

Research Article

Oxidative stability of refrigerated mackerel fillet as influenced by Maillard reaction products

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Abstract

Effect of Maillard reaction products (MRPs) prepared by heating the solution containing 2% mackerel sarcoplasmic protein and 2% galactose at 100°C, pH 8.0 for 12 h on the oxidative stability of mackerel (*Rastrelliger kanagurta*) fillet during 15 days refrigerated storage was investigated. Mackerel fillet soaked with MRPs at a ratio of 1:2 (w/v) prior to keep refrigeration retarded the oxidation of lipid and myoglobin effectively when compared to that soaked with 1% EDTA or distilled water at the same ratio. The lowest peroxide value (PV) of oil extracted from mackerel fillet and thiobarbituric acid reactive substances (TBARS) of mackerel muscle was found in MRPs treated sample throughout the storage period ($p < 0.05$). However, no difference in conjugated diene (CD) content was observed among treated and untreated samples throughout the storage time ($p > 0.05$). Metmyoglobin content of all samples sharply increased during the first 3 days of storage followed by continuous decrease till the end of storage time ($p < 0.05$). The red shift of the Soret peak of myoglobin, the indicator of metmyoglobin oxidation to form ferrylmyoglobin, was found in both treated and control fillets ($p < 0.05$). After 15 days of storage, the Soret peak shifted from 407.9 nm to 412.9 nm in control sample and it changed from 407.3 nm to 410 nm and from 406.6 nm to 410.6 nm in EDTA treated and MRPs treated samples, respectively. The lowered red shift in treated samples indicated that both EDTA and MRPs delayed such oxidation of metmyoglobin. As a consequence, the MRPs produced from mackerel sarcoplasmic protein and galactose can be used as a natural antioxidant to minimize the oxidation of lipid and myoglobin of mackerel fillet during refrigerated storage as a soaking agent.

Keywords: seafood, fish, myoglobin, lipid, oxidation, mackerel, Thailand

Introduction

Lipid and myoglobin deteriorations easily take place and limit the shelf-life of dark-fleshed fish during storage [1-3]. Both myoglobin and lipid oxidations in fish muscle are coupled and associated with the quality deteriorations especially off-flavor and discoloration [1, 4]. Myoglobin oxidation produces metmyoglobin and a reactive oxygen species such as hydrogen peroxide that undergoes further reaction with lipid to initiate lipid oxidation. This phenomenon is responsible for the rancid off-flavor and taste of fish and fish products [5].

The lipid components of *post-mortem* fish muscle tissue are prone to the oxidation because fatty acids of fish lipids are much more unsaturated than those of mammals and birds [6]. The lipid oxidation can be influenced by both intrinsic and extrinsic factors such as the fatty acid composition, the concentration of prooxidants, endogenous ferrous iron, myoglobin, enzymes, pH, temperature, ionic strength and oxygen consumption reaction [7-10]. Metmyoglobin formation is positively correlated with lipid oxidation [11-12]. Myoglobin and other heme compounds, at high concentration in red meats, function as prooxidants in muscle tissue [13]. Chaijan *et al.* [14] reported that the release of non-heme iron in sardine and mackerel during iced storage might be associated with the induced oxidation process in the muscle.

The Maillard reaction involved in the formation of brown pigments comprises the condensation between a carbonyl group of reducing sugars, aldehydes or ketones and an amine group of free amino acids (such as amino acids, peptides and proteins) or any nitrogenous compound [15]. The Maillard reaction is a complicated reaction that produces a large number of the Maillard reaction products (MRPs), such as aroma compounds, ultra-violet absorbing intermediates, and dark-brown polymeric compounds named melanoidins [16]. MRPs are known to have antioxidative effects *in vitro*, *in vivo* and in food systems [17]. Chuyen *et al.* [17] also reported that peptide-glucose reaction products, Amadori rearrangement products, melanoidins, modified protein and its hydrolysate, brown pigments isolated from Miso and other foodstuffs showed strong scavenging activity against reactive oxygen species including hydroxyl radical and superoxide anion. It was considered that the amino reductone structures, the electron donor, the chelating properties for metals and the reducing properties of melanoidins are the main causes for the antioxidative effect of MRPs [17]. Kirigaya *et al.* [18] reported that the MRPs from arginine, histidine or cysteine with glucose had stronger antioxidative activity than that of other amino acid MRPs.

