http://www.egejfas.org
Ege Journal of Fisheries and Aquatic Sciences, 35(3): 295-304 (2018)

Su Ürünleri Dergisi (2018)
DOI: 10.12714/egejfas.2018.35.3.09

RESEARCH ARTICLE **ARAŞTIRMA MAKALESİ**

The effect of frozen storage on chemical and sensory quality of horse mackerel (*Trachurus trachurus*) coated whey protein isolate enriched with thyme essential oil

Dondurarak depolamanın kekik esansiyel yağı ile zenginleştirilmiş peynir altı suyu protein izolatı ile kaplanan istavrit (*Trachurus trachurus*)'in kimyasal ve duyusal kalitesine etkisi

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Received date: 26.02.2018 *Accepted date: 17.05.2018*

How to cite this paper:
Tokur, B. & Aksun, E.T. (2018). The effect of frozen storage on chemical and sensory quality of horse mackerel (*Trachurus trachurus*) coated whey protein isolate enriched with thyme essential oil. *Ege Journal of Fisheries and Aquatic Sciences, 35*(3), 295-304. DOI:10.12714/egejfas.2018.35.3.09

Abstract: In this study, the effectiveness of whey protein isolate (WPI) and WPI enriched thyme essential oil coating to improve the quality of horse mackerel during frozen storage at -18 °C±2 were evaluated for nine month. Whey protein isolate without thyme essential oil (WPI0) and three different proportion thyme essential oil (3, 5 and 7 %, v/v) in whey protein isolate (WPI3, WPI5 and WPI7) coating solutions were applied to horse mackerel. As a control, non-coated and distillated water coating (DW) horse mackerel were used. The results showed that the lowest peroxide value (PV) (meq of peroxide oxygen kg−1) and thiobarbutiric acid value (TBA) (mg malonaldehyde/kg muscle) were determined in WPI coatings enriched with 3% thyme essential oil treated group ($p<0.05$) while the highest PV and TBA values were determined in WPI coatings enriched with 5% and 7% thyme essential oil treated groups ($p<0.05$). Among the treatment, DW and the WPI coatings enriched with 7% thyme oil gave significantly higher protein solubility than other treatments during frozen storage ($p<0.05$). Electrophoretic studies in the presence and absence of β-mercaptoethanol showed that high molecular weight polymers via non-disulfide and disulfide cross-linking occurred in the horse mackerel proteins extracted in 5% NaCl in all groups during frozen storage. Sensory assessment showed that the horse mackerel coated WPI enriched with 5 and 7% thyme essential oil could not be stored for more than 9 months.

Keywords: *Whey protein isolate, coating, thyme essential oil, frozen storage, horse mackerel*

Öz: Bu çalışmada, 18 ±2 °C'de dondurularak depolanan istavritin kalitesini geliştirmek amacıyla peynir altı suyu protein izolatı (WPI) ve kekik esansiyel yağı ile zenginleştirilmiş WPI kaplamanın etkisi 9 aylık süre için değerlendirilmiştir. İstavritlere, kekik esansiyel yağı ilave edilmemiş (WPI0) ve üç farklı oranlarda kekik esansiyel yağı (%3, %5 ve %7, v/v) içeren peynir altı suyu protein izolatlı (WPI3,WPI5 ve WPI7) kaplama solüsyonu uygulanmıştır. Kontrol grubu olarak, herhangi bir kaplama uygulanmayan ve saf su glazeli (DW) istavritler kullanılmıştır. Araştırmanın sonucunda, en düşük peroksit (PV) ve tiyobarbitürik asit (TBA) değerleri %3 oranında kekik esansiyel yağı uygulanan grupta bulunurken ($p<0.05$), en yüksek PV ve TBA değerleri %5 ve %7 oranında kekik esansiyel yağı uygulanan gruplarda bulunmuştur ($p<0.05$). Gruplar arasında DW ve %7 oranında kekik esansiyel yağı uygulananlar, dondurularak depolama boyunca diğer gruplara göre önemli oranda daha yüksek protein çözünürlüğüne sahip olmuştur ($p<0.05$). β-mercaptoethanol varlığında ve yokluğunda yapılan elektroforetik çalışmalar sonucunda, dondurarak depolama boyunca tüm gruplarda disülfit olan ve disülfit olmayan çapraz bağların oluşmasıyla yüksek moleküler ağırlıklı polimerlerin %5 NaCl'da ekstrakte edilen istavrit proteinlerinde meydana geldiği görülmüştür. Duyusal değerlendirmeye göre, % 5 ve %7 kekik esansiyel yağı ile zenginleştirilmiş WPI kaplı istavritlerin dokuz aydan daha uzun süre dondurularak depolanamayacağı belirlenmiştir.

Anahtar kelimeler: *Peynir altı suyu protein izolatı, kaplama, kekik esansiyel yağı, dondurularak depolama, istavrit*

INTRODUCTION

Freezing, one of the oldest methods of food preservation, is still the most common and effective technique for providing a significantly extended shelf life. Although freezing and frozen storage are able to control or decrease biochemical changes, degradation of lipid and protein cannot terminate, which causes deteriorative quality changes in flavour, odour and texture. Application of edible film and coatings are a good alternative for suppressing quality changes during frozen storage by, for instance, delaying moisture, aroma and oil loss or gain and reducing lipid oxidation. They also can be used as a potential biopolymer for carrying and holding antioxidants and antimicrobials at food surfaces (Janjarasskul and Krochta,

2010).

