www.trjfas.org
ISSN 1303-2712
Turkish Journal of Fisheries and Aquatic Sciences 17:1397-1403(2017) DOI: 10.4194/1303-2712-v17_6_33

RESEARCH PAPER

Prolonged Fish Lipid Stability with Albedo Fragments of Bitter Orange

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Received 13 December 2016
Accepted 12 April 2017

Abstract

The influence of albedo extracts of bitter orange peel on the oxidation of fish lipids was evaluated. The ethanol extracts of albedo fragments of bitter orange were added into fish lipid in the concentrations of 0, 0.5, 1.0, 2.0, 5.0 and 10.0mg/g and samples were stored at 25°C. The phenolic content and antioxidant activity determined in albedo fragments of bitter orange were 8.31±0.00 gGAE/100g and 0.537±0.004 µM trolox, respectively. 1.0 mg/g and 2.0 mg/g extract treated lipid samples were still in the range of good quality after the storage period by means of peroxide value. The lowest para-anisidine levels were recorded with the extract treatments of 2.0 mg/g, 1.0 mg/g and 0.5 mg/g with the findings 17.51±0.16, 17.81±0.05 and 18.68±0.28, respectively. The highest TBARS values were recorded in control samples (7.24±0.81 mg MDA/kg), whereas the lowest values were determined in fish lipids treated with 2.0 mg/g extracts (5.11±0.02 mg MDA/kg) at the end of the storage. The increase in the concentration of the extracts such as 5.0 and 10.0 mg/g had negative effect on oxidation stability. The choosing of appropriate antioxidant concentration is important in suppressing lipid oxidation.The most successful results were obtained with 1.0 mg/g and 2.0 mg/g extracts.

Keywords: Bitter Orange, albedo, fish oil, oxidation.

Introduction

Revealing the positive health effects of flavonoids such as reducing the risk of coronary heart disease and protection of lipid membrane from oxidation, enhanced the appeal of natural antioxidants (Plumb, Pascual-Teresa, Santos-Buelga, Rivas-Gonzalo & Williamson, 2002; Kumaran & Karunakaran, 2006). The total phenolic content and antioxidant activity of the parts of citrus such as fruit, juice, seed, peel and pulp were studied by many researchers (Bocco, Cuvelier, Richard & Berset, 1998; Mantley & Grohmann 2001; Jeong *et al*., 2004; Tumbas *et al*., 2010; Barreca, Bellocco, Caristi, Leuzzi & Gattuso, 2011).

Bitter orange (*Citrus aurantium*) is a hybrid citrus species between pomelo (*Citrus maxima*) and mandarin (*Citrus reticulata*). They are mostly used for their essential oil for making up of perfume, flavoring and solvent. Also, marmelade and jam of the bitter orange is preferred in food industry. Bitter orange is a demanded fruit with its content of bioactive compounds such as sineprin alcholoids, limonoids, fytosterols and flavanoids including hesperidin, neohesperidin, naringin and tangeretin (Barreca *et al*., 2011; Peixoto *et al*., 2012). Karoui, Wannes and Marzouk (2010) refined corn oil aromatization by bitter orange and reported that hydrocarbon compounds were retained on lipid matrix with a large contribution of monoterpene hydrocarbons such as limonene, α-pinene, ß-pinene, sabinene and α-terpinene ranging from 96% to 99%. The peels of ripened bitter orange has 2.4-2.8% neohesperidin, whereas unripened bitter orange has 14% which can be used as artificial sweetener (Morton, 1987).

The antioxidative activity of citrus extracts and their effects on stabilization of lipid oxidation is a focused the attention of researchers. Refined corn oil was protected from lipid oxidation with the tereatments of citrus peels extracted in various solvents. The best results were obtained with methanolic extract (Rehman, 2006). Bitter orange peel extract was applied to refined corn oil (Karoui *et al*., 2010). It was reported that the results of total phenolic content and antioxidant activity are higher in the de-fatted peel extracts of two kinds of orange species (Abd El-aal & Halaweish, 2010).

Bitter orange is too sour to be popular for eating. Flavonoids which do not have taste are called

hesperidin while limonene causes the bitter taste. Bitter orange is a wild culture of citrus and its fruits are not collected often from the branches, drop on the ground and left to rot. The peel of the citrus is composed of two fragments; the inner part of the citrus fruit is called albedo (inner white layer of the peel) and the external part of the fruit is flavedo (outer colored part of the peel). Essential oil relative to the skin, especially the flavedo fragment and leaves have a very characteristic pleasant odor and used in industrial areas (Moufida & Morzouk, 2003). The albedo fragment is regarded as waste. It is important to evaluate the waste part of citrus fruits in order to regain the worthy compounds, evaluate as natural antioxidant sources, contribute to economy, protection of environment. In this research, it was aimed to extend the fish lipid stability with the treatments of different concentrations of bitter orange peel albedo extracts.