Although many of the antioxidants presently used by the food industry are effective in preventing rancidity, their safety is often questioned by the consumer. Many foods contain MRPs, so the possibility of utilizing these as natural preservatives is attractive and should not be overlooked. Lipid foods have been relatively stable when the Maillard reaction was involved [19-23]. The antioxidative effect of MRPs was found in potato chips [19], biscuit and cookies [20], sausages [21], ground pork patties [22] and cooked ground beef [23]. The objective of this study was to investigate the effect of MRPs prepared by heating the solution containing 2% mackerel sarcoplasmic protein and 2% galactose at 100°C, pH 8.0 for 12 h on the oxidation of lipid and myoglobin of mackerel (*Rastrelliger kanagurta*) fillet during 15 days refrigerated storage.

Materials and Methods

Chemicals

Sodium thiosulphate, potassium iodide, trichloroacetic acid, anhydrous sodium sulfate and isooctane were obtained from Merck (Damstadt, Germany). Chloroform was purchased from BDH (Poole, England). 2-thiobarbituric acid and 1,1,3,3-tetramethoxypropane were procured from Fluka (Buchs, Switzerland). All chemicals were of analytical grade.

Fish samples

Mackerel (*Rastrelliger kanagurta*) with an average weight of 70-75 g were caught from Thasala-Nakhon Si Thammarat coast along the Gulf of Thailand during August, 2008. The fish, off-loaded approximately 12 h after capture, were placed in ice with a fish/ice ratio of 1:2 (w/w) and transported to the Department of Food Technology, School of Agricultural Technology, Walailak University within 1 h. The fish were immediately washed and filleted.

Extraction of sarcoplasmic protein

The whole muscle of mackerel (100 g) was coarsely minced and mixed with 300 ml of cold distilled water. The mixture was homogenized for 1 min using an IKA Labor Technik homogenizer (Selangor, Malaysia). After centrifugation at 4,000 rpm for 30 min at 4°C using the RC-5B plus centrifuge (Sorvall, Norwalk, CT, USA), the supernatant was collected and subjected to ammonium sulfate fractionation. The precipitate obtained with 0-50% saturation was dissolved in a minimal volume of cold 0.1 M phosphate buffer, pH 8.0. The mixture was then dialyzed against 10 volumes of same buffer with 5 changes at 4°C. The dialysate obtained was referred to as 'mackerel sarcoplasmic protein'. The protein concentration of sarcoplasmic protein solution was determined by the Biuret method [24] using bovine serum albumin as standard.

Preparation of MRPs

MRPs were prepared by heating the solution containing 2% mackerel sarcoplasmic protein (based on protein content in the solution) and 2% galactose in 0.1 M phosphate buffer, pH 8.0 at 100°C for 12 h according to the method of Lertittikul *et al.* [25] and Matmaroh *et al.* [26]. The heated samples were cooled immediately in iced water. MRPs sample obtained was kept at 4°C until used.

Effect of MRPs on oxidative stability of mackerel fillet

To study the effect of MRPs on oxidative stability of mackerel fillet, the mackerel fillet was soaked with different soaking agents including MRPs, 1% EDTA and distilled water at the ratio of 1: 2 (w/v) prior to keep refrigeration. After 60 min soaking at 4°C, the samples were taken out and subjected to dewatering using the nylon screen at 4°C for 30 min. Thereafter, the treated samples (500 g) were packaged in polyethylene bags individually, and stored at 4°C for 15 days. During storage, 3 bags of fish were randomly taken at days 0, 3, 6, 9, 12, and 15 for analyses. The flesh was chopped to uniformity and used for analyses.

Lipid extraction

Lipid was extracted by the Bligh and Dyer method [27]. Sample (25 g) was homogenized with 200 ml of a chloroform:methanol:distilled water mixture (50:100:50) at the speed of 9,500 rpm for 2 min at 4°C using an IKA Labor Technik homogenizer (Selangor, Malaysia). The homogenate was treated with 50 ml of chloroform and homogenized at 9,500 rpm for 1 min. Then, 25 ml of distilled water were added and homogenized again for 30 sec. The

homogenate was centrifuged at 3,000 rpm at 4°C for 15 min using a RC-5B plus centrifuge (Sorvall, Norwalk, CT, USA), and transferred into a separating flask. The chloroform phase was drained off into the 125 ml Erlenmeyer flask containing about 2-5 g of anhydrous sodium sulfate, shaken very well, and decanted into a round-bottom flask through a Whatman No.4 filter paper. The solvent was evaporated at 25°C using an EYELA rotary evaporator N-100 (Tokyo, Japan), and the residual solvent was removed by flushing nitrogen.