Edible films and coatings are usually made from proteins, lipids and polysaccharides. One consisting of protein, whey protein isolate (WPI) created by filtering milk protein, is of particular interest for coating owing to its excellent oxygen, aroma and oil barriers: it has good mechanical properties, excellent gloss and transparency. Although the use of edible coatings made of different types of biodegradable polymers during frozen fish storage has been researched (Duan et al.,2010; Kilincceker et al.,2009; Sathivel et al., 2007), few studies have been published to date about whey protein isolate coating (Motalebi et al.,2010;Stuchell and Krochta, 1995; Rodriguez-Turienzo et al., 2011).

Bioactive edible coatings can be enriched with plant essential oils (EOs), thus further improving food quality and safety (Ojagh et al.,2009). Thyme EOs have phenolic compounds responsible for the high antioxidant capacity due to their biologically active compounds such as carvacrol and thymol and offer a promising alternative to synthetic antioxidants in minimizing rancidity (Altiok et al.,2010;Burt et al., 2005). The protective effect of thyme oil against lipid oxidation in frozen seafood has been thoroughly reviewed (Çoban, 2012; Erkan and Bilen, 2010). However, there is no information about the use of edible coatings enriched with essential oil on frozen horse mackerel.

Horse mackerel (*Trachurus trachurus)*, a semi-pelagic fish distributed mainly over the continental shelf of the Northeast Atlantic from Norway to Senegal, and in the Mediterranean and Black Seas, has recently attracted great commercial attention because of its moderate price and large quantities captured (Aubourg et al., 2004; Özden, 2010). Horse mackerel is highly prone to oxidation because it contains high levels of ω3 polyunsaturated fatty acids (PUFAs), which can have a negative effect on the commercial value (Gimenez et al.,2011). The aim of this work was to determine the effects of whey protein isolate coatings enriched with different concentrations of thyme essential oil on chemical and sensory quality of horse mackerel during frozen storage at -18 ±2 °C.

MATERIALS AND METHODS

Materials

Horse mackerel (*Trachurus trachurus*), 13.31 ±0.68 cm length and 17.36 ± 1.59 g weight, were caught in eastern Black Sea Coast in December and transported in crushed iced till arrival to the laboratory within 10 hours. The whey protein isolate (WPI) was obtained from Davisco Foods International Inc. (BİPRO, La Sueur, MN, USA). According to the manufacturer, the composition of the product was 97.9 % protein (dry weight basis), 4.6% moisture, 1.8 % ash, 0.2 % fat and 6.9 pH (10%@20C). Candelilla wax was supplied from Strahl and Pitsch Inc. (West Babylon, N.Y., U.S.A.). Food grade glycerol and NaOH were purchased from a commercial food ingredient grocery store in Istanbul. Commercial thyme oil (Karden, Kardelen Tarım Ürünleri Ltd., Ankara) was supplied from a local herbal store. All chemicals and reagents used were of analytical grade and purchased from either Merck (Darmstadt, Germany) or Sigma (St. Louis, MO).

Methods

Preparation of coating solutions and application to fish

The WPI coatings solutions were prepared as described by Seydim and Sarikus (2006). Whey protein isolate (5% w/v) was dissolved in distilled water, and glycerol (5% w/v) was added. The pH was adjusted to 8.0 with 2 N NaOH. The solutions were heated to 90 ± 2 °C while being stirred continuously. Candelilla wax (0.8% w/v) was added during heating. The coating solutions were filtered through a layer of cheesecloth. Thyme oil 3%, 5%, and 7% ratios (v/v) were added to the coating solutions. After homogenizing for two minutes using an Ultra-turax homogenizer (IKA, Germany), the solutions were cooled to room temperature for 1.5 hours. Vacuum was applied for 30 minutes to remove dissolved air in the solutions.

After the fish arrived at the laboratory, A total of 48 kg of fresh horse mackerel (8 kg in each group) were washed with tap water and frozen at -80 °C for overnight storage. The coatings were applied after freezing. They were divided into six groups. One was directly packaged in polyethylene zip-lock freezer bags and stored at −18°C and considered as the non-coating group (Control). Others were dipped in distillated water (DW), whey protein isolate coating solution without thyme oil (WPI0) and WPI coating solution enriched with three different concentrations of thyme oil (WPI3%, WPI5% and WPI7%). The fish were dipped in the coating solution enriched with different concentrations of thyme essential oil for 30 seconds, drained for 15 seconds, packed in zip-lock freezer bags (Rodriguez-Turienzo et al., 2011) and then stored at – 18 ±2 °C for nine months.

Proximate analyses

The crude protein was determined by Kjeldahl's method (AOAC, 1984). Lipids were extracted by the method of Bligh and Dyer (1959). The moisture content (AOAC, 1990) and crude ash content (AOAC, 1998) were determined in an oven at 103°C and 550°C respectively until the weight became constant.

