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Original article

Effect of plant extracts on lipid oxidation during frozen storage of minced fish muscle

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Summary The aim of the study is to determine the effect of pomegranate seed extract (PSE) and grape seed extract (GSE) addition to chub mackerel minced muscle on lipid oxidation during frozen storage. Each extract was added to minced fish muscle at 2% concentration and then stored at −18 °C for 3 months. The effect of plant dietary fibres to control lipid oxidation was compared with untreated samples (control). Formation of lipid hydroperoxides and thiobarbituric acid-reactive substances (TBARS) was significantly inhibited by PSE and GSE addition when compared with control. Both extracts significantly retarded lipid oxidation according to the results of TBARS. A significant reduction of L* (lightness), a* (redness) and b* (yellowness) values was detected during frozen storage. GSE added samples had the highest redness and the lowest lightness and yellowness. However, samples with PSE showed the lowest redness and highest yellowness and h° (hue angle) values. The results from this study suggest GSE is a very effective inhibitor of primary and secondary oxidation products in minced fish muscle and have a potential as a natural antioxidant to control lipid oxidation during frozen storage of fatty fish.

Keywords Chub mackerel, frozen storage duration, grape seed extracts, lipid oxidation, pomegranate seed extracts.

Introduction

Seafoods are highly susceptible to oxidative deterioration because of the compositional nature of muscles. Fatty fish contain high concentrations of polyunsaturated fatty acids. During frozen storage, extensive autooxidation in fatty fish muscle occurs, resulting in decreased functionality of the muscle. Lipid oxidation is well recognised as a major form of deterioration in stored muscle foods. Besides, the lipid oxidation problem has extensive economic importance for the meat industry. Oxidative processes in meat are the most important factor responsible for quality loss including flavour, texture, nutritive value and colour. The rate and degree of the lipid oxidation depends on the fish species, its freshness prior to freezing, the muscle type, the presence or absence of activators and inhibitors, preservation methods and storage conditions (Petillo *et al.*, 1998; Undeland *et al.*, 1998; Richards & Hultin, 2002).

The use of antioxidants is an effective way to minimise lipid oxidation in lipid-based food products (Boyd *et al.*, 1993; Frankel, 1998). Antioxidant activity depends on many factors such as the lipid composition, antioxidant concentration, temperature, oxygen pressure, many common food components and processing conditions (Houlihan *et al.*, 1985; Giese, 1996; Alghazeer *et al.*, 2008). Both synthetic and natural antioxidants are used in food systems. Recently, there has been growing interest in using natural antioxidants in foods to replace synthetic antioxidants because of their potential to prolong the shelf life of food products. Some natural phenolic compounds, mostly flavonoids, tocopherols and phenolic acids, have antioxidant activities and have beneficial effects on lipid metabolism (Teissedre *et al.*, 1996; Fukumozo & Mazza, 2000). Grape and pomegranate seed have a great concentration of polyphenolic compounds and have antioxidant activities (Escribano-Bailo′n *et al.*, 1995; Mazza, 1995; Fukumozo & Mazza, 2000; Alonso *et al.*, 2002; Torres *et al.*, 2002). Grape and pomegranate seed extracts are extensively studied for their antioxidant properties (Pazos *et al.*, 2005a,b; Mielnik *et al.*, 2006; Luther *et al.*, 2007; Sánchez-Alonso *et al.*, 2007; Gokoğlu & Yerlikaya, 2008; Naveena *et al.*, 2008a,b; Kanatt *et al.*, 2010; Yerlikaya & Gokoglu, 2010).

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Chub mackerel (*Scomber japonicus*) is a fatty fish species and very susceptible to lipid oxidation during frozen storage. This work aimed to study the effect of grape and pomegranate seed extracts addition to minced chub mackerel muscle on lipid stability during frozen storage. The efficacy of the antioxidants added directly to meat before freezing was monitored throughout 3 months of frozen storage. The effectiveness of each natural antioxidant was evaluated by comparing the inhibition of oxidation products during frozen storage. An understanding of the reactions that occur during storage can help to achieve better quality products.