Measurement of peroxide value (PV)

Peroxide value was determined according to the method of Low and Ng [28]. The lipid sample (1.0 g) was treated with 25 ml of organic solvent mixture (chloroform:acetic acid mixture, 2:3). The mixture was shaken vigorously, followed by addition of 1 ml of saturated potassium iodide solution. The mixture was kept in the dark for 5 min and 75 ml of distilled water were added and the mixture was shaken. To the mixture, 0.5 ml of starch solution (1%, w/v) was added as an indicator. The peroxide value was determined by titrating iodine liberated from potassium iodide with standardized 0.01 N sodium thiosulfate solution. The PV was expressed as the milliequivalents of free iodine per kg of lipid.

Measurement of conjugated diene (CD)

Conjugated diene was measured according to the method of Frankel and Huang [29]. Oil sample (0.1 g) was dissolved in 5.0 ml of isooctane and the absorbance was measured at 234 nm using a UV-1601 spectrophotometer (Shimadzu, Japan).

Measurement of thiobarbituric acid-reactive substances (TBARS)

Thiobarbituric acid-reactive substances (TBARS) assay was performed as described by Buege and Aust [30]. Ground sample (0.5 g) was homogenized with 2.5 ml of a solution containing 0.375% thiobarbituric acid, 15% trichloroacetic acid and 0.25 N HCl using an IKA Labortechnik homogenizer (Selangor, Malaysia). The mixture was heated in a boiling water bath (95-100°C) for 10 min to develop a pink color, cooled with running tap water and centrifuged at 3,600×g at 25°C for 20 min using a RC-5B plus centrifuge (Sorvall, Norwalk, CT, USA). The absorbance of the supernatant was measured at 532 nm. A standard curve was prepared using 1,1,3,3-tetramethoxypropane at concentrations ranging from 0 to 10 ppm. TBARS was calculated and expressed as mg malonaldehyde/kg sample.

Myoglobin extraction

The extraction of myoglobin was performed by the method of Benjakul and Bauer [31]. A chopped sample of flesh (2 g) was weighed into a 50 ml polypropylene centrifuge tube and 20 ml of cold 40 mM phosphate buffer, pH 6.8, were added. The mixture was homogenized at 13,500 rpm for 10 sec, followed by centrifuging at 3,000×g for 30 min at 4°C using a RC-5B plus centrifuge (Sorvall, Norwalk, CT, USA). The supernatant was filtered with Whatman No. 1 filter paper. The supernatant obtained was referred to as 'myoglobin solution'.

Absorption spectra

The absorption spectra of myoglobin solution were taken using a V-530 UV/VIS double beam spectrophotometer (Jasco, Tokyo, Japan) as described by Chaijan *et al.* [14]. The spectra were recorded from 350 to 750 nm at the scanning rate of 1,000 nm/min using 40 mM phosphate buffer, pH 6.8 as a blank.

Determination of oxymyoglobin oxidation

The oxidation of oxymyoglobin during storage was determined spectrophotometrically with a V-530 UV/VIS double beam spectrophotometer (Jasco, Tokyo, Japan). The myoglobin solution was subjected to measure the A_{630} and A_{525} . A high A_{630}/A_{525} ratio indicates a high relative proportion of metmyoglobin [32].

Statistical analysis

Data were subjected to analysis of variance (ANOVA). Comparison of means was carried out by Duncan's multiple range test [33]. Statistical analysis was performed using the Statistical Package for Social Science (SPSS 11.0 for windows, SPSS Inc., Chicago, IL).

Results and Discussion

Changes in PV

Lipid oxidation is a complex process in which unsaturated fatty acids react with molecular oxygen, usually via a free radical mechanism, to form hydroperoxides, the primary oxidation products [34]. The lipid oxidation as measured by the primary lipid oxidation products, PV, occurred in mackerel muscle pre-soaked with distilled water rapidly during the first 3 days of refrigerated storage, probably due to the absence of antioxidants added (Figure 1). In the presence of natural antioxidant, MRPS, and synthetic antioxidant, EDTA, the PV of mackerel fillets were lowered. From the result, the sample treated with MRPs showed the lowest PV up to 9 days of storage when compared to that treated with EDTA and distilled water, respectively ($p < 0.05$). However, no differences in PV between treatments were observed at the end of storage period ($p > 0.05$).

