01	0.27±0.00	0.27±0.00
C20:3n6	0.12±0.01	0.12±0.01	0.12±0.01
C20:3n3	0.24±0.00	0.24±0.01	0.23±0.01
C20:4n6	1.12±0.03	1.12±0.02	1.08±0.03
C20:4n3	7.82±0.07	7.74±0.35	7.77±0.10
C20:5n3 (EPA)	14.08±0.59	14.72±0.26	15.08±0.71
C22:2	0.48±0.01	0.47±0.02	0.46±0.01
C22:4n6	0.09±0.01	0.09±0.02	0.10±0.00
C22:5n3	12.81±0.04	12.70±0.16	12.36±0.09
C22:6n3 (DHA)	1.00±0.01	0.96±0.05	0.98±0.01
PUFA total	45.00±0.41	45.23±0.69	45.02±060
unknown	6.67±0.27	6.78±0.19	6.85±0.14
total Fatty Acids	100	100	100

4.3 Lipid deterioration of mackerel fillets during cooking

The development of primary oxidation products in the mackerel fillets appeared to be highly affected by steaming (Figure 6). There was a significant increase of PV formation after steaming 30 minutes (P<0.05), which indicated lipid primary oxidation of mackerel after heating.

Secondary oxidation products, as estimated by TBARS and AV analysis are summarised in Figures 7 and 8. On the contrary, TBARS showed a significant decrease after cooking 30 minutes, which suggested MDA content was lower in cooked fillets, in accordance with lower linolenic acid shown in Table 1. But the AV results were well correlated with the PV regarding the influence of heating time, which indicated second oxidation produced after heating 30 minutes, which meant other aldehydes and second oxidation products than MDA generated.

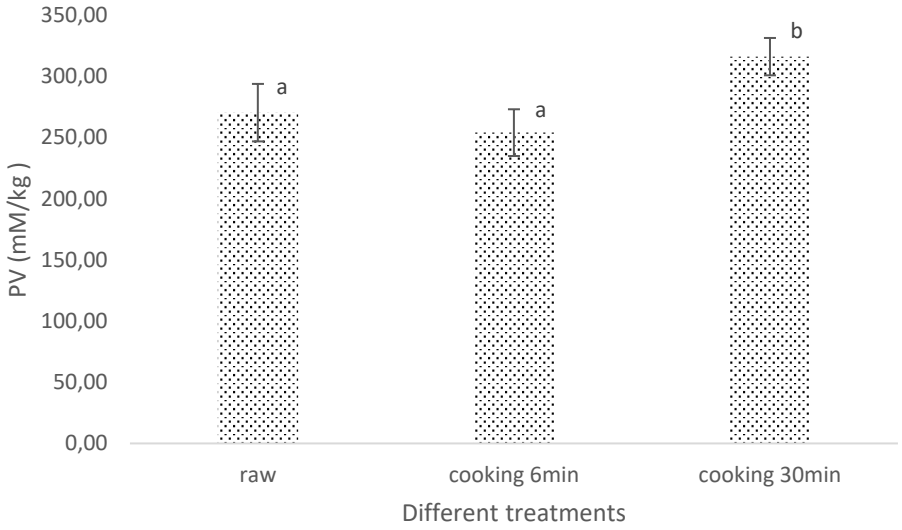


Figure 6. PV content of mackerel fillet affected by different cooking (lowercase letters completely different indicates that the difference is significant (P<0.05))

