www.trjfas.org
ISSN 1303-2712
Turkish Journal of Fisheries and Aquatic Sciences 16: 729-737 (2016) DOI: 10.4194/1303-2712-v16_3_27

RESEARCH PAPER

Antioxidant Effects of Wild Pistacia (*P. atlantica*), Rosemary (*Rosmarinus officinalis* L.) and Green Tea Extracts on the Lipid Oxidation Rate of Fish Oil-in-Water Emulsions

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Received 05 December 2015
Accepted 27 May 2016

Abstract

The objective of this study was to investigate the effectiveness of plant extracts on the oxidative stability of n-3 rich fish oil emulsion. The antioxidant efficiency, water extracts of wild pistachio, rosemary and green tea, were evaluated in 10 % fish oil-in-water emulsions. The fish oil emulsion was oxidatively unstable as measured by fatty acid profile, peroxide and p-Anisidine values and sensory analysis. Pre-emulsification of the fish oil with fish gelatin in water offered some protection towards oxidation, but addition of the extracts (200, 500 and 700 ppm) significantly reduced oxidative changes. Higher concentration of the extracts showed an antioxidant tendency in 10% fish oil-in-water emulsion. Moreover, the results of the experiment demonstrated the potential of wild pistachio leaf extract as a useful antioxidant.

Keywords**:**Green tea, wild pistachio, oil-in-water emulsion, oxidative stability, fish gelatin.

Introduction

Throughout recent decades, an increasing attention has been paid to the n-3 long-chain fatty acids and their incorporation in processed foods (Nielsen and Jacobsen. 2009; Pourashouri, Shabanpour and Razavi. 2014). Several studies have recommended the usual dietary consumption of the fatty acids (Riediger, Othman and Suh. 2009; Bao, Hu and Zhang. 2011; Ganesan, Brothersen and Fau–McMahon. 2014; Nascimento, Magnani and Sousa. 2015). Due to the evidence-based health benefits of n-3 long-chain fatty acids, marine oil as a rich source of the fatty acids is highly sought-after among people who pursue healthier lifestyles. Marine oil could be used as an essential fatty acids supplement in food emulsions (Takeungwongtrakul and Benjakul 2013). Oil-in-water (O/W) emulsions are extensively used as model food matrices to study the effect of compositional and process factors on lipid oxidation (Berton, Ropers and Genot. 2014). However, these fatty acids are highly susceptible to oxidative deterioration, which is a major challenge to enrich food by omega-3 PUFA. Therefore, a prerequisite for successful development is prevention of lipid oxidation or at least delay as long as possible (Jacobsen, Bruni and Nielsen. 2008). Addition of antioxidants is the most effective way to control or prevent of lipid oxidation in oil rich food systems (Shahidi and Zhong. 2011). However, consumers look for foods with fewer artificial additives. Therefore, the research continues to obtain new natural antioxidants, especially from plant origin, as an alternative to synthetic antioxidants (SabeenaFarvin and Jacobsen. 2015).

Formulated foods often contain a lipid phase dispersed in an aqueous medium. This form schematically described as oil-in-water (O/W) emulsions, because of the lipid exists in the emulsion form instead of bulk form (Berton-Carabin, Ropers and Genot. 2014; SabeenaFarvin and Jacobsen. 2015). Lipid oxidation and antioxidant mechanisms in multiphase food systems are very complex and many factors could influence the efficacy of different antioxidants in such systems. So, it is currently difficult to predict the behavior and efficacy of antioxidants in different multiphase systems (Jacobsen, Bruni and Nielsen. 2008).

Natural plant extracts may play vital roles in the inhibition of oxidation processes and decomposition of hydroperoxides in food products as well as in living tissues (Taheri, Motallebi and Fazlara. 2012). Many plants can be a source of natural antioxidants (Aksu, Alinezhad and Erdemir. 2015). Various sources of plant and fruit extract have been known as a potent antioxidant sources as well as synthetic antioxidants. Extracts of the seeds, skin and stem of