Changes in PV of mackerel fillet pre-soaked with MRPs and EDTA occurred with the same manner (Figure 1). From the result, it was noted that the PV of mackerel fillet treated with MRPs and EDTA tended to increase during the first 3 days and tended to decrease until day 9 of storage. Thereafter, the increase in PV was observed in MRPs treated samples throughout the storage period whereas the fluctuation of PV of EDTA treated sample was noticeable. The decreased PV was presumed to be due to the decomposition of hydroperoxide. Hydroperoxides break down in several steps, yielding a wide variety of decomposition products including aldehydes [35]. The release of non-heme iron with the disappearance of natural antioxidants in mackerel muscle during extended refrigerated storage might enhance the oxidation process in the muscle [14]. The result suggested that MRPs had an activity to retard the oxidation in mackerel fillet during refrigerated storage. The result was in agreement with Chiu *et al.* [36] who reported that the oxidation of sardine lipid and kamaboko-type sardine product was prevented by the addition of fructose-tryptophan MRPs. Antony *et al.* [37] reported that honey-lysine MRPs exhibited an antioxidative activity in turkey meat. In addition, Bailey *et al.* [23] found that MRPs prepared from histidine-glucose model system were effective inhibitors of oxidative rancidity in cooked ground beef. Furthermore, MRPs obtained by heating glucose with soy protein isolate can inhibit the lipid oxidation in cooked ground beef over 8 days of refrigerated storage [38].

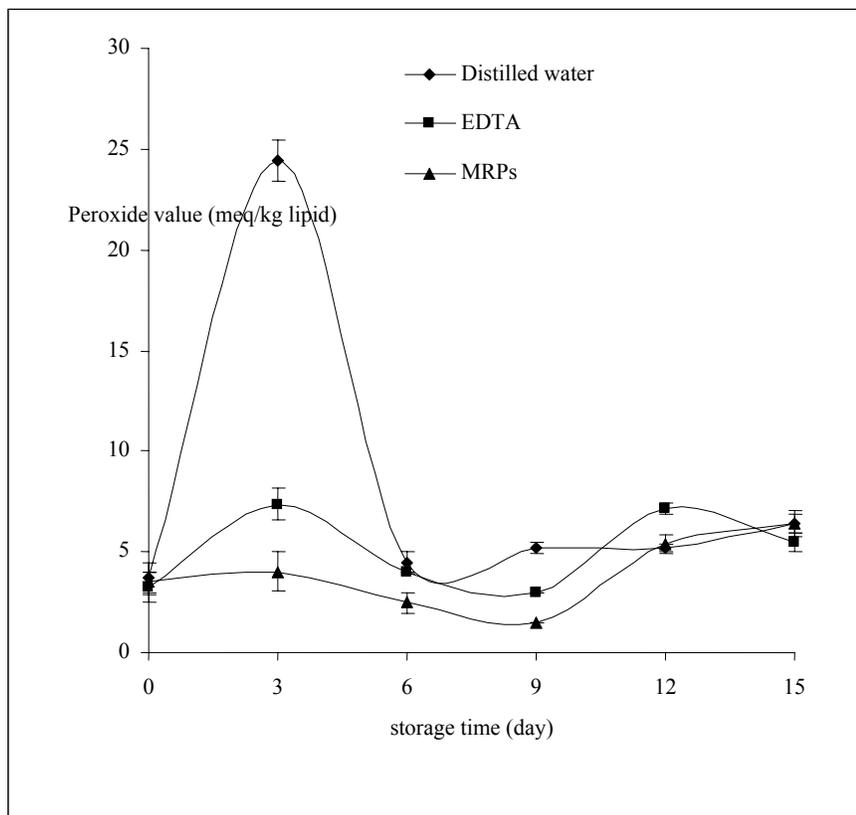


Figure 1. Changes in peroxide values of oil extracted from mackerel fillet pre-soaked with MRPs, 1% EDTA and distilled water during refrigerated storage. Bars indicate standard deviations from triplicate determinations.

Changes in CD

Almost immediately after peroxides are formed, the non-conjugated double bonds (C=C-C-C=C) that are present in natural unsaturated lipids are converted to conjugated double bonds (C=C-C=C) [39]. This is accompanied by increased UV absorption at 234 nm [40]. The increase in absorbance at 234 nm is an indicator of autooxidation and is reported to increase with uptake of oxygen and formation of peroxides during the early stages of oxidation [41].