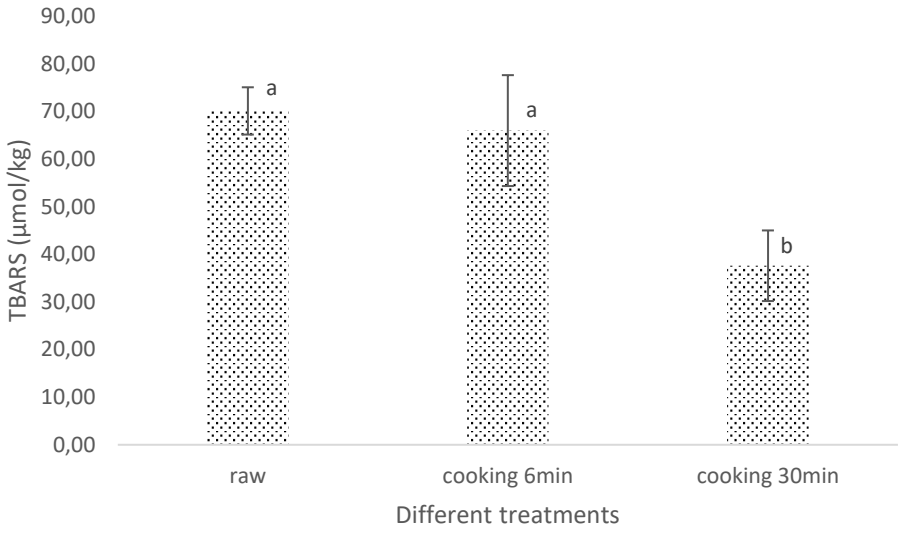


Figure 7. TBARS content of mackerel fillet affected by different cooking (lowercase letters completely different indicates that the difference is significant (P<0.05))

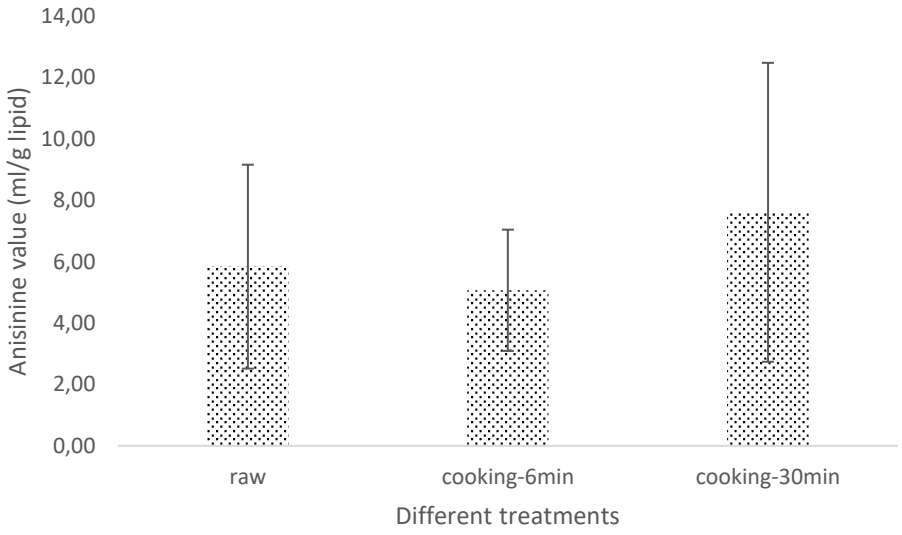


Figure 8. AV value of mackerel fillet affected by different cooking

4.3.1 HS-SPME-GC-MS methodology

A set of preliminary experiments was conducted to perform a good response for special volatiles associated to fish oxidation. The mixed external standards (including 1-penten-3-ol and 1-octen-3-ol) range from 0.125 to 2 ppm were incubated in PDMS/DVB fibre or DVB/Carboxen/PDMS fibre separately. As shown in Figures 9 and 10, PDMS/DVB fibre produced higher signal intensities for 1-penten-3-ol and 1-octen-3-ol than DVB/Carboxen/PDMS fibre. Therefore, PDMS/DVB fibre was selected as the fibre for the HS-SPME method. Additionally, the salting-out effect was evaluated and the higher sensitivity for mixed standards dissolved in saturated NaCl with both fibres were detected, as expressed in Figures 9 and 10. The addition of salt increases the ionic strength of the water sample by

lowering the solubility of analytes in the aqueous phase *(Iglesias & Medina, 2008)*, so fish samples were dissolved in saturated NaCl for further analysis.