Materials and Methods

Bitter orange (*Citrus aurantium*) was collected from the citrus trees located in the campus of Akdeniz University. Fish oil was obtained from a commercial firm (Ari Muhendislik, Ankara, Turkey). The initial quality parameters of refined fish oil were 0.75 mg KOH/g acid value, 275 g/100 g iodine number and 8.0 total oxidation value.

Extraction

The albedo fragments of the bitter orange were peeled manually and dried at 40°C for 17 h in an oven. Dried tissues were grinded into small pieces and were dissolved in ethanol:water 1:4 v:v (1:10 w:v). The solutions were first held in ultrasonic bath for 15 min at 40°C, and then extracted in a water bath with shaker at 40°C for 1 h 45 min. The extract was filtered and concentrated in a rotary evaporator at 40°C to get crude extracts. The dry matter of the crude extract was 61.61±0.32 g.

Treatment

Bitter orange albedo extracts were added into the fish lipids in the ratios of 0.5, 1.0, 2.0, 5.0 and 10.0 mg/g. The samples without extract was regarded as control group. The mixtures were pipetted (2ml) into eppendorf tubes and left in an incubator adjusted to 25°C. Analyses were performed weekly in order to follow the alteration of lipid oxidation. All assays were conducted on duplicate samples for each replication.

Analyses

Total phenolic content of bitter orange albedo extract

Total phenolic contents of the extracts were determined spectrophotometrically according to the Folin-Ciocalteu colorimetric method (Spanos & Wrolstad, 1990). Each extract of 0.1 ml was introduced to 5 ml Folin-Ciocalteu's reagent (0.2 N), 4 ml sodium carbonate (7.5 g/L) and 0.9ml distilled water. The mixture was allowed to stand for 2 hours before absorbance measurement against blank at 765 nm (Shimadzu UV 160A, Tokyo, Japan). Results were expressed as mg gallic acid equivalent (GAE)/100g of extract.

Antioxidant activity of bitter orange albedo extract

The antioxidant capacity of citrus peel extracts was determined by measuring the formation of the radical cation ABTS, according to the photometric method of Re *et al*. (1999). 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS•+) radical cations were prepared by adding 7 mM ABTS reagent including 2.45 Mm potassium persulphate and allowed to stand 12-16 hours at room temperature in darkness. The radical was adjusted to 0.700±0.02 at734 nm on each spectrophotometric measurement with ethanol. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), a water soluble analog of vitamin E, was used as an antioxidant standard. Diluted ABTS•+ solution (1.0ml) and increasing aliquots of plant extracts (10-30µL) were reacted for 6 min. The percentages of inhibitions at 734 nm were calculated as proportioning the slope of the curves. The results were expressed in mmol Trolox equivalents (mmol TE) as means of three consecutive measurements of the same sample.

Lipid oxidation analyses

Peroxide value was determined according to American Oil Chemists' Society (1985). The lipid samples were dissolved in glacial acetic acid: chloroform (3/2 v/v) and 1 ml KI solution (14 g KI/10ml distilled water) was added. The mixture was titrated against 0.01 N sodium thiosulphate with the precence of starch as an indicator. The result was calculated as;

$$PV\ (meq/kg) = (V\text{-}B \times Nf / W) \times 1000$$

V= spent thiosulphate, B= spent thiosulphate for the blank, W= weight of the sample (g), Nf= the factor for sodium thiosulphate

Thiobarbituric acid content determination was done according to AOCS (1998). The spectrophotometric determinations of thiobarbituric acid content were performed depending on the principle of colorization of malondialdhyde present in the lipids with TBA reagent. Lipid solved in n-buthanol was mixed with the same amount of TBA reagent. The absorbance of the samples were recorded at 530 nm after incubation at 95°C for 120 min in

water bath. Results were calculated as;

TBARS (mg MDA/ kg) = 50 x (The absorbance of lipid- The absorbance of blank) / sample weight (mg)

Para-anisidine value was determined by IUPAC method (1987a). Lipid sample was dissolved in n-hexane and the absorbance of the mixture was measured at 350 nm by a UV–Vis spectrophotometer (A1). Para-anisidine reagent (1 mL) was added to 5 mL of the mixture and held in dark for 10 min before absorbance reading (A2) at 350 nm. The result was calculated as p-Av = 25 (1.2 A2–A1) / m, where 'm' represents mass of sample oil (g).