Materials and methods

Preparation of frozen samples and storage

Chub mackerel (*Scomber japonicus*) were supplied by a local store overnight catching in Black Sea. They were packed in crushed ice and transferred to the laboratory within 6–8 h after catching. The fish was carefully deboned, eviscerated and filleted by hand. The fillets with skin were minced separately through a 3.0-mm plate using a kitchen-type mincer (Moulinex, France) to obtain homogeneous samples and divided into three groups. Commercial red grape seed extracts (GSE) and pomegranate seed extracts (PSE) (Balen, Arı Mühendislik Ltd, Ankara, Turkey) were obtained from the market. Concentrations of 2% GSE and PSE were separately added to the minced fish muscle and mixed for 3 min to get homogeneous samples. Minced fish muscle was formed by hand (80 g) to give average dimensions of 12 cm diameter and 1 cm thickness. Freezing process was conducted in a mobile air blast freezer (Frigoscandia Labofreeze, Sweden). Meat samples on stainless steel trays were placed in the freezer at −35 °C and 2.5 m s^{-1} air flow. Frozen temperature history was recorded by copper-constant thermocouples inserted into the centre, upper and bottom of the meat sample in centre of the tray. In order to balance the temperature throughout the samples as −18 °C, frozen samples were transferred to a thermo-container and then placed into cartoon trays and stored at −18 °C for 3 months.

Sampling

Samples were taken before freezing (at day 0) and at 1-month intervals during frozen storage at −18 °C for a period of 3 months. For each sampling time, samples were thawed at 4 °C for 3 h before analyses.

Antioxidant activity of plant extracts

The antioxidant activities of grape seed and pomegranate seed extracts were determined using trolox equivalent antioxidant capacity method according to Miller *et al*. (1997) and Re *et al*. (1999). ABTS was dissolved in water to a 7 mM concentration. ABTS radical cation ($ABTS^+$) was produced by reacting ABTS stock solution with 2.45 mM potassium persulfate (final concentration) and allowing the mixture to stand in the dark at room temperature for 12–16 h before use. Afterwards, the $ABTS^+$ solution was diluted with phosphate buffer saline to an absorbance of 0.7 ± 0.02 at 734 nm. After addition of 1.0 ml of diluted $ABTS^+$ solution to 10, 20 and 30 μL of samples, the absorbance reading was taken exactly 1 min after initial mixing and up to 6 min. Following the absorbance readings, per cent inhibition of oxidation was calculated for each sample.

$$\%\ Inhibition = \frac{(\text{Absorbance of sample at 0 min - Absorbance of sample at 6 min})}{(\text{Absorbance of sample at 6 min})} \times 100$$

Sample concentration vs. % inhibition was plotted, and the equation of curve was determined. Trolox concentrations were used for calibration, and the results were expressed as millimolar of trolox equivalent per gram extract.

Total phenolic content of extracts

Total phenolics in water extracts were determined using Folin-Ciocalteu reagent according to Singleton & Rossi (1965) by using gallic acid as the standard, and the results were given as gallic acid equivalents.

Lipid extraction

Total lipids were extracted from minced muscle according to the method outlined by Lee *et al*. (1996). A 5 g sample was homogenised with 50 mL chloroform/methanol mixture (2/1, v/v) for 1.5 min in an Eberbach blending jar (Eberbach Corp., Ann Arbor, MI, USA) on Waring blender base (Waring Products, New Hatford, CT, USA). The homogenate was filtered through fast-speed filter paper (12.5 mm id, Whatman No.1) into separatory funnel, and 20 mL of 0.5% NaCl was added to separate the filtrate into two phases. The mixture was gently shaken and allowed to stand until a clear separation was visible. The methanol–water phase was discharged, and the chloroform phase was used for

analysis. To obtain lipid fraction, chloroform phase was removed by using a vacuum evaporator. Fish lipid was used for the peroxide value and conjugated dienes analysis.

Lipid oxidation measurement

Development of lipid oxidation during frozen storage was evaluated by measuring peroxide values (PV), thiobarbituric acid-reactive substances (TBARS), conjugated diene (CD) values.