No differences in CD of mackerel fillets treated with MRPs, EDTA and distilled water were observed during 15 days of refrigerated storage ($p > 0.05$) (Figure 2). From the result, it was presumed that the formation and decomposition of CD in both treated and control samples took place at equal rates. From the result, the fluctuation of CD value was found in both treated and control samples during storage. For control, the highest CD value was found initially and it decreased rapidly within the first 3 days of storage. Thereafter, the CD of control tended to increase till day 12 of storage and decreased sharply at the end of storage time. For treated samples, the CD value of those tended to increase at the first 3 days of storage. However, the changes in CD value of treated samples during day 3-15 were found with the same trend as observed in control. The decrease in CD in all samples after 15 days of refrigerated storage was possibly due to the decomposition of CD. The rate of CD decomposition could be greater than the formation rate, leading to decrease in CD accumulated in the lipid fraction. The decrease in CD might be due to the transformation of primary oxidation products to the new form, secondary lipid oxidation products such as

aldehydes and ketones. From the result, the lipid oxidation could proceed to a great extent, as evidenced by the increase in measurable TBARS (Figure 3). Perez-Alonso *et al.* [42] reported that no changes in CD in dorsal muscle of Atlantic pomfret within the first 9 days of chilled storage, followed by a gradual increase up to 19 days of storage. For horse mackerel (*Trachurus trachurus*), CD formation had no clear trend during frozen storage with or without previous chilled storage [43]. For sardine (*Sardinella gibbosa*) muscle, no difference in CD was found within the first 12 d of iced storage and a slight increase in CD was observed at the end of storage (day 15) [44]. Rawdkuen *et al.* [45] reported the changes in CD value of oil extracted from both dorsal and ventral muscle of farmed giant catfish (*Pangasianodon gigas*) during refrigerated storage that the CD sharply decreased at the first period of storage and gradually increased throughout the storage time.

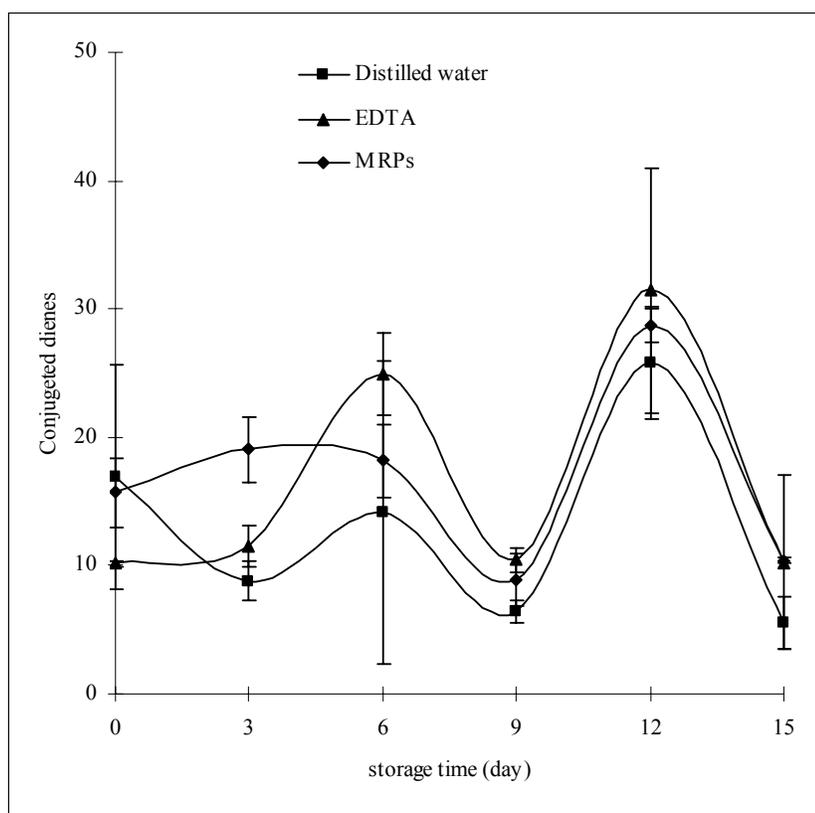


Figure 2. Changes in conjugated diene of oil extracted from mackerel fillet pre-soaked with MRPs, 1% EDTA and distilled water during refrigerated storage. Bars indicate standard deviations from triplicate determinations.