grape and peel of fig have high antioxidant properties (Hernández-Hernández, Ponce-Alquicira and Jaramillo-Flores. 2009; Tuncel and Yılmaz. 2013; AksuAlinezhad and Erdemir. 2015; Doshi,Adsule and Banerjee. 2015). Green tea extract (GTE) has been known as an important source of bioactive compounds, mainly natural tea polyphenol antioxidants. Tea catechins are one of the major components of polyphenol antioxidants (Lante and Friso. 2013; Goh, Gao and Ananingsih. 2015). The GTE effect in lowering of lipid oxidation in emulsion systems has been studied previously (O'Dwyer, O'Beirne and Eidhin. 2012). It is reported that the use of the GTE more effectively decreased the formation of peroxides in n-3 rich table spreads than α-Tocopherol (O'Dwyer,O'Beirne andEidhin. 2012). Also, rosemary extracts (ROS) have a high antioxidant potential wich is rich of phenol diterpenes such as carnosic acid, carnosol and rosmarinic acid (Hernández-Hernández, Ponce-Alquicira and Jaramillo-Flores. 2009). Thus, according to this worthy character, ROS extract has been used to preserve meat and fish in food industry (Gao, Feng and Jiang. 2014).

The resiniferous pistachio tree belongs to *Pistacia*, from the *Anacardiaceae* family have been distributed in throughout the western, central, and eastern parts of Iran (Rezaie,Farhoosh and Iranshahi. 2015). Bene tree (*Pistaciaatlantica* subsp. mutica) products (e.g. fruit, gum and leaf) have been used for a variety of medicinal and nutritional purposes according to their antioxidant capacities and phytochemical contents. Bene or wild pistachio (WPS) extract has been derived from the dried leaves (Peksel. 2008; Benhammou, Bekkara and Panovska. 2008; Gourine, Bombarda and Nadjemi. 2009). The leaves of *Pistaciaatlantica* have an excellent antioxidant activity and its essential fatty acids content is about 3-11 times more active than ascorbic acid. Also, its oil is rich in monoterpenes and oxygenated sesquiterpenes (GourineBombarda and Nadjemi. 2009). Furthermore, gallic acid, catechin, epicatechin, and gallic acid methyl ester were identified in leaves and galls of *P. atlantica*(Bozorgi,Memariani and Mobli. 2013). Thus, a mixture of fish oil emulsion containing fish gelatin and the plant extracts may be useful to enrich the foods with n-3 long-chain fatty acids and natural antioxidants, meanwhile, prevent rancidity in food industry. The objective of this study was to compare the antioxidant potential of the rosemary, wild pistachio and green tea in fish oil-in-water emulsion model system.

Materials and Methods

Chemicals

Cod (*Gadusmorhua*) liver oil and fish gelatin (gelatin from cold water fish skin), were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Other analytical-grade chemicals and reagents were purchased from Merck (Darmstadt, Germany).

Preparation of the Plant Extract

Air-dried aerial materials were partially smashed, manually before extraction. In preparation of decoction, 20 g of dried leaves boiled in 100 °C for 15 min with about 200 ml of twice-distilled water. The extract was rapidly filtered through a linen cloth and reduced in volume on a rotary evaporator (IKA, Germany). The extract stored at 4 ºC (Peksel. 2008). The freeze-dried extracts were used for antioxidant assays (Seo, Lee, Kim. 2012).

Total Phenolic Contents (TPC)

The TPC of the extracts was determined following the method of Seo *et al*., (2012). 1 mL of the diluted extract was mixed with 1 mL of 2% Na_2CO_3 and 1 mL of 50% Folin-Ciocalteu reagent, and centrifuged at 13,400×g for 5 min. After 30 min incubation at room temperature, the absorbance was measured at 750 nm. The TPC were expressed as gallic acid equivalents (GAE).

DPPH Radical Scavenging Activity

The DPPH radical scavenging activity of the extracts was determined according to the method of Seo *et al*., (2012). The extracts diluted with DMSO, and then 0.1 mL of each extract was mixed with 0.9 mL of 0.041 mM DPPH in ethanol for 30 min. The absorbance of the sample was measured at 517 nm. Radical scavenging activity was expressed as percentage.

$$\% \text{ DPPH radical scavenging activity} = [1-(A_{sample}/A_{control})] \times 100$$

Preparation of O/W Emulsion

Oil-in-water emulsion was prepared following the method of Pourashouri *et al*., (2014). Briefly, aqueous solutions of wall materials (e.g. fish gelatin and maltodextrin) were prepared by dispersing them in distilled water and keeping the solution in a shaking water bath (Memmert, GmbH, Schwabach, Germany) overnight to warrant hydration of the polymer molecules. Total concentration of dissolved solid was 40 % (w/v). Also, the plant extracts were added to obtain ten experimental treatments without any plant extract (as control) or with 200, 500 and 700 ppm of the each rosemary, wild pistachio and green tea extract. Then, 10 % fish oil was progressively added to the continuous phase during pre-emulsion preparation and stirred for 10 min by a laboratory mixer. During the latest step, the emulsions were kept in a beaker and submerged in a water/ice mixture. The

emulsions were then emulsified using a 22-mm-diameter ULTRA-TURRAX homogenizer (T25 Digital IKA-Werke Stuttgart Staufen, Germany) that operated at a speed of 13,500 rpm for totally 5 min (Takeungwongtrakul and Benjakul. 2012). In optimized emulsion, after ensuring their physical stability, the effect of antioxidant potential of plant extracts on lipid oxidation was studied under incubation conditions.