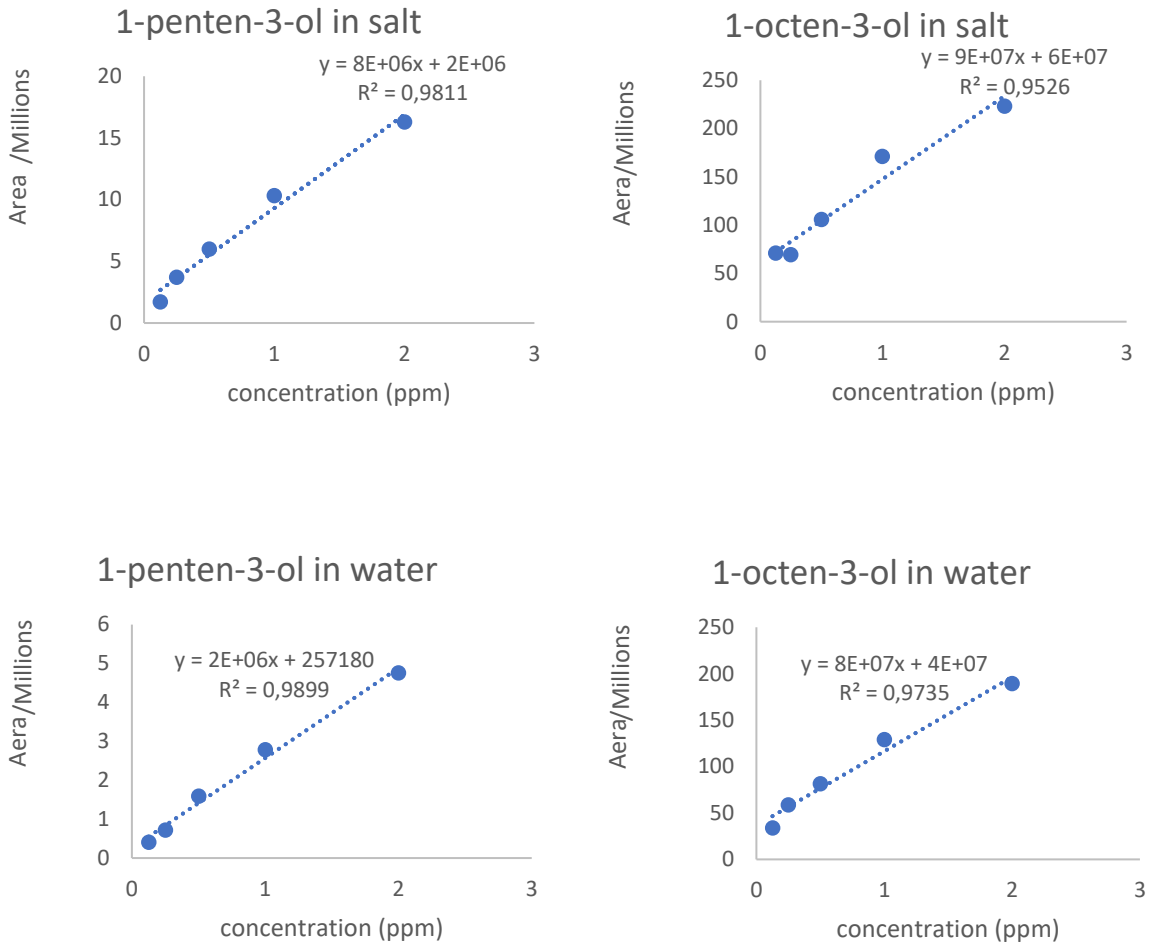
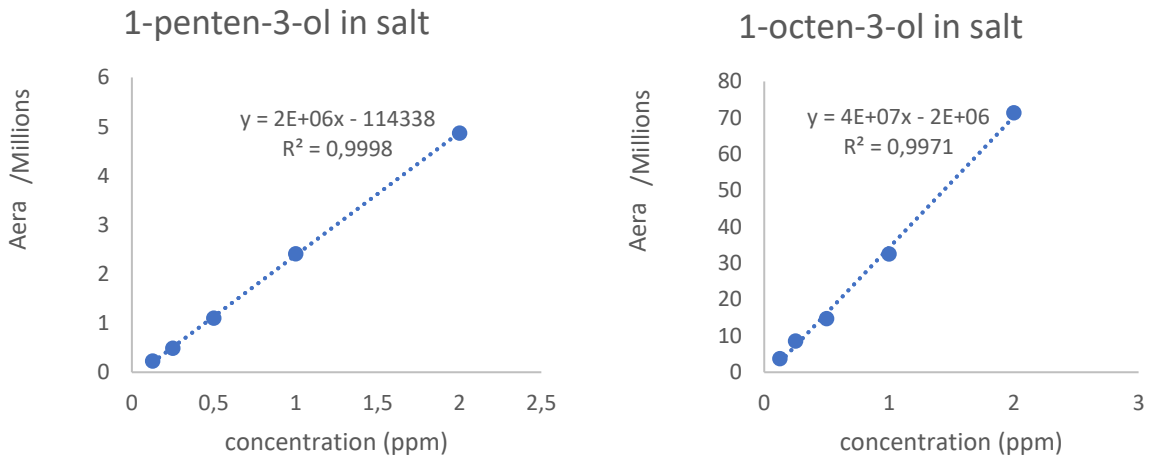