Changes in TBARS

Changes in TBARS of mackerel fillet pre-soaked with MRPs, EDTA and distilled water during 15 days refrigerated storage are shown in Figure 3. The increase in TBARS indicated formation of secondary lipid oxidation products [46]. TBARS has been used to measure the concentration of relatively polar secondary reaction products, especially aldehydes [35].

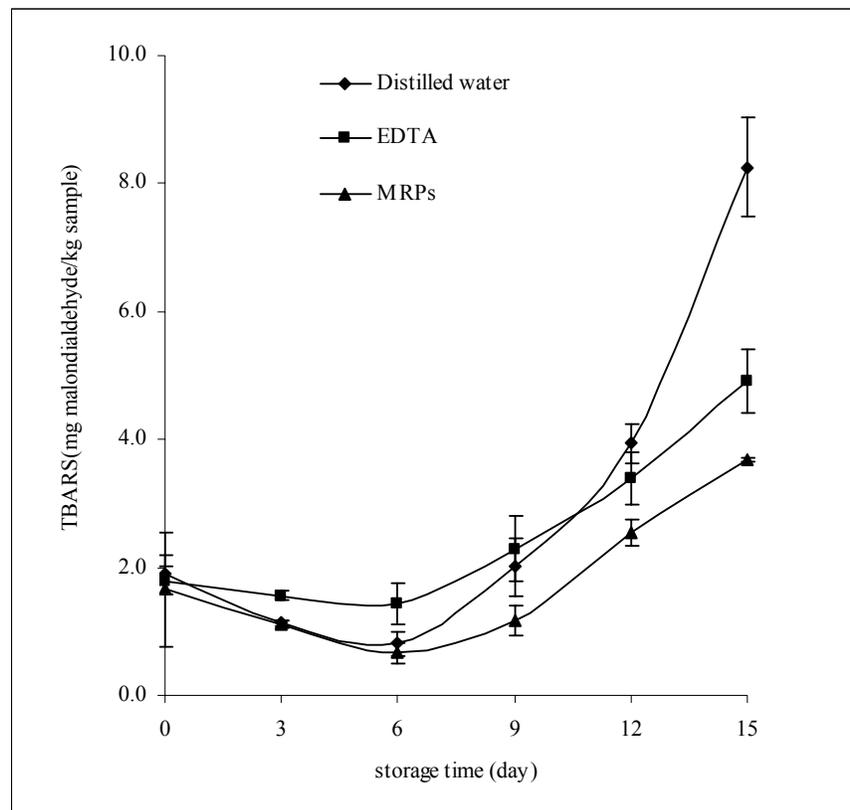


Figure 3. Changes in TBARS values of mackerel fillet pre-soaked with MRPs, 1% EDTA and distilled water during refrigerated storage. Bars indicate standard deviations from triplicate determinations.

TBARS values of all samples tended to increase throughout the storage time (Figure 3). From the result, the lowest TBARS value of mackerel muscle was found in MRPs treated sample throughout the storage period ($p < 0.05$) (Figure 3). This result was coincidental with the pattern of PV value as present in Figure 2. The marked increase in TBARS at the end of storage was found in control (pre-soaked with distilled water). This was most likely that a higher rate of lipid oxidation might be taking place in the sample without antioxidant added. Furthermore, the released free iron and other prooxidants from the muscle which was excessively degraded when storage time increased could enhance the oxidation of lipid in control fillet. The presence of EDTA and MRPs could prevent the oxidation initiated by metal ions because EDTA and MRPs served as a metal chelator. MRPs are known for their ability to retard lipid oxidation by several mechanisms including free radical-scavenging action [47], metal ion-chelating property [48, 16] and/or reducing activity [49]. Additionally, the loss in natural antioxidants during extended storage might contribute to the increased lipid oxidation. Thus, lipid oxidation became more pronounced in mackerel fillet when refrigerated storage time increased. This resulted in the deterioration and unacceptability of the mackerel meat.