Emulsion Stability

Each emulsion (10 ml) was placed in a test tube and stored at ambient temperature (20 °C) for 1 month in replicating. During this period, a daily evaluation to assess the depths (centimeter units) of a distinctive clear serum lower phase was achieved. The results were expressed as a creaming index (%) of total emulsion height in the tubes (Klaypradit and Huang. 2008).

Fatty Acid Composition in Fish Oil and Emulsions

Fatty acid methyl esters of samples were prepared according to the method of Metcalfe and Schmitz (1961) using boron-trifluride in methanol. The fatty acid composition was then analyzed by gas chromatography (Hewlett Packard 5890 series II, Ramsey, MN, USA) equipped with a flame ionization detector and a fused silica capillary (25 m×0.2 mm, film thickness, BPX70 SGE Australia Pty. Ltd., analytical products, Unicam 4600 gas chromatograph, England, UK). Operating conditions were as follows: temperature injection port, 250 °C, detector temperature, 300 °C, oven programmed from 160 to 200 °C at 20 °C/min. Helium was employed as carrier gas.

Oxidative Stability of Emulsions

In order to investigate the lipid oxidation in the 10% fish oil-in-water emulsions, primary and secondary oxidation products were measured, with their physical stability. Emulsions containing various plant extracts were transferred into the amber bottle and capped tightly. Sample without extracts incorporated was used as the control. The samples were stored at 30°C and were taken randomly for analyses on day 0, 2, 4, 6, 8, 10 and 12.

Peroxide Value

Peroxide value (PV) was determined according to the method of Hu *et al*. (2003) with slight modifications. The emulsion samples were separated into two phases; two ml of chloroform/methanol (2:1, v/v) were added to 1 ml of the emulsion sample and mixed using a vortex mixer. Then, 50 µL of 30 % ammonium thiocyanate (w/v) and 50 µL of 20 mM ferrous chloride solution in 3.5 % HCl (w/v) added to the organic solvent phase. After 20 min, the absorbance of the colored solution was read at 500 nm using a spectrophotometer (Biochrom, Libra s12, England). The PV was calculated and expressed as mg cumenehydroperoxide/ liter of the emulsions. A standard curve was prepared using cumenehydroperoxide with the concentration range of 0.5-2 ppm.

P-Anisidine Values

An adaptation of the IUPAC Method No. 2.504 was used to analyze p-Anisidine values (p-Av). The emulsions were destabilized: 2 ml ethanol was added, then 5 ml iso-octane. Tubes were capped, vortexed for 10 s, and centrifuged for 10 min at 4000 rpm, at 15 ◦C. Absorbance of the solution was measured. Analysis was carried out in triplicate, in duplicate samples (O'Dwyer, O'Beirne and Eidhin. 2012).

Sensory Evaluation

The sensory panel consisted of five persons (3 female/2 male; aged between 20 and 35 years) trained in sensory analysis. Prior to sensory analysis, the panelists trained with respect to the sensory assessment of oil. The odor attributes identified for fresh and stored fish oil. 10 ml of bulk fish oil or fish oil emulsion were evaluated in odorless, blue sensory glasses, covered with plastic Petri dishes. The panelists were asked to open sensory glass and to subsequently uncap the glass and sniff the headspace of the sample with short sniffs and to replace the cover of the glass quickly. The panelists cleared their nasal passage between samples by 1 min resting. The intensities of the rancidity odor attributes were evaluated by an intensity scale (0–5); where 0 and 5 represent no rancidity and the strongest rancidity, respectively. The Sensory factor was assessed at day 0, 4, 8 and 12 (Serfert, Drusch and Schwarz. 2010; Yarnpakdee, Benjakul and Nalinanon. 2012).