UV spectrum was also determined by the method of IUPAC (International Union of Pure and Applied Chemistry) (1987b). Oil samples were dissolved in iso-octane and the value of absorptivity at 232 nm (K232-conjugated dienes) and 270 nm (K270-conjugated trienes) was measured by spectrophotometer.

All oxidation assays were conducted on duplicate samples of homogenates. Analysis was conducted using the SAS software (Statistical Analysis System, Cary, NC, USA). When main effects or interactions were significant, Duncan's multiple range test was applied.

Results and Discussion

The phenolic content and antioxidant activity determined in albedo fragments of bitter orange were 8.31±0.00 g GAE/100g and 0.537±0.004 µM trolox, respectively. The total phenolic content of bitter orange (*C. aurantium* L.) was found 0.51 mg GAE/100g dietary fiber (Garau, Simal, Rossello & Femenia, 2007). The antioxidant activity of enzyme maseration applied ripened bitter orange albedo fragments were 1276 µM trolox/g extract arising from ascorbic acid, naringin and other organic compounds content (Zapata, Montoya, Cavalitto, Hours & Rojano, 2012). Differences in extraction parameters such as time, temperature, used solvent and ultrasound assistance, ripening levels of the raw material and the method for antioxidant activity determination cause differences in the findings of the researches.

Peroxide value of the lipid samples increased gradually (Figure 1). Fish lipids treated with 1.0 mg/g and 2.0 mg/g had low levels of peroxide at the end of the storage period with the results of 4.78±0.59 and 4.49±0.13, respectively ($P<0.01$). It was reported that peroxide value of good quality fish lipid should be less than 5 (Hamilton, Kalu, McNeill, Padley & Pierce, 1998). The lipid samples of 1.0 mg/g and 2.0 mg/g were still in the range of good quality after the storage period. High extract concentrations such as 5.0 mg/g and 10.0 mg/g were ineffective in protecting the fish lipid. Moreover, it seems that 0.5 mg/g bitter orange albedo extract was not enough to inhibit the oxidation. The peroxide values for those samples were close to the peroxide values of control samples. Rehman (2006) used citrus peel methanol extracts in the concentrations of 1.200 ppm, 1.600 ppm and 2.000 ppm to protect corn oil from oxidation. Peroxide value reached to 4.8±0.17 meq/kg, 3.1±0.11 meq/kg and 2.5±0.2 meq/kg, respectively at the end of two months at 24°C. The results of the study showed that the increase in the concentration had better protection against oxidation. Similar findings were reported by Abd El-aal and Halaweish (2010) for soybean oil treated with different concentrations (400, 800, 1.200 and 1.600 ppm) of orange peel ethanol extract. The findings of our study supports this phenomenon up to a point. It is obvious that high concentrations (5.0 and 10.0 mg/g) of the citrus peel albedo extracts had solubility problem or had pro-oxidative effect on lipids. It was reported that some antioxidants may have pro-oxidant activity under certain conditions such as high doses or in the presence of metal ions. O-cumaric acid, p-cumaric acid and m-cumaric acid which present in bitter orange are reported to have pro-oxidant effects (Yordi, Perez, Matos & Villares, 2012).

The p-anisidine test is often used in conjunction with the peroxide analysis to calculate the so-called total oxidation value or totox value (Labrinea, Thomaidis & Georgiou, 2001). Para-anisidine value essentially reflects how the lipid has been handled and stored versus peroxide value, which measures current oxidation. The changes of para-anisidine values of the fish lipids during storage can be seen from Figure 2. Para-anisidine value revealed that the increase in the concentration of the extracts ensures the lipid stability against oxidation, except for high concentrations such as 5.0mg/g and 10.0 mg/g. The lowest levels were recorded with the extract treatments of 2.0 mg/g, 1.0 mg/g and 0.5 mg/g with the findings such as 17.51±0.16, 17.81±0.05 and 18.68±0.28, respectively. The extract treatment of 1.0 mg/g and 2.0 mg/g concentrations were significantly different from the other treatments ($P<0.01$) at the end of the storage. Those lipids were in the range of good quality referring to the limit of 20 as an acceptable level (Gokoglu, Topuz, Buyukbenli & Yerlikaya, 2012a). The level of para-anisidine value of refined soybean oil was recorded as 5.97 with the 1.200 ppm orange peel extract and 5.61 in the 1.600 ppm extract treatments after incubation at 65°C for 1 week. These findings are reported to be better than both control group and synthetic antioxidant applied samples (Abd El-aal & Halaweish 2010).