Gas chromatography mass spectrometry (GC/MS) analyses of thyme essential oil

Thyme EO was analyzed using gas chromatography mass spectrometry (GC/MS). GC/MS analyses were performed on a Perkin Elmer Clarus 500 capillary gas chromatograph directly coupled to the mass spectrometer system (Japan). SGE non polar fused silica capillary column (60 m x 0.25 mm, ID. BPX5 0,25um, USA) was used under the following conditions: oven temperature program from 60°C (10 min) to 250°C at 4°C/min, and the final temperature kept for 10 min; injector temperature 220°C; helium as carrier gas, flow rate 1.5 mL/min. The volume of injected sample was 1 µl of diluted oil in hexane; splitless injection technique; ionization energy 70eV, in the electronic

ionization (EI) mode; ion source temperature 200°C; scan mass range of m/z 35-425 and interface line temperature 250°C. The constituents of essential oils were identified and calculated in relation to the retention time of a series of alkanes (C4- C28) as reference products and the similarity of their mass spectra with those gathered in the NIST-MS and WILEY-MS library, or reported in the literature. As a result of GC–MS analysis, the main components in the commercial thyme oil showed that it contained carvacrol (78.12%), trans-Caryophyllene (4.27%), beta Bisabolene (3.34%), dinopol NOP (2.86%), thymene (1.91%), gamma terpinene (1.59%) and caryophyllene oxide (1.18%).

Chemical analyses

Peroxide value (PV), expressed in milliequivalents of peroxide oxygen kg−1 of fat, was determined according to AOCS (1994). The value of thiobarbituric acid (TBA) was determined according to the method of Tarladgis et al. (1960), and results were expressed as TBA value, mg malonaldehyde/ kg muscle.

The protein solubility was measured according to Dyer et al. (1950). 0.5 g fish muscle were homogenized with 5 % NaCl solution using a homogenizer (Ultra-turrax, Ika T8, 1KA Labortechnik, Staufen, Germany) for 1 min at speed 5 (20.000 rpm) in ice. Homogenate was then centrifuged at 5.000 × *g* at 4°C for 20 min using a Hettich Rotina 420 R bench centrifuge (DJB Labcare Ltd., Buckinghamshire, UK). The supernatant was used for SDS-PAGE and for the determination of protein concentrations. The protein content of the extract was determined by the Lowry method (Lowry et al., 1951). Bovine serum albumin was used as a standard. Percentage of soluble protein was expressed as the ratio of the quantity of soluble protein after frozen storage to that of original soluble protein from fresh samples.

Sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis was used to monitor polymerization and fragmentation of proteins using a BioRad Mini vertical gel electrophoresis (Laemmli, 1970). To determine the polymerization of myosin heavy chain (MHC), samples prepared with sample buffer with and without β-mercaptoethanol were compared. SDS-PAGE was performed in a 10% (w/v) resolving gel and 4% (w/v) stacking gel run at 240 volt for 40 minute. Gels were stained overnight with 0.025% (w/v) Coomassie blue R-250 in 40% aqueous methanol and 7% acetic acid. Destaining was achieved by sequential treatment of gels first with an aqueous solution consisting of 40% methanol and 7% acetic acid, followed by 5% aqueous methanol and 7% acetic acid, and finally distilled water. Protein molecular weight standards were obtained from Sigma Ltd. (St. Louis, MO) consisted of myosin (200 kDa), β-galactosidase (116 kDa), phosphorylase b (97 kDa) bovine serum albumin (66 kDa), glutamic dehydrogenase (55 kDa), ovalalbumin (45 kDa) and glyceraldehydes-3-phosphate dehydrogenase (29 kDa).

Sensory Analysis

For sensory analysis, each assessment was carried out by a six to eight trained panelists. Sensory analyses of fish (appearance, odour, flavour and texture) were assessed according to the method of Paulus et al. (1979) with modification. modification. Fish were cooked in a microwave oven for 3 min (450 w) and then served to the panelists to assess. A hedonic scale from 9 to 1 was used to evaluate frozen horse mackerel. A score of 9 represents 'very good quality', a score of 7–8, 'good quality', a score of 5–6 'acceptable', while a score of 1–4 was regarded as 'bad or unacceptable'.

Statistical analyses

Experiment statistics were performed using SPSS for Windows software program (SPSS 18.0 for Windows). The results were expressed as the mean and standard deviation. Data analysis for each treatment was carried out in triplicate and the mean of each sample for each group was analysed three times. One-way analysis of variance (ANOVA) was used to check the variance homogeneity, normality and compared by using Duncan's multiple range test at 95% confidence interval. The treatment effect was performed using Duncan's multiple range test in the general linear models procedure (GLM). Sensory analyze was performed using non-parametric test and differences between in groups were evaluated analysis of variance.

RESULTS AND DISCUSSION

Proximate analyses

The moisture, lipid, crude protein and crude ash content of raw horse mackerel were found to be 76.4 ± 0.64 % , 6.86 ±0.12 %, 21.74 ± 0.48 % and 1.22±0.03%, respectively. The proximate composition of the horse mackerel showed similarities to the findings of Bandarra et al. (2001) and Vareltzis et al. (1997) with little differences. However, some researchers have found different results in lipid content of horse mackerel (Aubourg et al., 2002, Aubourg et al.,2004; Boran and Karaçam, 2011). These differences may be related to size, food, migration and sexual changes and geographic region (Bandarra et al., 2001; Losada et al., 2005; Çelik, 2008; Boran and Karaçam, 2011). The moisture, lipid, crude protein and crude ash content of horse mackerel coated with WPI were found to be 73.70±1.04 %, 5.86 ±0.06 % and 24.48 ±0.45 % and 1.14 ±0.05 %, respectively. There was an apparent net increase in protein content of horse mackerel coated with WPI compared to the raw ones but a decrease in the moisture, lipid and crude ash. The increasing the crude protein content in it was related to coating of whey protein isolate. The decreasing the moisture, lipid and crude ash can be explained by the increase in the protein content.

Chemical quality

Peroxide value (PV) of horse mackerel coated whey protein isolate enriched with thyme oil is given in Table1.