Statistical Analysis

The experiment arranged in completely randomized design and analysis of the data was carried out by analysis of variance using SPSS statistical software (version 16.0 for windows, SPSS, Chicago, IL, USA). The values of various samples were compared to each other on each particular day tested during storage. Each particular sample was also compared as storage time increased. Comparison among means were made using the Duncan's multiple range analysis at $P<0.05$. Statistical analysis was carried out on triplicate samples in duplicate, on each day tested after storage.

Results and Discussions

TPC of the Extracts

Phenols are important constituents of plant extract; because of their radical scavenging ability. The amount of phenolic compounds may contribute directly to the antioxidative effect (Bidchol *et al*., 2011). The amount of TPC determined in extracts has shown in Table 1. A significant difference has been found between total phenol contents of the extracts ($P<0.05$). The highest amount of total phenols was given by GTE and WPS, while ROS had the least total phenolic content. The results are in agreement with the results of antioxidant activity assay. A correlation was found between phenolic contents and antioxidant activities, this fact was also observed by Chahmi *et al* (2015). Due to differences in plant types, concentrations, extraction and drying method, solvent polarity and solubility of components, each extract acts differently from others and have a significant effect on total phenols (Anwar, Kalsoom and Sultana. 2013).

DPPH Radical Scavenging Activity of the Extracts

The antioxidant activity of the extracts was assessed, employing established in vitro systems, such as 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity and total phenolic compounds. The DPPH radical scavenging activity of the extracts and L-ascorbic acid (a positive control) were shown in Figure 1. The antioxidant compounds in plants have different polarities. The antioxidant activity depends on the selected solvent (Water, methanol, ethanol, and acetone) in extraction processes (Aksoy, Kolay, Agilonu. 2013; Seo, Lee, Kim. 2012). Freeze-dried aqueous extract of GTE, WPS, and ROS showed notable inhibitory effects. Results (Figure 1) showed that GTE and WPS extracts had the highest scavenging activity. SEO *et al*., (2014) has reported preparation method of extracts might affect DPPH radical activity. Most concentrations of GTE and WPS extract used equally showed higher scavenging activity than ascorbic acid; which means that increasing the concentration of the extract increase its scavenging activity. Lower concentration of ROS extract did not increase the radical scavenging activity.

Emulsion Stability

The O/W emulsion stabilized with fish gelatin by low creaming index values. The embedded fish oil in protein particles was stable enough without any coalescence or flocculation. The protein in the emulsion is an agent to prevent oxidation (Hu, McClements and Decker. 2003). Gelatin as a surface active could be used as an emulsifier in the O/W emulsions (Karim and Bhat. 2009).

Fatty Acids Composition

Fatty acid analysis was considered according to the composition of saturated (SFA), monounsaturated (MUFA), and polyunsaturated (PUFA) fatty acids (Table 2). The most fatty acids in starting fish oil were C18:1n9 and C16:0, followed by C20:5n3 and C22:6n3. The higher levels of MUFA and PUFA groups were observed in the oil than the SFA (Table 1). During the storage (at 30 ◦C) period, the control treatment showed markedly higher values of SFA than in their counterparts corresponding to plant extracts, while MUFA and PUFA provided the opposite behavior (Table. 1). In this study, the type of plant extract had significant effect on fatty acid composition, while a previous study reported no effect on fatty acid composition of spread (O'Dwyer,

Table 1. Total phenolic content of green tea, rosemary and wild pistahio extract (unit:

Sample	Total phenol content
green tea	50.26 ± 2.5^{a}
rosemary	25.73 ± 4.6^{b}
wild pistahio	52.18 ± 1.84^{a}

Values in the same column followed by different letters are significantly different ($P<0.05$)

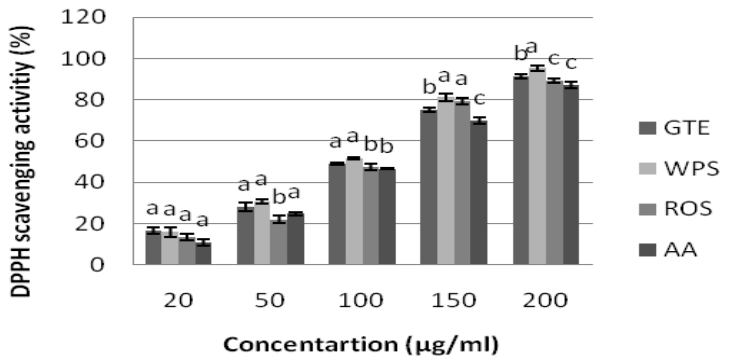

Figure 1. DPPH radical scavenging activities of extracts of green tea (GTE), wild pistachio (WPS), rosemary (ROS) and Ascorbic acid (AA).