TBARS value is the indicator of secondary oxidation products. The alteration of primary oxidation products resulted in enhancement in secondary oxidation products which can be follow up by determination of TBARS levels. The alteration of TBARS values of the lipids can be seen from Figure 3. The highest TBARS values were recorded in control samples (7.24±0.81 mg MDA/kg), whereas the lowest values were determined in fish lipids

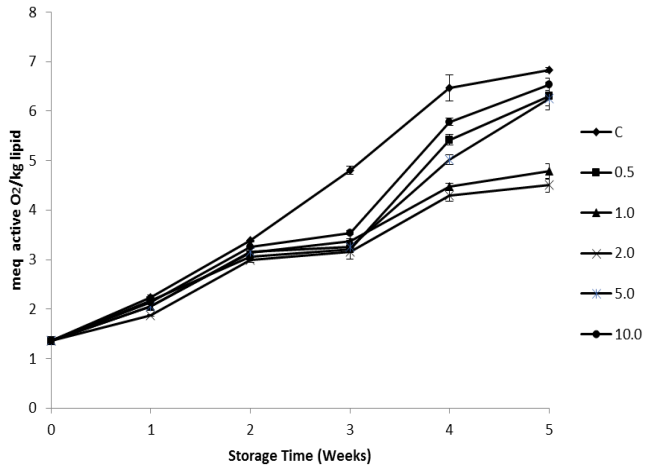

Figure 1. Peroxide values of the fish lipids treated with bitter orange albedo extracts.

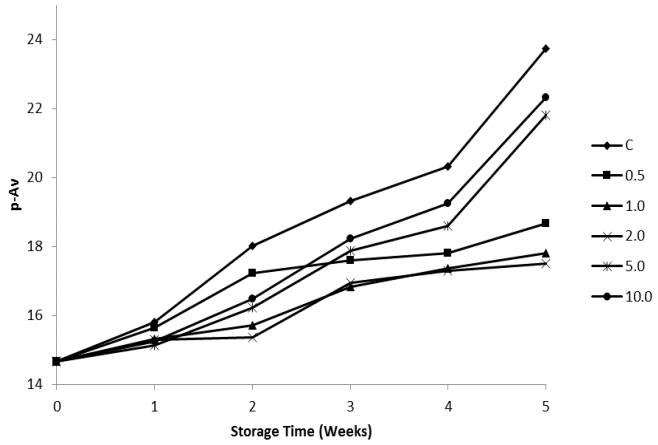

Figure 2. Para-anisidine values of the fish lipids treated with bitter orange albedo extracts.

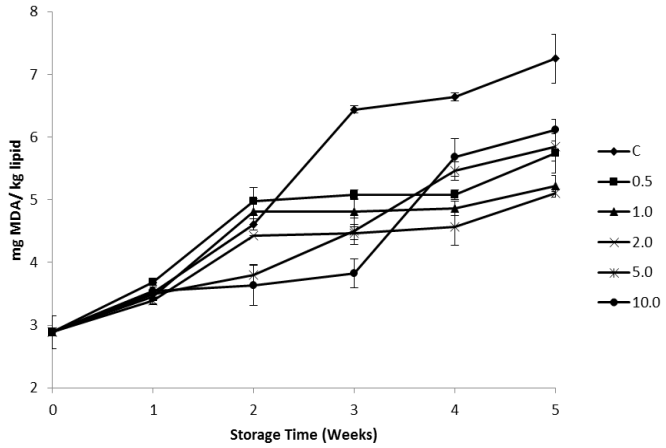

Figure 3. TBARS values of the fish lipids treated with bitter orange albedo extracts.

treated with 2.0 mg/g bitter orange albedo extracts (5.11±0.02 mg MDA/kg) at the end of the storage. The samples of 10.0 mg/g extract treatment were in good condition up to 3rd week. However, the TBARS scores of those samples increased sharply on the last two weeks and reached to 6.10±0.18 mg MDA/kg. The extract concentrations of 1.0 mg/g and 2.0 mg/g were effective in retarding the increase of TBARS levels of fish lipids.