Peroxide value
Peroxide value was determined according to the method of the International Dairy Federation (IDF) (Shantha & Decker, 1994). To determine the peroxide value, the lipid sample (0.01–0.30 g, depending on the extent of peroxidation) extracted as described earlier was mixed with 9.8 mL dichloromethane-methanol in a disposable glass tube and vortex-mixed for 2–4 s. Ammonium thiocyanate solution (0.05 mL) was added, and the sample was vortex-mixed for 2–4 s. Then, 0.05 mL iron (II) solution was added and the sample was again vortex-mixed for 2–4 s. After 5-min incubation at room temperature, the absorbance was measured at 500 nm using an UV-VIS double-beam spectrophotometer (Labomed UVD-3200, Culver City, CA, USA). The results were expressed in milliequivalents of peroxide per kg of fat.

Measurement of conjugated diene hydroperoxides
Diene conjugation was determined by the procedure of Buege & Aust (1978). Conjugated hydroperoxides were measured from chloroform phase resulting from lipid extraction. The chloroform phase (0.1 mL) was placed in a test tube, and the upper layer was removed by stream of nitrogen. Then, 1.5 mL of hexane was added and mixed on a vortex. For diene conjugation, the absorbance of the resulting layer was read at 234 nm against a blank. The amount of dienes expressed as nmol of hydroperoxides per mg of lipid using a molar extinction coefficient of 25 200 M^{-1} cm^{-1}.

Thiobarbituric acid-reactive substances
TBARS indicate the oxidative changes in muscle foods during frozen storage. The amounts of TBARS in samples were determined according to the method of McDonald & Hultin (1987). The minced sample (1 g) was homogenised with 10 mL deionised water, and an aliquot of the sample (1 mL) was added to 2 mL of TCA/TBA stock solution consisting of 15% TCA (w/v) and 0.375% TBA (w/v) in 0.25 M HCl and 3 mL 2% butylated hydroxytoluene (BHT) (w/v) prepared in 2 mL absolute ethanol in a test tube and immediately mixed thoroughly with a vortex mixer. The mixture was exposed to incubation in a 90 °C water bath for 15 min to develop pink colour. After cooling in ice-bath, the sample was centrifuged at 1000 ***g*** for 10 min. The absorbance of the supernatant was measured at 532 nm. The amounts of TBARS were expressed as mg of malondialdehyde per kg of meat using a molar extinction coefficient of 1.56×10^5 $M^{-1}cm^{-1}$.

Antioxidant effectiveness

The antioxidant effectiveness for thiobarbituric acid-reactive substances and conjugated diene hydroperoxides was calculated as per cent inhibition of oxidation (%I), as described by Frankel (1998): $\%I = [(c-s)/c]^* 100$, where c = high increment of control in the experiment and s = increment of sample with added different extracts at the same time. High levels of %I indicate greater antioxidant effectiveness (Sánchez-Alonso *et al*., 2007).

Colour measurement

Colour measurements were performed using a Minolta Chroma Reflectance Colorimeter CR-300 (Minolta, Osaka, Japan). The colorimeter had an 8-mm-diameter viewing area. The instrument was calibrated with a white reference tile before the measurements. The L*, a* and b* colour values were measured at six locations of the surface of minced meat. Results were expressed as L* (Lightness), a* (Redness), b* (Yellowness), C (Chroma) and h° (hue angle); $C = (a^{*2} + b^{*2})^{1/2}$, h° = arctan (b*/a*).

Statistical analyses

The statistical model was designed completely at random. All values obtained from measurements were analysed by a two-factor factorial design. The factors were natural antioxidant sources (GSE, pomegranate seed extract, control) and duration of storage at −18 °C (0–3 months). Two replications were carried out for all samples. The data obtained were subjected to statistical analyses using MINITAB for Windows Release 13.1® (Minitab, 2000). Duncan Multiple Range Test was used to determine significance of the mean values for multiple comparison (Mstat, 1986).