O'Beirne and Eidhin. 2012).

Moreover, the results showed that fish oil emulsions containing WPS and GTE had significantly greater percentages of EPA and DHA compared with the control emulsion ($P<0.05$). After 12 days of storage, the EPA content of the GTE, WPS, and ROS emulsions respectively increased numerically about 11.74, 12.21 and 3.57 % when compared to the control emulsions. The DHA content of the GTE and WPS emulsions respectively increased 9.24 and 11.09 % than control treatment, while this value decreased in the ROS (0.61 %). Shen,Bhail and Sanguansri (2014) found that stabilized krill oil emulsions with fish gelatin or Maillard reaction products had high percentages of PUFA than bulk oil.

Oxidative Stability of fIsh Oil-in-Water Emulsion Without Plant Extracts

In this study, we used fish liver oil containing 40 % long chain n-3 PUFA and fish gelatin as an emulsifier to prepare the O/W emulsion. The results showed that the oxidative stability of control samples was 48 h at 30 °C, whereas the emulsion containing plant extracts were continued to remain stable after this time (Table 3). This observation proposed the

Table 2. Fatty acid compositions of starting fish oil and O/W emulsions containing various plant extracts

Fatty acid	Starting fish oil	Storage 12 days at 30°C			
		Emulsion (Control)	Emulsion+ GTE	Emulsion+ WPS	Emulsion+ ROS
C14:0	8.97	8.12	8.01	7.97	7.96
C16:0	17.51	19.32	17.01	17.42	17.32
C18:0	3.42	3.14	3.37	3.52	3.68
SFA	29.9	30.58^{a}	28.39^{e}	28.91^{d}	28.96^{c}
C16:1	9.94	8.15	9.23	8.52	8.69
C18:1	12.67	12.4	12.86	13.02	12.48
C20:1n	1.24	1.33	1.56	1.64	1.34
MUFA	23.85	21.88^{e}	23.65^{b}	23.18^{c}	22.51^{d}
C18:2n6	2.54	1.82	1.87	1.73	1.69
C18:3n6	1.04	0.79	0.87	0.99	0.86
C18:3n3	1.23	1.09	1.17	1.13	1.18
C20:3n3	4.01	5.12	4.56	4.58	4.47
C20:4n6	6.47	4.23	5.09	5.38	4.91
C20:5n3 (EPA)	13.52	10.64	11.89	11.94	11.02
C22:6n3(DHA)	11.45	9.73	10.63	10.81	9.67
PUFA	40.26	33.42^{e}	36.08^{c}	36.56^{b}	33.8^{d}

Total fatty acid (FA), saturated fatty acid (SFA), monounsaturated (MUFA), polyunsaturated (PUFA)
Values are based on all fatty acids detected. Results were carried out in triplicate, on duplicate samples.
a-e Different uppercase letters in the same row indicate significant differences ($P < 0.05$).
Control, no antioxidants; GTE, green tea extract; WPS, wild pistachio; ROS, rosemary

Table 3. Peroxide value of fish oil -in-water emulsion (O/W) containing various plant extracts during storage at 30°C