Measuring UV absorption of fish oil is a simple

and rapid method to monitor lipid oxidation. Natural occurrence of non-conjugated double bonds tends to change into conjugated double bonds just after formation of peroxides in unsaturated fatty acids (Chaijan, Benjakul, Visessanguan & Faustman, 2006). Measurement of the absorbance value of oil at 232 nm is a sensitive method in following the early stages of lipid oxidation under conditions in which hydroperoxides undergo little or no decomposition. Conjugated triene value is an index of secondary oxidation products and has strong UV absorption at 270 nm. UV absorbance values of the samples increased during storage period (Figure. 4a and Figure. 4b). As reported in the other oxidation parameters, the lowest levels were obtained with 1.0 mg/g and 2.0 mg/g orange peel albedo fragment extract, whereas the highest values were recorded for control samples. Iqbal and Bhanger (2007) stabilized sunflower oil by adding garlic acid extract and noted higher conjugated dienes in control samples. Lower absorbance values at 232 and 270 nm in the fish oil emulsion samples treated with tomato and garlic extracts were found compared with the control samples indicating the protective effect of plant extracts on lipid oxidation (Gokoglu *et al*., 2012b).

Conclusion

Protective effect of bitter orange peel albedo extract on lipid oxidation was proved by oxidation parameters. Most of the studies reported that the increase in the concentration of the natural extracts appears to be more effective. This phenomenon is true up to a point. In some cases, high concentrations cannot be as effective as lower concentrations like in this study due to some problems such as solubility of the plant extracts or their pro-oxidative effects. The point to be emphasized is choosing the right extract and the proper concentration.

Acknowledgements

The Scientific Research Projects Administration Unit of Akdeniz University supported this research.

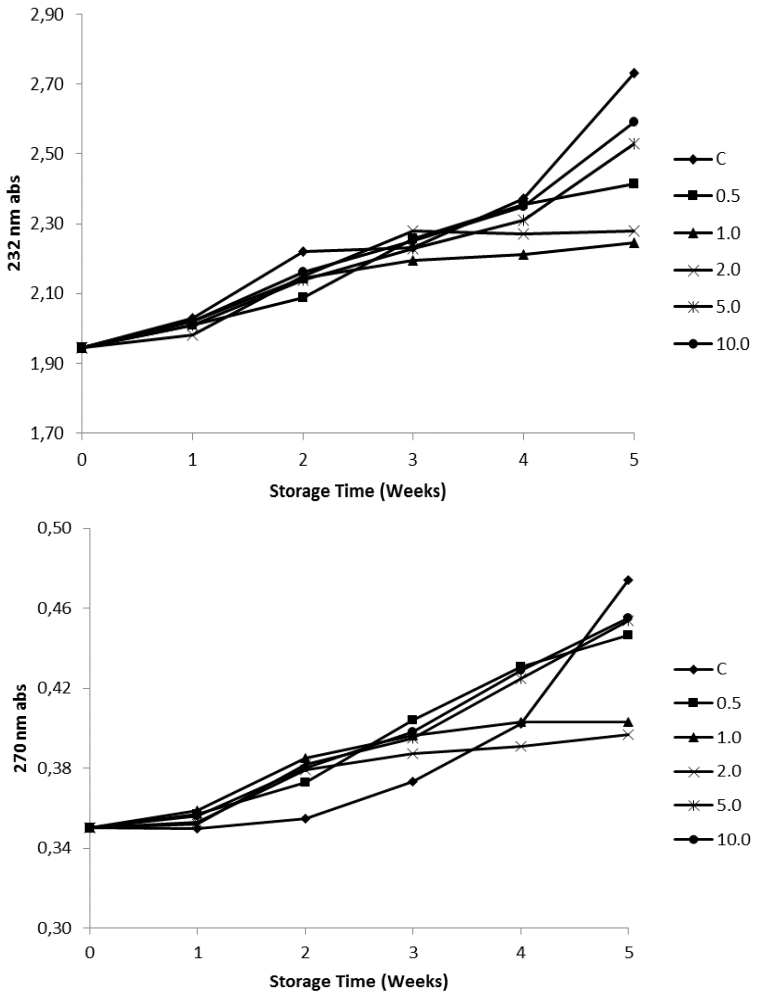

Figure 4. UV spectrum values of the fish lipids treated with bitter orange albedo extracts; (a) 232 nm conjugated-dienals and (b) 270 nm conjugated-trienals.

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