Table 1. PV (meq of peroxide oxygen kg−1) of horse mackerel coated whey protein isolate enriched with thyme oil[1,2,3]

Months	Control	DW	WPI0	WPI3	WPI5	WPI7
0	0.55±0.03^{c1}	0.44±0.01^{b1}	0.41±0.01^{ab1}	0.34±0.03^{a1}	0.84±0.06^{d1}	0.97±0.02^{e1}
1	0.55±0.01^{c1}	0.45±0.03^{b1}	0.59±0.01^{d2}	0.39±0.02^{a1}	0.89±0.02^{e1}	0.96±0.03^{f1}
2	1.23±0.03^{b2}	1.11±0.03^{ab2}	1.35±0.01^{bc3}	0.71±0.55^{a12}	1.49±0.02^{bc2}	1.71±0.03^{c2}
3	1.23±0.03^{c2}	1.14±0.04^{b2}	1.34±0.01^{d3}	1.02±0.02^{a2}	1.44±0.02^{e2}	1.71±0.02^{c2}
4	3.63±0.02^{c3}	3.54±0.02^{b3}	3.67±0.02^{d4}	3.46±0.01^{a3}	5.18±0.02^{e3}	5.29±0.02^{f3}
5	6.38±0.03^{b4}	6.53±0.02^{c4}	6.54±0.02^{c5}	6.34±0.01^{a4}	6.96±0.02^{d4}	7.03±0.02^{e4}
6	7.06±0.02^{b5}	7.05±0.03^{b5}	7.16±0.02^{c6}	6.97±0.02^{a5}	7.82±0.02^{d5}	7.96±0.03^{e5}
7	11.03±0.02^{a6}	10.98±0.02^{a6}	11.04±0.02^{a7}	11.27±0.44^{a6}	11.21±0.04^{a6}	11.37±0.03^{a6}
8	14.04±0.02^{b7}	14.07±0.02^{b7}	14.08±0.02^{b8}	14.00±0.01^{a7}	14.81±0.03^{c7}	14.98±0.01^{d7}
9	16.12±0.01^{b8}	16.15±0.03^{b8}	16.30±0.06^{c9}	16.01±0.01^{a8}	16.61±0.01^{d8}	16.77±0.05^{e8}
GLM	6.18^{b}	6.15^{b}	6.25^{c}	6.0530^{a}	6.7253^{d}	6.8739^{e}

[1] Values are mean ± standard deviation.
[2] Means within the same column (1,9) and the same row (a,f) with different letters are different ($P < 0.05$).
[3] GLM, genel linear model DW, distillated water; WPI0, whey protein isolate coating without thyme oil; WPI3, whey protein isolate coating with 3% thyme oil; WPI5, whey protein isolate coating with 5% thyme oil; WPI7, whey protein isolate coating with 7% thyme oil

Table 2. Thiobarbutiric acid (TBA) (mg malonaldehyde / kg muscle) value of horse mackerel coated whey protein isolate enriched with thyme oil[1,2,3]

Months	Control	DW	WPI0	WPI3	WPI5	WPI7
0	0.56±0.01^{c1}	0.59±0.01^{d1}	0.49±0.00^{b1}	0.46±0.02^{a1}	0.46±0.01^{e1}	0.47±0.00^{f1}
1	0.73±0.01^{d2}	0.64±0.00^{c2}	0.56±0.00^{a2}	0.56±0.00^{a2}	0.62±0.00^{b2}	0.61±0.02^{b2}
2	0.73±0.01^{d2}	0.65±0.01^{c3}	0.56±0.00^{a2}	0.57±0.00^{a2}	0.62±0.00^{b2}	0.62±0.00^{b2}
3	0.85±0.04^{a3}	0.87±0.00a^{b4}	0.86±0.00^{ab3}	0.85±0.00^{a3}	0.88±0.00^{b3}	0.92±0.01^{c3}
4	0.96±0.00^{b4}	0.96±0.00^{b5}	0.87±0.01^{a4}	0.86±0.00^{a3}	1.04±0.02^{c4}	1.10±0.00^{d4}
5	1.11±0.00^{c5}	1.12±0.00^{d6}	0.96±0.00^{b5}	0.88±0.00^{a4}	1.17±0.00^{e5}	1.22±0.00^{f5}
6	1.52±0.02^{c6}	1.48±0.00^{b7}	1.40±0.00^{a6}	1.40±0.00^{a5}	1.56±0.00^{d6}	1.57±0.00^{d6}
7	1.56±0.00^{a7}	1.58±0.01^{b8}	1.56±0.00^{a7}	1.56±0.00^{a6}	1.80±0.00^{c7}	1.97±0.00^{d7}
8	3.51±0.05^{b8}	3.48±0.00^{b9}	3.31±0.00^{a8}	3.31±0.00^{a7}	3.43±0.12^{b8}	3.70±0.00^{c8}
9	3.46±0.03^{b9}	3.48±0.00^{b9}	3.31±0.00^{a8}	3.31±0.00^{a7}	3.75±0.00^{c8}	3.84±0.03^{d8}
GLM	1.499277^{d}	1.483677^{c}	1.386586^{b}	1.375003^{a}	1.549548^{e}	1.625110^{f}