	Time (Day)						
Treatment	0	2	4	6	8	10	12
Emulsion(control)	± 0.27 ^{D} 1.92	± 0.08 $^{a, D}$ 5.94	±0.96 $^{a, E}$ 8.23	±1.14$^{a, C}$ 14.28	±$^{a, BC}$ 2.53 18.16	±3.84$^{a, B}$ 21.73	±$^{a, A}$1.31 27.39
+GTE 200	± 0.01 ^{C} 0.23	± 0.16$^{ab, BC}$ 0.70	±$^{ab, BC}$ 0.26 3.14	± 0.67$^{b, BC}$ 3.26	± 0.12 $^{b, AB}$ 5.61	$^{bc, A}$ 8.85±1.27	±$^{cd, A}$ 0.55 9.06
+GTE 500	± 0.003 ^{C} 0.06	± 0.001$^{b, C}$ 0.01	±0.05 $^{b, BC}$ 0.34	0.59^{b,} 1.58±^{ABC}	±$^{b, AB}$ 0.30 5.01	± 1.17$^{bcd, B}$ 5.42	±$^{cdf, A}$0.94 6.28
+GTE 700	± 0.006 0.02	± 0.003^{b} 0.01	± 0.02 ^{b} 0.39	2.23 ± 0.55^{b}	± 0.53 ^{b} 3.15	4.05 ±1.18^{cd}	±^{df} 1.04 4.10
+WPS 200	± 0.18 ^{B} 0.02	±0.63$^{b, B}$ 0.45	±0.45 $^{b, B}$ 1.17	± 1.40$^{b, AB}$ 5.00	± 0.49 $^{b, AB}$ 3.97	± 1.82$^{d, B}$ 2.73	±0.87 $^{cd, B}$ 8.93
+WPS 500	±0.13 0.02	0.33 ±0.30^{b}	0.63 ±^{b}0.25	1.28 ± 0.11^{b}	± 0.87 ^{b} 2.86	4.29 ± 0.31^{cd}	± 1.79^{ef} 3.09
+WPS 700	± 0.03 0.03	± 0.16^{b} 0.03	± 0.30 ^{b} 0.28	1.25 ± 0.20^{b}	± 0.17 ^{b} 2.22	0.48 ±0.21^{d}	± 0.41 ^{f} 2.71
+ROS 200	± 0.001 ^{C} 1.67	±0.18$^{ab, C}$ 2.27	± 0.14 $^{ab, C}$ 3.09	±0.69$^{a, B}$ 12.85	±$^{a, B}$ 1.21 13.20	± 0.22$^{a, A}$ 21.95	±2.02 $^{b, A}$ 21.33
+ROS 500	± 0.001 ^{C} 0.35	±0.26$^{ab, C}$ 1.68	± 0/12$^{b, C}$ 2.00	±0.25$^{b, BC}$ 3.99	±0.25$^{b, BC}$ 5.44	± 0.22$^{bc, AB}$ 8.75	±$^{c, A}$1.31 10.96
+ROS 700	± 0.01^{B} 0.18	±0.005$^{b, B}$ 0.55	± 0.06 $^{b, B}$ 1.83	±0.24$^{b, B}$ 2.59	± 0.33 $^{b, B}$ 4.04	±0.09$^{b, A}$ 8.91	±$^{cde, A}$0.43 8.38

high stability of fish oil-in-water emulsions improved by antioxidants. A previous study reported that protein as emulsifier could reduce or eliminate the need for antioxidants (Hu, McClements and Decker. 2003). Also, it's reported that emulsifying of fish oil by sodium caseinate protects it against oxidation. Casein by adsorbing into the oil droplets form a physical barrier and protect the oil droplets in the emulsion from air (Nielsen and Jacobsen. 2009). They suggested addition of the chelating agent to O/W emulsion decreased the energy bars of oxidative stability in comparison of energy bars in emulsified fish oil and bulk oil.

The Effect of Plant Extracts on Lipid Oxidation of O/W Emulsion System

Due to droplet surface, partitioning of emulsifier between lipid and aqueous phases, PUFA rich oils behaves differently when emulsified in water and there is controversy about the oxidative stability of O/W emulsions (Sorensen, Nielsen and Jacobsen. 2010). Thus, to evaluate lipid oxidation in n-3 oil-in-water emulsions, primary and secondary oxidation products were measured in the emulsion system with their physical stabilities. Hydroperoxides are intermediate products of the first stage of lipid oxidation; which is measured by peroxide value that is one of the most widely used tests for measuring the oxidation in oils and fats (Nascimento, Magnani and Sousa. 2015). The formation in the different emulsion systems is shown in Table 3. The content of peroxides increased during 12 days of the storage.

The highest oxidative stability was shown in the wild pistachio and green tea, followed by the rosemary containing emulsions. We observed that WPS and GTE were more effective in the oxidative stabilization of the fish oil-in-water emulsions than ROS at application levels of 500 and 700 ppm. The WPS (e.g. 500 and 700 ppm) containing n-3 HUFA rich oil-in-water emulsions exhibited pro-oxidative effects toward the formation of hydroperoxides, confirmed by the higher values of the intermediates from the beginning of the storage period (Table.3). In contrast, rosemary had significantly higher lipid hydroperoxide values. During the storage, the control and emulsions containing rosemary (e.g. 200 and 500 ppm) had significantly higher lipid hydroperoxide values than the other treatments (Table. 2). The ROS (e.g. 200 and 500 ppm) and GTE (e.g. 200 ppm) treatments did not have any significant effect on lipid hydroperoxide values. Addition of the low amounts of the plant extracts in emulsion increased primary lipid oxidation product formation, and had no effect on secondary lipid oxidation product formation.