Figure 9. Standards curves with PDMS/DVB fibre



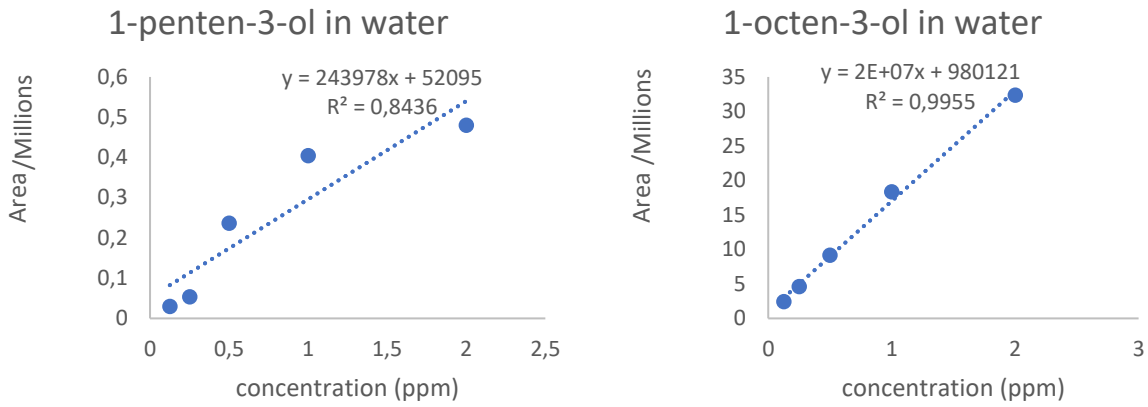


Figure 10. Standards curves with DVB/Carboxen/PDMS fibre

4.3.2 Volatile compounds analysis

Volatile compounds of mackerel fillets from raw material, steaming 6 minutes group and steaming 30 minutes group were analysed. Figure 11 highlights the overall volatiles produced by the three group（black lines for raw, red lines for cooking 6 minutes, blue lines for cooking 30 minutes). It was found volatiles from raw group had a higher content in generally, but there were some new compounds produced after steaming compared with raw material.

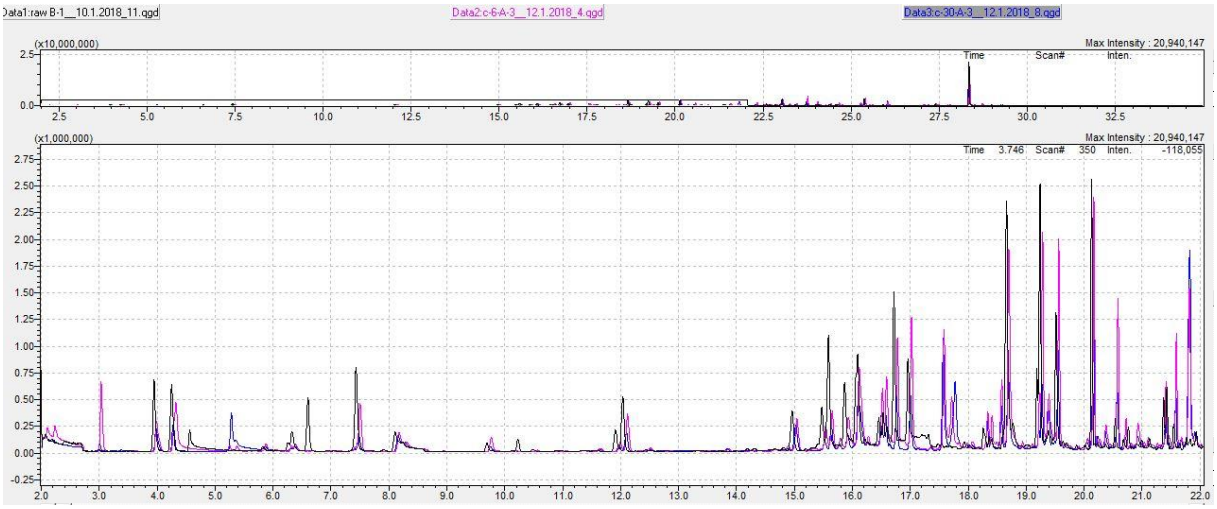


Figure 11. Scan chromatogram obtained from mackerel fillets by different treatments