Changes in metmyoglobin content and absorption spectra

A high A_{630}/A_{525} ratio indicates a high relative proportion of metmyoglobin [32]. Metmyoglobin content of all samples sharply increased during the first 3 days of storage followed by continuous decrease till the end of storage time ($p < 0.05$) (Figure 4). The increase in metmyoglobin content revealed that the oxidation of myoglobin occurred progressively

even though the antioxidant was applied. Thereafter, the decrease in metmyoglobin content in all samples was observed throughout the storage period ($p < 0.05$). The result indicated that the metmyoglobin might be oxidized to form ferrylmyoglobin. The result was in agreement with the red shift of the soret peak of myoglobin, the indicator of metmyoglobin oxidation to form ferrylmyoglobin (Table 1). After 15 days of storage, the soret peak shifted from 407.9 nm to 412.9 nm in control sample and it changed from 407.3 nm to 410 nm and from 406.6 nm to 410.6 nm in EDTA treated and MRPs treated samples, respectively (Table 1). The lowered red shift in treated samples indicated that both EDTA and MRPs delayed such oxidation of metmyoglobin.

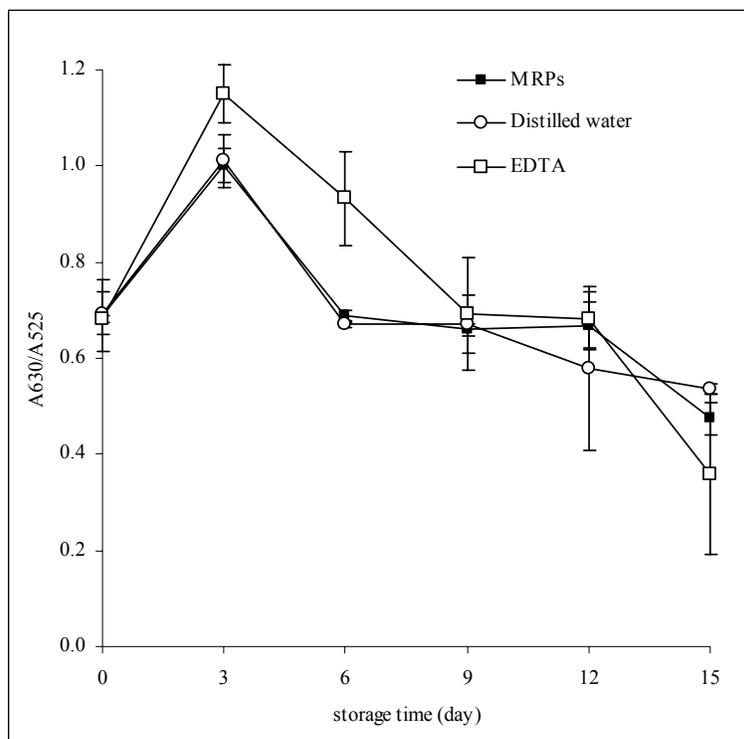


Figure 4. Changes in metmyoglobin content of mackerel fillet pre-soaked with MRPs, 1% EDTA and distilled water during refrigerated storage. Bars indicate standard deviations from triplicate determinations.

The strong absorption of myoglobin from mackerel was located in the blue region (350-450 nm) or soret band (Figure 5A and 5B). During storage, higher peak was observed in muscle pre-soaked with MRPs, compared with that in muscles pre-soaked with EDTA and distilled water, respectively. This was probably due to the cross-linking ability of MRPs that can stabilize the heme-globin structure. From the result, the soret peak in all samples decreased when the storage time increased. This disappearance of the soret absorption band indicated the destruction of the heme protein. Baron *et al.* [50] reported that heme protein degradation was monitored by changes in the soret absorption band, known to be very sensitive to detachment of the porphyrin moiety from the globin. The result of metmyoglobin content all together with the changes in absorbance spectra supported that during refrigerated storage, the degradation and oxidation of heme proteins in both treated and control mackerel muscles occurred with varying degrees.

Table 1. Changes in absorption maxima (nm) in the sorlet region of myoglobin extracted from mackerel fillet pre-soaked with MRPs, 1% EDTA and distilled water during refrigerated storage.

Storage time (day)	Absorption maxima (nm) in the sorlet region		
	Distilled water	1% EDTA	MRPs
0	407.9±0.12* ab [#]	407.3±0.00a	406.6±0.69a
3	407.5±0.12a	407.3±0.31a	407.6±0.00bc
6	408.3±0.42ab	408.0±0.35a	408.3±0.31c
9	408.9±0.81b	409.5±0.23b	408.0±0.35c
12	408.7±0.31ab	407.5±0.81a	407.1±0.31ab
15	412.9±1.29c	410.0±0.60b	410.6±0.31d

*Values are given as mean ± SD from triplicate determinations.