Results and discussion

Total phenolic contents and antioxidant activity of plant extracts

Total phenolic contents and antioxidant activity of GSE and pomegranate seed extracts (PSE) are presented in Table 1. Total phenol content was determined for the aqueous extracts using the Folin-Ciocalteu method. Water was chosen as an extracting solvent providing

Table 1 Total phenolic contents (TPC, g gallic acid/kg extract) and antioxidant activity (AA, mM trolox/g extract) of grape seed extracts (GSE) and pomegranate seed extracts (PSE)

	Grape seed extract	Pomegranate seed extract
TPC	65.95a	47.21b
AA	156.14a	77.05b

[a,b]Different letters in the same line indicate significant differences ($P < 0.01$).

similarity to industrial practise. The total phenolic contents of GSE and PSE were 65.95 g gallic acid equivalents per kg extract and 47.21 g gallic acid equivalents per kg extract, respectively. Different phenolic contents of plant extracts are reported in the literature. Total phenolic concentration of 56 mg gallic acid/100 g for pomegranate juice concentrate (Gokoglu *et al*., 2009) and 131.1 mg g^{-1} for pomegranate peel extract and 7.25 mg g^{-1} for pomegranate seed extract (Kanatt *et al*., 2010) was reported. Baydar *et al*. (2004) determined phenolic content of GSE were found as 67 ± 1 g GAE per 100 g. Total phenolic contents of chardonnay seed and black raspberry seed extracts were found 99.3 mg gallic acid equivalents per gram extract and 11.8 mg gallic acid equivalents per gram extract, respectively (Luther *et al*., 2007). Differences in the plant variety, plant part, climatic conditions, maturity, solvent and extraction method cause differences in results (Shi *et al*., 2003; Yerlikaya & Gokoglu, 2010).

Phenolic compounds are responsible for the antioxidant activity of many fruits and vegetables (Kanatt *et al*., 2010). GSE with high phenolic contents indicate relatively high antioxidant activity as measured by ABTS methods. Antioxidant activity of GSE and PSE was found to be 156.14 mM trolox per gram and 77.05 mM trolox per gram, respectively. Antioxidant activity determined in GSE was higher than PSE depending on their phenolic contents. Antioxidant activity of red grape dietary fibre was found to be 153 ± 9 μmol trolox per gram by DPPH method and 525 ± 28 μmol trolox per gram by FRAP method by Sánchez-Alonso *et al*. (2007). They reported that white grape dietary fibre contained high amounts of extractable polyphenols and had relatively high antioxidant activity, which was 466 μmol trolox per gram dry matter by FRAP method and 284 μmol trolox per gram dry matter by ABTS method (Sánchez-Alonso *et al*., 2008). Antioxidant activity of GSE was found to be 1.605 ± 0.045 mM trolox Yerlikaya & Gokoglu, 2010). These differences may result from differences in defatting process before extraction, moisture content of material, solvent used for extraction, time and temperature for extraction, method for determination of antioxidant activity.

Development of lipid oxidation products

Lipid oxidation increased in all minced muscle samples as storage time prolonged. PV of fish samples are presented in Fig. 1. PV increased from 1.28 to 3.42 meq peroxides per kilogram lipid in the control samples, 1.38 to 4.88 meq peroxides per kg lipid in PSE-treated samples and 1.27 to 2.29 meq peroxides per kilogram lipid in GSE-treated samples after 3 months of storage. PSE-treated samples had a higher PV than GSE-treated and control samples (Fig. 1). During frozen storage, no significant increase was observed in the samples with GSE ($P > 0.01$). However, significant increases were detected in the control and PSE-treated samples after 1 month of storage ($P < 0.01$). The increase was probably due to the faster rate of formation of peroxides during early times of storage than degradation of peroxides into secondary oxidation products. Peroxide analysis is not enough to assess the oxidation because peroxides form initially and react to form secondary oxidation products.

The results of TBARS analyses are given in Fig. 2. The oxidative rancidity as measured by TBARS increased from 38.87 to 126.84 mg MDA per kg meat in control samples, 18.24 to 32.87 mg MDA per kg meat in samples treated with PSE, and 32.40–54.08 mg MDA per kg meat in samples treated with GSE after 3 months of storage. Variance analysis of TBARS showed significant effects of antioxidant source, storage time and interactions between antioxidant source and storage time ($P < 0.01$). There was an increase in the level of TBARS during frozen storage, particularly in control samples compared with natural antioxidant sources. After 2 months of frozen storage, significantly highest TBARS value was seen in the control sample where no