From all the volatiles, 12 volatile compounds closely related with lipid oxidation were detected here, which are listed in Table 2. Amongst these, propanal, (*Z*)-4-Heptenal, (*E*,*E*)-2,4-Heptadienal, (*E*,*Z*)-2,6-Nonadienal are important second volatiles derived from n-3 PUFA. It was found that the content of propanal and (*E*,*Z*)-2,6-Nonadienal increased dramatically after steaming, especially for (*E*,*Z*)-2,6-Nonadienal, whose content after cooking was much more 19 fold than raw material. Except these compounds, the content of other volatiles decreased depending on the cooking time, shown in Table 2.

Table 2. Selective compounds from lipids oxidation in mackerel by different treatment

Name	Ret.Time	Area （10^6）		
		raw	cooking-6min	cooking-30min
Propanal	2.073	----	0.88±0.20	0.71±0.18

1-Penten-3-ol	3.947	2.02±0.04	1.00±0.11	0.78±0.04
2,3-Pentanedione	4.251	2.05±0.06	----	0.72±0.15
Hexanal	7.435	2.77±0.11	1.52±0.20	0.57±0.08
(*E*)-2-Hexenal	9.689	----	0.48±0.06	----
(*Z*)-4-Heptenal	11.911	0.73±0.02	0.28±0	----
Heptanal	12.04	1.75±0.08	1.20±0.12	0.66±0.08
1-Octen-3-ol	15.865	2.54±0.08	1.01±0.41	0.65±0.06
2-pentyl-Furan	16.219	0.39±0.02	0.53±0.14	----
(*E*,*E*)-2,4-Heptadienal	16.961	2.96±0.21	2.88±1.10	1.88±0.47
3,5-Octadien-2-one	19.237	5.48±0.47	4.00±0.41	1.53±0.28
(*E*,*Z*)-2,6-Nonadienal	20.537	0.74±0.03	2.55±0.34	14.31±0.18

"----" means not detected

4.4 Changes of chemical composition and lipid deterioration of mackerel lipids after cooking

4.4.1 Chemical composition of mackerel lipids

In order to remove any potential interference from fillets, pure lipids from fillets were heated and analysed again. Lipids from raw material were divided into 4 groups, and heated for 0 minutes, 5minutes, 15 minutes and 25 minutes by 100 ℃ water bath, separately. After that, the FFA and phospholipids content were investigated. It was shown FFA content had least change after heating, as illustrated in Figure 12, suggested heating short time did not induce FFA decrease or oxidation. Compared with Figure 4, lower FFA content in cooked fillets probably was caused by enzyme deactivation.

Figure 12. FFA content of mackerel lipids affected by different cooking

In the pure lipids system, phospholipids content was a significantly constant decrease from raw material lipids to lipids heated 25 minutes (see Figure13). Phospholipids were reduced by hydrolysis or oxidation after heating. Compared with changes of phospholipids in fillets, it further proved phospholipids content increased from protein denaturation during cooking.

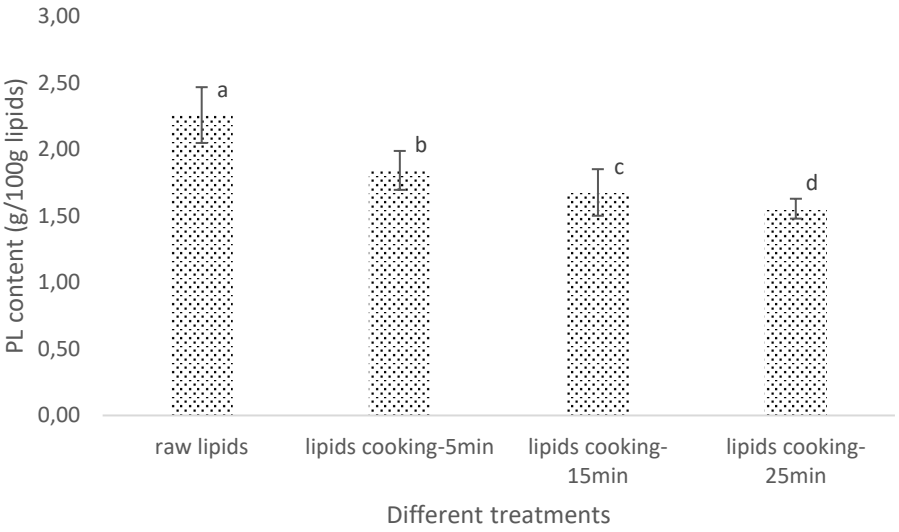


Figure 13. PL content of mackerel lipids affected by different cooking (lowercase letters completely different indicates that the difference is significant ($P<0.05$))