[1] Values are mean ±standard deviation.
[2] Means within the same column (1,9) and the same row (a,f) with different letters are different ($P < 0.05$).
[3] GLM, genel linear model DW, distillated water; WPI0, whey protein isolate coating without thyme oil; WPI3, whey protein isolate coating with 3% thyme oil; WPI5, whey protein isolate coating with 5% thyme oil; WPI7, whey protein isolate coating with 7% thyme oil

The secondary lipid oxidation products were measured by means of thiobarbituric acid values (TBA) expressed as mg malonaldehyde /kg muscle. As seen in Table 2, the initial TBA for the raw non-coated horse mackerel was found to be 0.55 mg malonaldehyde /kg muscle. This value was higher than finding for horse mackerel by other researchers (Simeonidou et al.,1997; Aubourg et al., 2002**;** Aubourg et al.,2004). The reason of higher TBA value could be resulted from higher lipid content and iced storage (10 hour) before freezing (Aubourg et al., 2002). During frozen storage, TBA value showed an increase in all groups. WPI coating without thyme oil caused a decrease in TBA value when compared with noncoated and water coated groups. Similarly, coating with WPI solution/antioxidant overspray of King salmon have been found to be effective in controlling lipid oxidation after 77 days of frozen storage (Stuchell and Krochta,1995). Similar results have also been reported by Sathivel et al. (2007). As well as the present PV, the higher TBA value were found in samples coated WPI with enriched thyme oil 5% and %7 than others one, while the lowest TBA value was found in samples coated with WPI enriched with thyme oil 3%. The prevention of lipid oxidation for frozen horse mackerel by using antioxidant have been reported previously (Aubourg et al., 2004). But antioxidant effect of thyme oil alone or combined with edible coating for

horse mackerel during frozen storage did not evaluated before. It has been observed that 1% tyhme oil-treated chub mackerel (Erkan and Bilen, 2010) and rainbow trout (Çoban, 2012) had lower TBA value during frozen storage. In the current study, the using higher than 3% concentration thyme oil combined with WPI showed prooxidant acvtivity during frozen storage for horse mackerel. Several authors claim that a variety of testing systems is required when assessing the antioxidant potential of a substance, since a substance exhibiting high antioxidant activity in one system may have a pro-oxidant effect in another system (Laughton et al.,1989; Pearson et al., 1997.) Recent works indicate that high essential oils can have pro-oxidant effects (Mennen et al., 2005; Bakkali et al., 2008). Undeger et al. (2009) observed that high concentrations (100 µM) carvacrol showed a pro-oxidant effect and high concentrations of thymol (25 µM) increased DNA damage. This might explain why the higher thyme oil treated groups had a higher TBA value than the lower thyme oil treated groups.

Effect of whey protein isolate coating enriched with thyme oil on protein solubility (%) in 5% NaCl during frozen storage of horse mackerel is given in Table3.

Table 3. Protein Solubility (%) of horse mackerel coated whey protein isolate enriched with thyme oil

Months	Control	DW	WPI0	WPI3	WPI5	WPI7
0	100[1]	100[3]	100[2]	100[4]	100[3]	100[4]
1	96.80±0.14^{a3}	103.50±0.17^{e1}	102.76±0.22^{e1}	103.12±0.12^{d1}	101.63±0.07^{c1}	100.37±0.15^{b3}
2	97.02±0.24^{a3}	101.37±0.28^{c2}	100.29±0.30^{b2}	101.04±0.20^{c2}	101.13±0.13^{c2}	104.48±0.16^{d1}
3	98.86±0.55^{a2}	101.31±0.21^{d2}	99.75±0.19^{b2}	100.43±0.14^{c3}	101.13±0.23^{d2}	103.02±0.20^{e2}
4	86.02±0.17^{a7}	89.49±0.37^{b6}	92.8±02.62^{c3}	93.43±0.25^{c4}	88.14±0.14^{b4}	91.82±0.11^{c5}
5	85.10±0.17^{a8}	88.47±0.14^{d7}	90.31±0.24^{e4}	87.88±0.15^{c67}	87.22±0.32^{b5}	91.18±0.17^{f6}
6	93.98±0.19^{a4}	86.00±1.36^{d8}	89.63±0.21^{e4}	86.54±0.13^{c8}	87.28±0.19^{b5}	88.29±0.19^{f9}
7	93.98±0.18^{e4}	93.66±0.34^{e4}	93.31±0.15^{d3}	85.58±0.19^{a9}	86.33±0.21^{b6}	88.39±0.29^{c9}
8	90.12±0.16^{d6}	92.20±0.29^{e5}	86.28±0.17^{a5}	88.12±0.12^{b6}	88.25±0.21^{b4}	89.55±0.17^{c8}
9	91.62±0.38^{d5}	91.88±0.15^{d5}	85.58±0.17^{a5}	87.70±0.13^{b7}	87.51±0.16^{b5}	90.05±0.11^{c7}
GLM	93.35^{c}	94.79^{a}	94.15^{b}	93.38^{c}	92.86^{d}	94.72^{a}

[1] Values are mean ± standard deviation.
[2] Means within the same column (1,9) and the same row (a,f) with different letters are different ($P < 0.05$).
[3] GLM, genel linear model DW, distillated water; WPI0, whey protein isolate coating without thyme oil; WPI3, whey protein isolate coating with 3% thyme oil; WPI5, whey protein isolate coating with 5% thyme oil; WPI7, whey protein isolate coating with 7% thyme oil