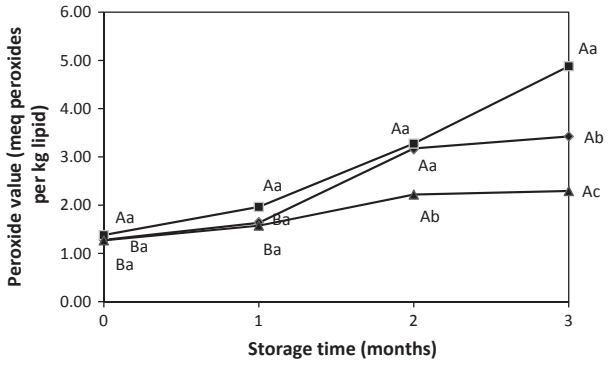

Figure 1 Formation of peroxides in minced fish muscle added grape and pomegranate seed extracts during frozen storage at −18°C [Control (♦), grape seed extract (▲), pomegranate seed extract (■)].
[A,B] Different letters in the same line indicate significant differences ($p < 0.01$).
[a,c] Different letters in the same column indicate significant differences ($p < 0.01$).

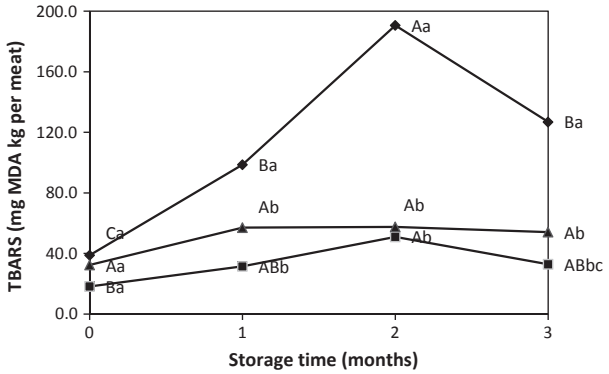

Figure 2 Formation of malondialdehydes in minced fish muscle added grape and pomegranate seed extracts during frozen storage at −18°C [Control (◆), grape seed extract (▲), pomegranate seed extract (■)].
[A,C] Different letters in the same line indicate significant differences ($p < 0.01$).
[a,c] Different letters in the same column indicate significant differences ($p < 0.01$).

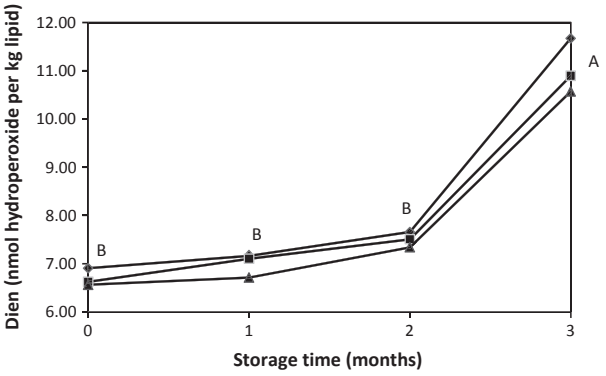

Figure 3 Formation of conjugated dienes in minced fish muscle added grape and pomegranate seed extracts during frozen storage at −18°C [Control (◆), grape seed extract (▲), pomegranate seed extract (■)].
[A,B] Different letters in the same line indicate significant differences ($p < 0.01$).

antioxidants added. This result showed that natural antioxidant sources were effective in retarding the increase in TBARS levels of minced muscle during frozen storage. After 2 months of frozen storage, there was a decrease in the level of TBARS of control samples. This situation can be explained by TBA-reactive substances cross-linking with proteins (Saeed *et al.*, 1999; Alghazeer *et al.*, 2008). At the end of the frozen storage (3 months), TBARS value of control samples was significantly ($P < 0.01$) higher than the values of antioxidant-treated samples. Alghazeer *et al.* (2008) found the highest TBARS values in mackerel without antioxidant treated and stored at −10°C on the 8-week frozen storage. It was reported that chub mackerel treated with ascorbic acid, BHT, butylated hydroxyanisole (BHA) and BHT/BHA combination were more effective in preventing oxidation than control samples (Soyer & Sahin, 1999). Addition of GSE into mackerel muscle was an effective way for delaying lipid oxidation (Pazos *et al.*, 2005a). Yerlikaya & Gokoglu (2010) have reported similar results for bonito fillets treated with green tea and GSE.