[#]Different letters in the same column indicate significant differences ($p < 0.05$).

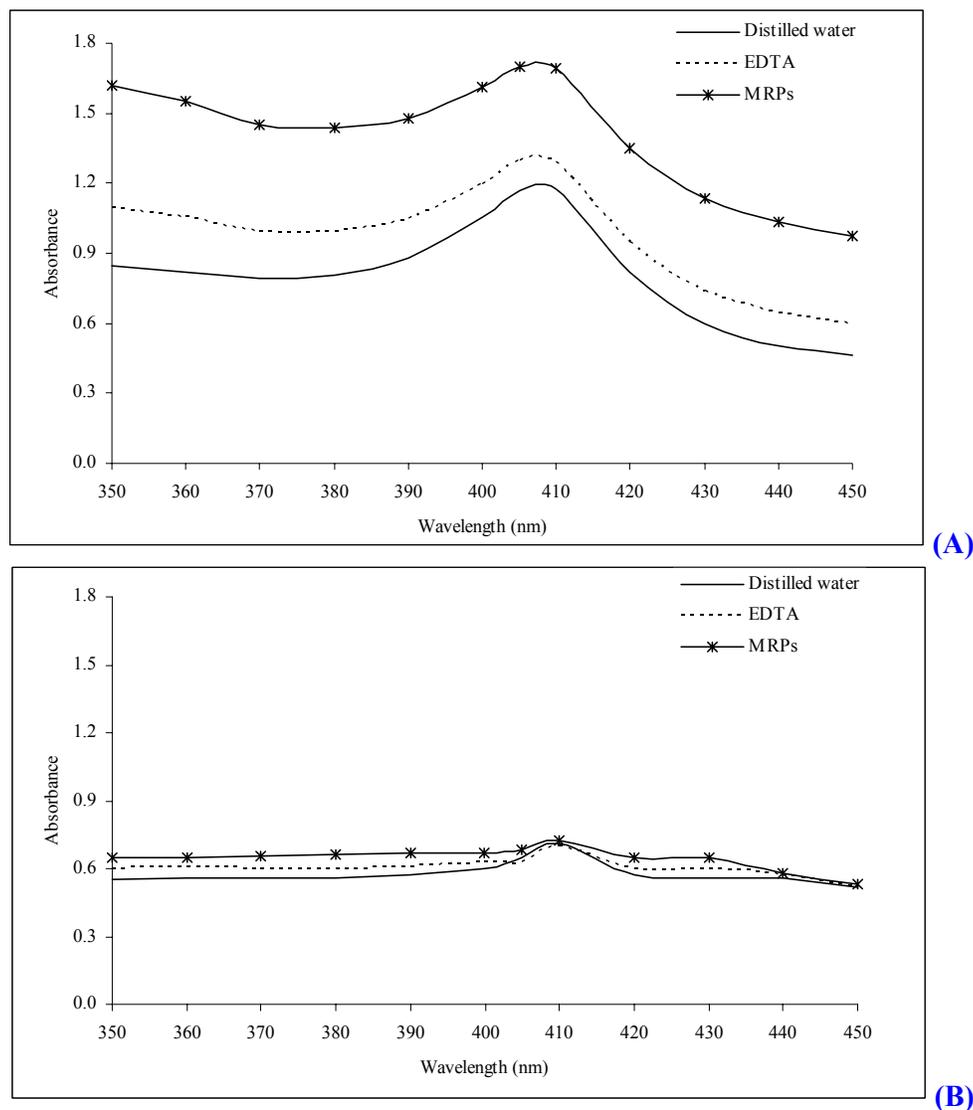


Figure 5. Absorption spectra of myoglobin extracted from mackerel fillet pre-soaked with MRPs, 1% EDTA and distilled water at day 0 (A) and day 15 (B) of refrigerated storage.

Conclusion

Mackerel fillet soaked with MRPs, prepared by heating the solution containing 2% mackerel sarcoplasmic protein and 2% galactose at 100°C, pH 8.0 for 12 h, at a ratio of 1:2 (w/v) prior to keep refrigeration retarded the oxidation of lipid and myoglobin effectively when compared to that soaked with 1% EDTA or distilled water at the same ratio. Therefore, the MRPs produced from mackerel sarcoplasmic protein and galactose can be used as a natural antioxidant to minimize the oxidation of lipid and myoglobin of mackerel fillet during refrigerated storage as a soaking agent.

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