4.4.2 Fatty acid profile of mackerel lipids

Similar with fatty acid composition in fillets, only small changes were found in pure lipids system, even after heating 25 minutes, shown in Table 3. C22:0 was found increased after cooking compared with raw material, which was 0.09% in raw material and 0.74% after heating 5 minutes, but it decreased to 0.43% after 25 minutes of heating. For PUFA, C18:3n3 and C22:5n3 reduced after heating, which suggested n-3 PUFA slightly oxidized or degraded after heating in pure lipids.

Table 3. Fatty acid composition of mackerel lipids after cooking

Fatty acid	Content in total fatty acids (%)			
	Raw fillets	Oil cooking 5min	Oil cooking 15min	Oil cooking 25min
C11:0	0.15±0.00	0.15±0.00	0.15±0.00	0.15±0.00
C14:0	6.90±0.02	6.73±0.04	6.73±0.04	6.75±0.12
C15:0	0.41±0.00	0.41±0.00	0.41±0.00	0.41±0.01
C16:0	11.89±0.01	11.57±0.01	11.57±0.03	11.70±0.21
C17:0	0.25±0.01	0.24±0.01	0.24±0.01	0.25±0.01
C18:0	8.50±0.05	8.23±0.02	8.23±0.03	8.36±0.15
C20:0	10.43±0.16	10.03±0.09	9.99±0.12	10.26±0.22
C21:0	0.37±0.00	0.37±0.00	0.37±0.00	0.38±0.01
C22:0	0.09±0.00	0.74±0.02	0.73±0.01	0.43±0.39
C24:0	1.23±0.05	1.21±0.05	1.21±0.05	1.22±0.03
SFA total	40.24±0.06	39.69±0.04	39.64±0.06	39.91±0.30
C14:1	0.23±0.01	0.22±0.00	0.22±0.00	0.23±0.01
C16:1n7	3.76±0.09	3.65±0.12	3.69±0.12	3.72±0.05
C17:1	0.55±0.00	0.55±0.01	0.55±0.01	0.55±0.01
C18:1n9	1.64±0.05	1.61±0.04	1.61±0.04	1.63±0.02
C18:1n7	1.72±0.03	1.70±0.03	1.70±0.03	1.72±0.00
C20:1n11	0.18±0.02	0.16±0.01	0.16±0.01	0.16±0.01
MUFA total	8.08±0.19	7.89±0.21	7.93±0.21	8.02±0.07
C16:2n4	0.29±0.00	0.28±0.00	0.29±0.00	0.29±0.01
C16:3n4	0.09±0.00	0.08±0.00	0.08±0.00	0.08±0.01

C18:2n6	0.27±0.01	0.26±0.01	0.26±0.01	0.27±0.01
C18:3n6	1.55±0.08	1.53±0.06	1.53±0.07	1.55±0.05
C18:3n3	5.24±0.03	5.12±0.04	5.13±0.03	5.15±0.11
C20:2	0.27±0.01	0.27±0.01	0.27±0.01	0.27±0.00
C20:3n6	0.12±0.01	0.12±0.00	0.12±0.00	0.12±0.00
C20:3n3	0.24±0.00	0.23±0.00	0.24±0.00	0.24±0.00
C20:4n6	1.12±0.03	1.08±0.02	1.09±0.04	1.12±0.02
C20:4n3	7.82±0.07	7.61±0.10	7.61±0.11	7.65±0.06
C20:5n3 (EPA)	14.08±0.59	15.67±0.39	15.57±0.43	15.03±0.47
C22:2	0.48±0.01	0.47±0.00	0.47±0.00	0.48±0.01
C22:4n6	0.09±0.01	0.10±0.01	0.09±0.00	0.09±0.01
C22:5n3	12.81±0.04	12.50±0.05	12.46±0.04	12.52±0.19
C22:6n3 (DHA)	1.00±0.01	0.98±0.01	0.97±0.00	1.00±0.01
PUFA total	45.00±0.41	45.83±0.21	45.71±0.19	45.38±0.33
unknown	6.67±0.27	6.59±0.07	6.72±0.06	6.69±0.19
total Fatty Acids	100	100	100	100