The conjugated diene provides a marker of the early stages of lipid peroxidation (Halliwell & Gutteridge, 1985). The change of conjugated diene values in samples treated with/without antioxidants during frozen storage is shown in Fig. 3. Duration of storage had a significant effect on conjugated dienes of samples. The conjugated dienes increased from 6.91 to 11.67 nmol hydro peroxides per kilogram lipid in the control samples, 6.63 to 10.90 nmol hydro peroxides per kg lipid in PSE-treated samples and 6.56–10.57 nmol hydro peroxides per kilogram lipid in GSE-treated samples after 3 months of storage. The conjugated dienes increased significantly over 2 months of storage ($P < 0.01$). GSE- and PSE-treated samples had lower diene values than control samples, and diene values of control samples were significantly higher than extract-treated samples at the end of storage ($P < 0.01$). Similar results were reported by Sánchez-Alonso *et al.* (2007, 2008) that control minced horse mackerel samples showed a high increase in the development of conjugated dienes while grape dietary fibre added samples showed a smaller increase during 6 months of frozen storage at −20°C.

Table 2 shows the per cent of inhibition on the formation of diene hydroperoxides and malondialdehydes. Formation of TBARS was inhibited by 74.08% with PSE and by 57.43% with GSE. In the case of conjugated dienes, the percentage of inhibition was higher in GSE-treated samples than PSE one. The development of conjugated dienes was inhibited by 6.67% with PSE and by 9.44% with GSE. Higher

Table 2 Percentage of inhibition[a] of formation of conjugated dienes and malondialdehydes in minced fish muscle (means ± SD)

Sample	Dienes (nmol hydroperoxide per kg lipid) Month 3	Thiobarbituric acid-reactive substances (nmol MA per g meat) Month 3
Pomegranate seed extract	6.67 ± 0.99(ns)	74.08 ± 0.36A
Grape seed extract	9.44 ± 0.45(ns)	57.43 ± 2.68B

[a]$\%I = [(c-s)/c]*100$, where c = high increment of control in the experiment and s = increment of sample with added different extracts at the same time.
[A,B]Different letters in the same column indicate significant differences ($P < 0.05$).
ns, not significant.

antioxidant activity and lower inhibition rate of TBARS in GSE added samples may be attributed to different phenolic compositions of plant extracts or antioxidative mechanism (Majsood & Benjakul, 2010). The inhibition of formation of malondialdehydes was higher than that of hydroperoxides, the same effect has been described by Decker *et al.* (2005) and Sánchez-Alonso *et al.* (2007). In a previous study, TBARS inhibition values in samples with 2% and 4% grape antioxidant dietary fibres (GADF) at 90 days of frozen storage were 47.5% and 70.1%, respectively. The rate of conjugated dienes inhibition at 90 days of storage was 18.2% in samples with 2% GADF and 26.7% in samples with 4% GADF (Sánchez-Alonso *et al.*, 2008). In the cooked chicken hamburgers, the rate of TBARS inhibition at 5 days of storage was 13% for the 1% GADF sample while this index was 26% for the samples with 2% added antioxidant fibre (Sáyago-Ayerdi *et al.*, 2009).

Changes in colour

Table 3 shows the effects of colour alteration by the addition of PSE and GSE in minced chub mackerel muscle. The lightness (L*) values of samples treated with GSE were significantly ($P < 0.01$) lower than those of the other samples. GSE-treated samples had higher a* values than those of the other samples. Addition of PSE resulted in significantly high b* values ($P < 0.01$). During frozen storage, there was a decrease in L*, a* and b* values. Losses of redness, lightness and yellowness of the samples with control and PSE samples during storage could be attributed to lipid oxidation. The addition of GSE showed a noticeable increase in C* compared with other groups. Significant decreases in b* and C* values were observed during frozen storage ($P < 0.01$). It is well known that fatty fish contain oxidation catalysts such as haemoglobin, an activator of lipid oxidation (Undeland *et al.*, 1998). The degree of colour change depends on the colour and level of extract (Luther *et al.*, 2007). Many plant extracts have a dark colour and may effect the visual perception of final products. It should be noted that some other factors could have an effect on colour parameters such as fineness of mincing and consequently surface reflection properties (Naveena *et al.*, 2008b).