Protein solubility in 5% sodium chloride is used to characterize the degree of protein denaturation during frozen storage. In present study, protein solubility showed slightly decrease in all samples during frozen storage, similar to previous research on frozen horse mackerel (Undeland et al., 2003). There are published data indicating that a reduction in protein solubility during frozen storage was due to denaturation and aggregation of myofibrillar proteins in frozen fish (Geirsdottir et al., 2007). Hurling and McArthur (1996) studied frozen storage effects in cod and they found that myofibrillar protein solubility decreased from 70 to 22% of the total protein after 9 month storage at –30°C. They also indicated that decline in protein solubility was not caused by complete protein unfolding. Insolubilization of proteins during frozen storage is caused by formation of intermolecular hydrogen and/or hydrophobic bonds as well as disulfide bonds and ionic interaction (Akahane,1982). Montecchia et al.(1997) observed a slight increase in salt-soluble protein from hake after 15 day frozen storage at –20°C followed by gradual decrease until the end of frozen storage. This slightly increased protein solubility might be caused by modifications of chemical groups, especially sulfhydryls. These changes would cause a transitory modification of molecular conformation as was suggested by *in vitro* experiments with carp actomyosin, myosin, and heavy meromyosin during short term frozen storage (Matsumoto, 1980). Comparison among the different treatments showed that coating process was helpful to protect the decrease in protein solubility till first three months (Aubourg et al., 2004). Among the treatment, DW and the WPI coated enriched with 7% thyme oil caused significantly higher protein solubility than other treatments during frozen storage ($p<0.05$). However, the reduction of solubility did not change regularly. These fluctuations during frozen storage could be caused by interactions of proteins with other compounds such as fatty acids, decomposition products of lipids and amine compounds (Saeed and Howell 2002, Siddaiah et al., 2001).

SDS-PAGE

The electrophoretic profiles of the salt-soluble proteins extracted with 5% NaCl from groups during frozen storage are shown in Figure 1.

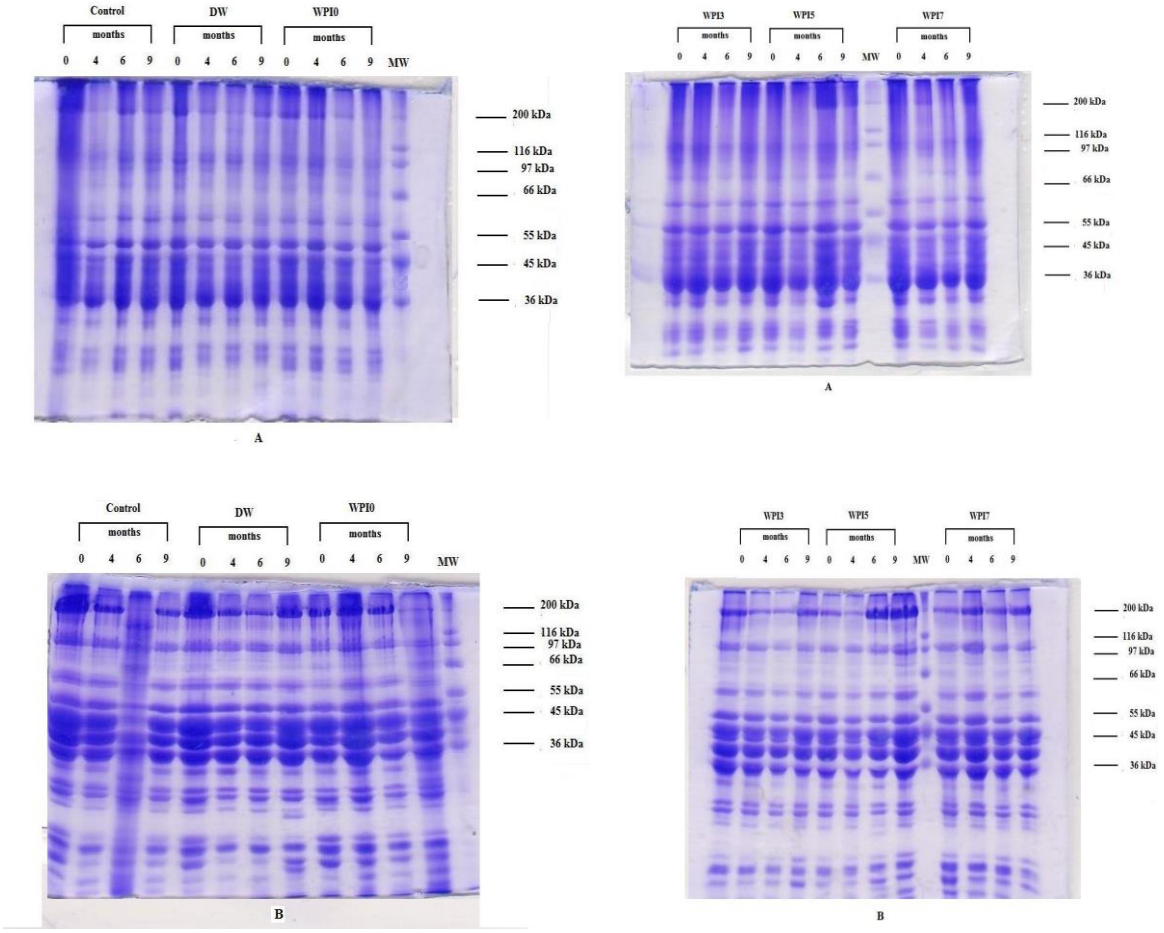


Figure 1. The electrophoretic profiles of the salt-soluble proteins extracted with 5% NaCl from groups prepared without (A) and with β-mercaptoethanol (B) during frozen storage (DW, distillated water; WPI0, whey protein isolate coating without thyme oil; WPI3, whey protein isolate coating with 3% thyme oil; WPI5, whey protein isolate coating with 5% thyme oil; WPI7, whey protein isolate coating with 7% thyme oil, MW; molecular weight)