4.4.3 Lipid deterioration of mackerel lipids during cooking

In pure lipid system, oxidation induced by heating was evaluated by PV and AV. As shown in Figure 14, PV decreased along with heating time significantly from 1896.8 mM/kg to 950.6 mM/kg, which suggested primary oxidation products steadily consumed after heating. But in this system, AV could not be detected. After 100 ℃ water bath, the lipid colour became darker than raw lipid, so it could be speculated that another oxidation production might be generated, such as oligomer.

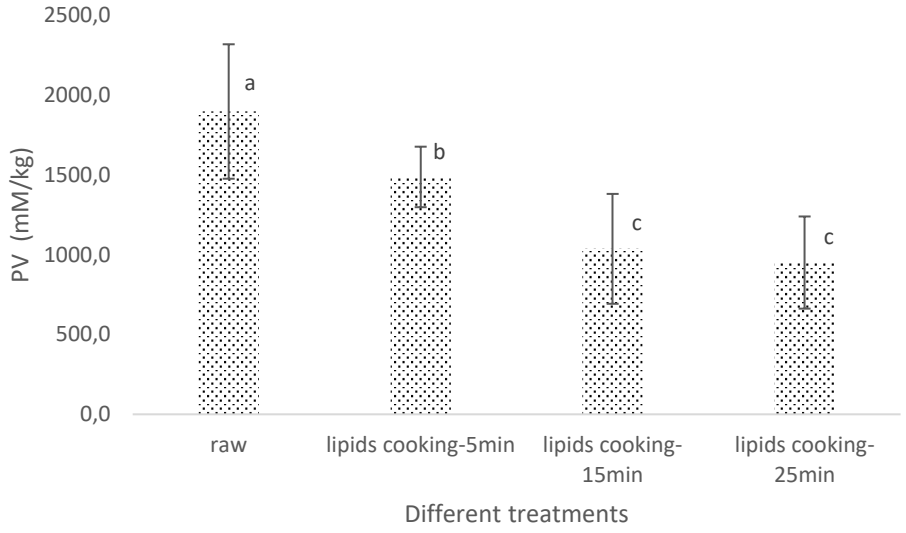


Figure 14. PV content of mackerel lipids affected by different cooking (lowercase letters completely different indicates that the difference is significant (P<0.05))

5 DISCUSSION

Atlantic mackerel, one kind of fatty fish, is a great source of unsaturated fatty acids, especially PUFA. Fat in mackerel can be affected by seasonal migration and feed resources (Romotowska, Karlsdottir, Gudjonsdottir, Kristinsson, & Arason, 2016). Though the lipid content and

composition varies seasonally, it has been reported a 1:3 ratio of saturated lipids to PUFAs (Leu, Jhaveri, Karakoltsidis, & Constantinides, 1981). EPA and DHA are the predominant fatty acids in PUFA. In general, DHA content is much higher than EPA, but it was found DHA (1.00±0.01%) is lower, less than EPA (14.08±0.59) here, but C22:5n3 (12.81±0.04) was relatively higher, which may be due to different feed sources. More PUFA has been related to nutrition and health, showing beneficial effects on neural development and cardiovascular system, but PUFA with up to 6 double bonds are very susceptible to oxidation, which can easily happen in cooking and storage.