Table 3 Effects of grape and pomegranate seed extracts on colour values of minced fish muscles during frozen storage

Colour values	Storage time (months)	Treatments			
		Control	Grape seed extract	Pomegranate seed extract	Average
L*	0	46.28 ± 3.55	41.47 ± 0.94	44.01 ± 1.20	43.93 ± 1.33A
	1	48.99 ± 0.16	42.94 ± 0.83	46.81 ± 0.43	46.24 ± 1.15A
	2	37.14 ± 1.72	33.46 ± 0.65	35.73 ± 0.77	35.44 ± 0.85B
	3	37.55 ± 1.62	32.36 ± 0.81	35.35 ± 0.83	35.08 ± 1.08B
Average		42.49 ± 2.14a	40.47 ± 1.93ab	37.55 ± 1.80b	
a*	0	4.80 ± 0.74	9.77 ± 0.92	4.39 ± 0.02	6.32 ± 1.14A
	1	4.38 ± 0.25	10.14 ± 0.33	4.45 ± 0.05	6.32 ± 1.21A
	2	3.17 ± 0.18	6.48 ± 0.51	2.85 ± 0.19	4.17 ± 0.75B
	3	3.12 ± 0.16	6.36 ± 0.42	2.79 ± 0.02	4.09 ± 0.73B
Average		3.87 ± 0.32b	3.62 ± 0.31b	8.19 ± 0.71a	
b*	0	8.09 ± 1.63	9.54 ± 1.21	12.34 ± 1.06	9.99 ± 0.99A
	1	9.58 ± 0.05	10.53 ± 0.33	13.48 ± 0.05	11.19 ± 0.75A
	2	6.76 ± 0.04	6.65 ± 0.34	8.46 ± 0.11	7.29 ± 0.38B
	3	6.97 ± 0.14	6.51 ± 0.44	8.65 ± 0.28	7.38 ± 0.43B
Average		7.85 ± 0.52b	10.73 ± 0.86a	8.31 ± 0.72b	
C	0	9.43 ± 1.77	13.66 ± 1.51	13.10 ± 0.99	12.06 ± 1.06A
	1	10.54 ± 0.15	14.62 ± 0.49	14.20 ± 0.03	13.19 ± 0.83A
	2	7.48 ± 0.11	9.29 ± 0.59	8.93 ± 0.17	8.57 ± 0.39B
	3	7.66 ± 0.06	9.13 ± 0.59	9.09 ± 0.27	8.63 ± 0.35B
Average		8.77 ± 0.59b	11.33 ± 0.91a	11.67 ± 1.00a	
h°	0	59.21 ± 1.17	44.17 ± 0.95	70.34 ± 1.61	57.91 ± 4.83B
	1	65.36 ± 1.09	46.10 ± 0.08	71.72 ± 0.27	61.06 ± 4.88A
	2	64.86 ± 1.08	45.84 ± 0.77	71.26 ± 1.03	60.65 ± 4.85A
	3	66.07 ± 1.49	46.12 ± 0.43	72.04 ± 0.60	61.41 ± 4.97A
Average		63.88 ± 1.13b	71.34 ± 0.45a	45.55 ± 0.39c	

[A,B]Different letters in the same column indicate significant differences ($P < 0.01$).
[a,b]Different letters in the same line indicate significant differences ($P < 0.01$).

Conclusion

Natural plant extracts have substantial amount of phenolic compounds. These natural antioxidants may stabilise food products, particularly fatty fish muscles, by reducing lipid oxidation during frozen storage. The present study clearly demonstrated that grape seed and pomegranate seed extracts affected the stabilisation of lipids in minced chub mackerel muscle during frozen storage. These results indicate that grape seed and pomegranate seed could be used as an antioxidant source to prevent oxidation in minced mackerel muscle during frozen storage. The rate of lipid oxidation and the amount of oxidation products formed depend on antioxidant source. The food industry should take a note to use various plant extracts as a potential source of phenolics. Plant extracts can be used instead of synthetic antioxidants.

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