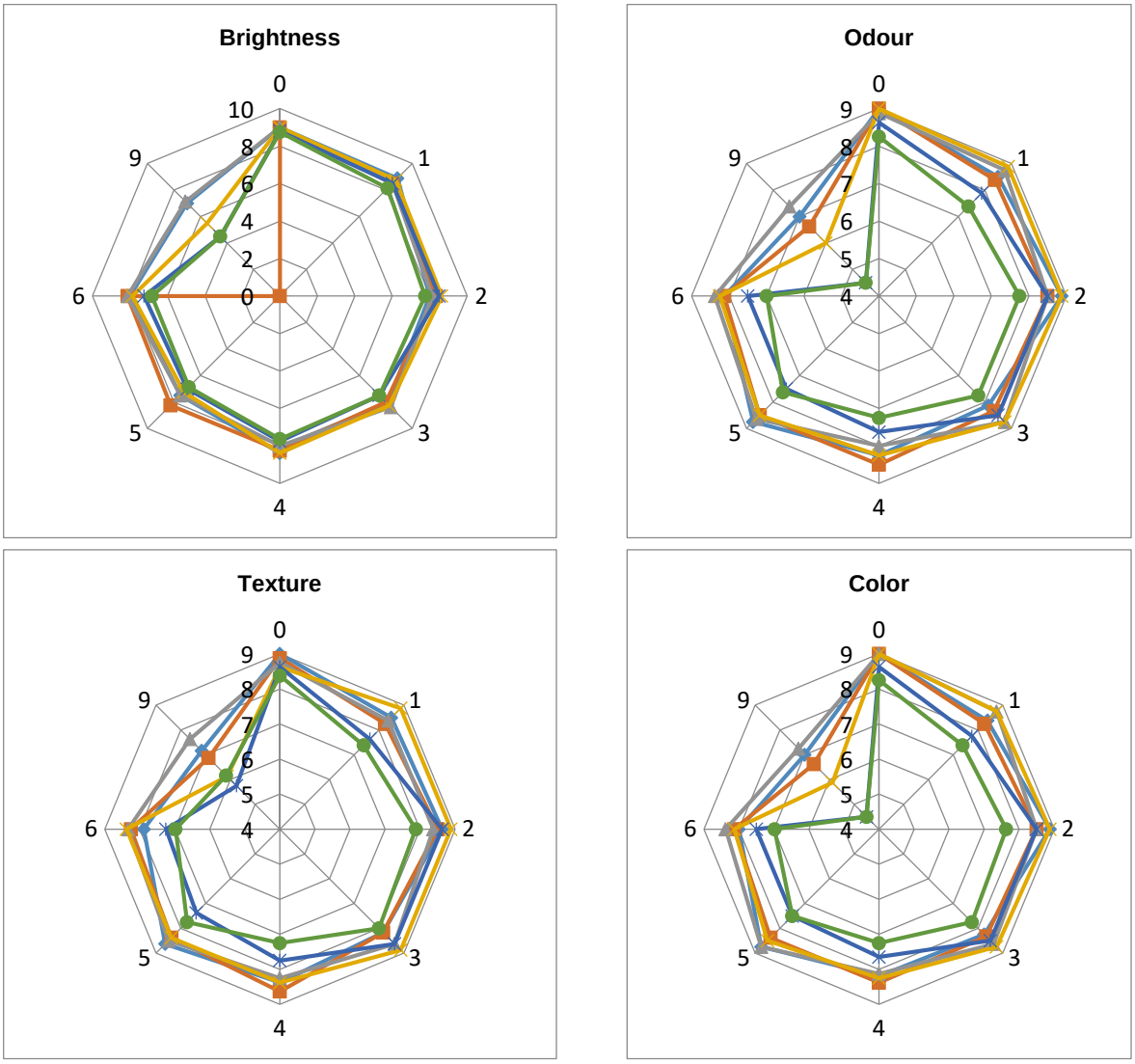
In this study, electrophoretic protein profiles in groups showed that the remarkable changes seen in the protein band around 200 kDa in the absence of β-mercaptoethanol, indicating the absence of intermolecular disulfide bridges (Figure 2). In control, DW, WPI0 and WPI7, the intensity of this band decreased and/or increased throughout the frozen storage period of 9 months at – 18 ^{o}C ±2, while it didn't seen in WPI3 from at the beginning of the storage to at the end of the storage and it didn't seen in WPI5 at the beginning four month and then appeared at month 6 and 9. A double protein bands with molecular weight around 97 kDa did not seen in the groups coated with thyme oil during storage. No significant differences were observed in other protein bands both between samples and during frozen storage. The protein profiles of samples treated with β-mercaptoethanol as reducing agent showed that there were many new bands in all samples when compare with the protein profiles of samples treated without β-mercaptoethanol during frozen storage. The new bands in the presence of reducing agent were attributed to polymerization high molecular weight via disulfide cross-linking (Le Blanc and Le Blanch 1989). Formation of disulfide band also contributes to decrease in salt soluble protein during frozen storage (Owusu-Anash and Hultin 1986). In this study, the decrease in protein solubility observed in all samples during storage. These decreases could be resulted from cross-linking of protein (Keyvan et al., 2008). No significant differences between the electrophoretic profiles obtained within samples but the appearance of new bands of low moleculer weight (<36 kDa) were seen in all samples during frozen storage. Main differences within groups were observed in control samples at six month of storage, the protein bands near 200 kDa completely disappeared and new bands occurred near 116 and 97 kDa. At the interface of stacking and resolving electrophoresis gels, there was a proteins that did not penetrate the resolving gel, with a higher molecular weight than MHC, probably formed by the covalent linkage during frozen storage of muscle (Careche et al., 2002). In frozen fish, high molecular weight protein aggregate accumulated by hydrophobic interaction as well as by disulfide bonds and other covalent cross-links (Haard, 1992).