Mackerel is very popular in Iceland and China because of its high nutritional value and low cost. It is often cooked by steaming. Changes and oxidation of mackerel fillets with different cooking time were evaluated according to the different customs. Similar with most results of cooking, steaming caused increase of lipid content due to loss of water, such as frying, baking, grilling, and boiling (Moradi, Bakar, Motalebi, Muhamad, & Man, 2011; Nieva-Echevarría, Manzanos, Goicoechea, & Guillén, 2017). After cooking, content of FFA decreased but PLs increased. This is in agreement with the observation of Weber *et al.* who found that decreased FFA content probably because of loss of volatile FFA and deactivation of hydrolysis enzyme occurred during heating (Weber J. , Bochi, Ribeiro, Victorio, & Emanuelli, 2008). It is possible that steaming deactivated hydrolysis enzyme, which still hydrolysed lipids in raw material. Compared steaming 6 minutes and 30 minutes, no significant difference was found, which further suggested FFA could not be generated more only by heating without enzyme. It was found that PL content increased after cooking, and cooking 30 minutes generated most PL within the three group, which also was found in mackerel during storage, explained by extractability of phospholipids resulting from the protein denaturation during cooking, similar with extended frozen storage (Romotowska, Karlsdottir, Gudjonsdottir, Kristinsson, & Arason, 2016). Compared with pure lipid heated (shown in Fig. 13), phospholipids tended to decreased gradually along the heating time, which could prove phospholipids were not stable in high temperature, and more phospholipids were obtained from other components during heating.

Lipid changes and oxidation induced by cooking have been reported, including decomposition of fatty acids and formation of volatile compounds. Frying of mackerel with canola oil or sunflower oil decreased PUFA content, especially DHA and EPA (Marichamy, Raja, Veerasingam, Rajagopal, & Venkatachalapathy, 2009). But hot-smoking at 60 ℃ did not cause significant reduction of long chain PUFAs (Chung, Choi, Cho, & Kim, 2011), which have been observed in our study. Least fatty acids changes were found during steaming, only C22:0 increased dramatically and C18:0 increased responding to C18:3n3 and C22:5n3 reduced. But the slight variety still accompanied oxidation products. As shown in results, PV and AV in mackerel fillets raised after cooking, but TBARS reduced. Since MDA generated from C18:3n3, it could be explained low MDA due to low linolenic acid. The volatile components, major products from degradation of lipid in mackerel have been investigated in raw and cooked fish. More volatiles were detected during broiling, compared with raw mackerel, in which aldehydes, alcohols and benzothiazole were significant enhanced (Chung, Choi, Cho, & Kim, 2011).

In this study, 12 volatile compounds closely related with lipid oxidation were detected, which were listed in Table 2. It was found that the content of propanal and (E,Z)-2,6-Nonadienal increased dramatically after steaming, especially for (E,Z)-2,6-Nonadienal, whose content after cooking was more 19-fold that of the raw material. Since these compounds were derived from

n-3 PUFA, it could be assumed that some PUFA were degraded and oxidized, although only C18:3n3 and C22:5n3 was detected decreased from FAC analysis. (E,Z)-2,6-Nonadienal has been reported to attribute to the interaction with amino acids to form pyrroles, which would decrease (E,Z)-2,6-Nonadienal content (Lu, Nielsen, Baron, & Jacobsen, 2012a). Since high (E,Z)-2,6-Nonadienal were found after steaming 30 minutes, it was suggested Strecker derived volatiles had not generated within 30 minutes of steaming.

In order to understand lipid oxidation clearly in mackerel fillets, pure lipids were heated and analysed. Comparing the cooking fillets and heating oil, except similar fatty acid profile, results were definitely different. FFA content did not change much, and PL content decreased significantly in pure lipids, which meant PLs decomposed by heating. The result of stable FFA content was similar with marine PL emulsions, in which stability of FFA were confirmed during the storage at room temperature and 2 ℃ for 32 days (Lu, Nielsen, Baron, Jensen, & Jacobsen, 2012b). The activity of hydrolysis enzymes during heating should be analysed in the future, which would help to understand the mechanism. After pure lipids were heated, the colour of the oil became darker than raw material, but no more oxidation products detected by PV and AV, so the further analysis of oxidation should be studied in the future, such as volatiles from GC-MS and oligopolymers from HPLC.

6 CONCLUSION

The study demonstrated relatively stable lipids in mackerel fillets for 6 minutes and 30 minutes steaming based on lipid, FFA, PL, FAC and volatiles. Compared with 30 minutes of cooking, 6 minutes of cooking could maintain better lipid characteristics. Cooking 30 min caused more lipid oxidation according to PV and AV, but still higher content of PL, DHA, and EPA, in which not much more nutrient loss of lipid. Therefore, it can be recommended both cooking ways can be accepted, people could choose cooking time within 30 min depending on personal preferences. Furthermore, PL in fish oil was thermal sensitivity, even heated for 5 minutes induced PL loss significantly.

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