At the end of storage period, pAvs generally were significantly lower at WPS treatments (e.g. 700 ppm) (Table. 4). In the present study, GTE and ROS at high concentration showed the highest efficacy in the retardation of pAvs in emulsion during the storage. The p-Aanisidine levels of the control treatment increased continuously ($P<0.05$), and reached the plateau at day 10. The rate of the secondary lipid oxidation products formation was lower than the primary ones.

Several researchers have studied the antioxidant properties of rosemary extract as *in-vitro* experiment (Almela, Sánchez-Muñoz and Fernández-López. 2006; Moreno,Scheyer and Romano, 2006; Upadhyay and Mishr. 2014). The antioxidant activity of oleoresin from rosemary and Sage (*Salvia Officinalis* L.) was evaluated, the sage showed significantly higher antioxidant activity than rosemary in sunflower oil. Our findings are consistent with the results of Almela*et al*. (2006) who studied the effects of plant extracts from different raw materials and found distilled rosemary had the lowest antioxidant activity. Concentration of phenol compounds (i.e. rosmarinic acid, carnosol and carnosic acid), extraction method and solvent could affect the antioxidant abilities of plant extracts (Hernández-Hernández *et al*. 2009). The literature confirmed that the natural plant extracts are sources of several antioxidative phytochemicals. In the current study, WPS was the most effective, spathulenol, camphene and p-cymene, gallic acid, catechin, epicatechin, and gallic acid methyl ester has been known as the active antioxidants in leaves essential oil of WPS (Benhammou, Bekkara and Panovska. 2008, Gourine, Bombarda and Nadjemi. 2009; BozorgiMemariani and Mobli. 2013).

Green tea extract has been known is efficient at diminishing lipid oxidation in O/W emulsion (Liu and Yang. 2008; O'Dwyer, O'Beirne and Eidhin. 2012). Wanasundara and Shahidi (1998) reported that the GTE exhibited excellent antioxidant activity in bulk menhaden and seal blubber oils when compared to the synthetic antioxidants. Protein-bound catechin related to the antioxidant activity of these systems (O'Dwyer, O'Beirne and Eidhin. 2012). On the other hand, O'Dwyer *et al*. (2012) found that green tea extract had lower lipid hydroperoxide value through the storage period than α- Tocopherol; also antioxidant addition had no effect on p-Anisidine values of the O/W emulsions. However, in the present study, positive relation was found among antioxidant efficiency and increased levels of extracts in the emulsion which was in agreement with Gramza-Michałowska *et al*. (2008). The lower rate formation of volatile compounds and oxidation products were reported in emulsion containing natural plant extracts than ascorbic acid (Jayasinghe,Gotoh and Wada. 2013).

Different behaviors of antioxidants, which are due to the concentrations and their physical locations in phases, could affect antioxidants effectiveness in food and biological systems. According to the polar paradox, rosmarinic acid (e.g. a polar antioxidant) may not be an effective antioxidant in n-3 rich oil-in-water emulsion (Frankel,Huang and Aeschbach. 1997;

O'Dwyer, O'Beirne and Eidhin. 2012). However, the GTE and WPS contain water soluble compounds which are lipid antioxidants, even though they cannot access the lipid core.

Sensory Evaluation

Sensory assessment of emulsions generally increased during 12 days of the storage (Table 5). At the beginning of the storage, control treatment had significantly higher score after 4 days. At day 0, no significant differences in rancidity found among the treatments. In early days of the storage, control, GTE (e.g. 200 ppm) and ROS (e.g. 200 ppm) treatments had a higher off-flavor, which was evaluated by the assessors. Low percentage of extracts addition had no effects on fish oil emulsion sensory evaluation. There were no significant increases in rancidity scores of the samples treated by the GTE (e.g. 700 ppm), WPS (e.g. 700 ppm) and ROS (e.g. 500 ppm) during the storage.