Sensory Quality

The results of the sensory analyses of horse mackerel coated WPI enriched thyme oil during frozen storage are shown in Figure 2.

Panelists scored for brightness, color, odor, flavor, texture and general acceptability using a nine-point hedonic scale (1, dislike extremely to 9, like extremely) for the assessment of sensory quality in samples. Results of the sensory evaluation obtained reveal that samples coated WPI enriched with %5 and %7 thyme oil showed the most marked changes ($p<0.05$).

Significantly higher scores for color, odor and flavor were given to control, DW, WPI and WPI3, while WPI7 scored the significantly lowest for all parameters at the beginning of the storage ($p<0.05$). When they stored longer, lower scores were given subsequently for all groups by the panelists. However, rancid flavor was estimated in samples coated WPI enriched with 5% and 7% thyme oil after nine month storage and the scores samples reached unacceptable levels. But, other groups received scores above the acceptability limit of 5. These conclusions were also supported by the results for lipid quality analyses. The higher PV and TBA value were noticed at the later stage of storage days and these could be due to the production of lipid oxidation products, resulting in the unacceptable flavor. Similar trends were also observed in brightness, color, odor, texture and general acceptability (Figure 2). Similarly, Rodriguez-Turienzo et al. (2011) found that no significant differences ($p>0.05$) between whey protein coated samples and controls and the sensory properties of salmon samples were not affected negatively by these coatings.



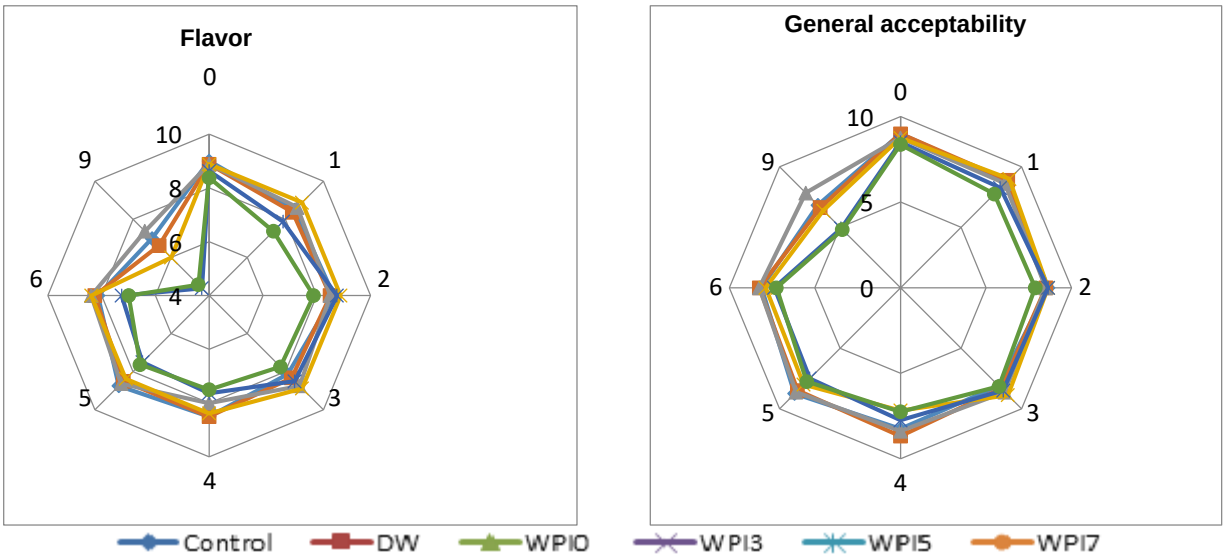


Figure 2- Sensory evaluation of horse mackerel coated whey protein isolate enriched with thyme oil during frozen storage

No significant ($p > 0.05$) effects of protein coating on a^*, b^*, and whiteness values for cooked pink salmon after 3 mo frozen storage were also reported by Sathivel (2005). The changing of brightness and color with addition of essential oil in edible coatings have been reported previously by researchers who claimed that addition of EOs in edible films may change the native color of edible films and the degree of change is concentration-dependent (Du et al., 2011; Rhim et al., 2000). Those results showed that the horse mackerel coated WPI enriched with 5 and 7% thyme oil could not be stored for more than 9 months.

CONCLUSION

In conclusion, when thyme oil used higher concentration than 3%, it can be play as a pro-oxidant for horse mackerel over the course of frozen storage and caused unacceptable level of fish. As a result of protein solubility and SDS-PAGE, whey protein isolate and enriching whey protein isolate with thyme oil showed no remarkable changes in protein quality.

ACKNOWLEDGMENTS

The authors want to thanks to Çukurova University, Scientific Research Projects Coordinating Office (ÇÜ-BAP-SÜF2012BAP13) for supporting this research.

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