The results suggest that there was a slight increase in the rancid odor when used 700 ppm of the extract. The results are in agreement with the p-Anisidine values (Table 4). Therefore, the addition of the plant extracts had a preventing effect on off-odor in fish oil emulsion. A previous study reported that addition of EDTA + tannic acid or EDTA + tannic acid + lecithin + α-tocopherol could prevent off-odor in emulsified shrimp oil (Takeungwongtrakul and Benjakul. 2013). Another study reported that the addition of the EDTA (100–2000 p.m.) to fish oil

Table 4. P-Anisidine value of fish oil -in-water emulsion (O/W) containing various antioxidants during storage at 30°C

	Time (Day)						
Treatment	0	2	4	6	8	10	12
Emulsion(control)	± 0.09 ^{D} 1.71	2.05±0.24 $^{ac, D}$ 7.30	±0.50 $^{a, C}$ 6.01	± 0.46$^{b, C}$ 8.91	±0.33 $^{a, B}$ 9.20±0.09 $^{a, A}$ 10.24		±0.86 $^{a, A}$
+GTE 200	±0.23 ^{C} 1.75	± 0.47 $^{a, AC}$ 2.91	±0.20 $^{bd, AC}$ 2.80	±0.30$^{c, BC}$ 2.62	±0.01$^{ce, AB}$ 3.37	±0.07 $^{bcd, A}$ 4.14	±0.08 $^{de, BC}$ 2.33
+GTE 500	± 0.09 ^{D} 0.77	±0.06$^{bcd, D}$ 1.19	±0.18 $^{de, CD}$ 1.57	± 0.03$^{c, BC}$ 2.77	±0.36 $^{bc, AB}$ 4.22	±0.39 $^{bc, A}$ 4.81	±0.03 $^{de, AB}$ 3.27
+GTE 700	±0.10 ^{D} 0.61	±0.05 $^{cd, CD}$ 0.87	0.24 ^{de,} 1.72±^{ACD} 2.52	±0.07 $^{c, AB}$ 2.12	±0.03 $^{ef, ABC}$ 2.93	±0.40 $^{de, A}$ 2.76	±0.15 $^{de, A}$
+WPS 200	± 0.02 ^{D} 0.65	±0.04 $^{acd, CD}$ 1.57	±0.35 $^{b, AB}$ 3.53	±0.08 $^{c, ABC}$ 2.83	±0.13 $^{ef, ABD}$ 2.21	±0.13 $^{cde, ABD}$ 3.44	±0.22 $^{cd, A}$ 3.70
+WPS 500	± 0.18 ^{CD} 0.76	±0.06 $^{d, D}$ 0.53	±0.25 $^{e, ACD}$ 1.41	±0.37 $^{c, ABC}$ 2.13	±0.42 $^{f, ABD}$ 1.85	±0.13 $^{f, ABD}$ 1.26	±0.15 $^{de, A}$ 2.43
+WPS 700	±0.02 ^{B} 0.63	±0.04 $^{bcd, B}$ 1.38	±0.10 $^{de, B}$ 1.71	±0.17 $^{c, B}$ 1.44	±0.20 $^{f, B}$ 1.31	±0.30 $^{ef, A}$ 2.54	±0.19 $^{e, A}$ 2.15
+ROS 200	±0.25 ^{D} 1.55	±0.22 $^{ab, C}$ 2.49	0.40 ^{bc,} 3.24±^{CE} 8.31	±0.13 $^{a, A}$ 4.90	±0.34 $^{b, B}$ 5.26±0.10 $^{b, B}$ 5.87		±0.28 $^{b, B}$
+ROS 500	±0.17 ^{C} 1.44	±0.13 $^{bcd, BC}$ 1.34	±0.06 $^{cde, C}$ 1.93	±0.02 $^{d, A}$ 4.31	±0.08 $^{bcd, A}$ 4.48	±0.27 $^{cd, A}$ 4.92	±0.10 $^{bc, A}$ 5.29
+ROS 700	±0.12 ^{C} 1.65	±0.08 $^{bcd, C}$ 1.42	±0.20 $^{de, C}$ 1.44	±0.32 $^{c, BC}$ 2.24	±0.15 $^{bcd, A}$ 4.75	±0.09 $^{ef, BC}$ 2.26	±0.21 $^{de, AB}$ 3.35

Values are given as means ± SD (n = 3). Control, no antioxidants; GTE, green tea extract; WPS, wild pistachio; ROS, rosemary
A-C Different uppercase letters in the same row indicate significant differences (P < 0.05).
a-f Different lowercase letters in the same column indicate significant differences (P < 0.05).

Table 5. Rancid odour of fish oil -in-water emulsion (O/W) containing various antioxidants during storage at 30°C

	Time (day)			
Treatment	0	4	8	12
mulsion(control)	2.00 ± 0.44 ^{B}	3.80 ±0.20 $^{a, A}$	3.80 ±0.37^{A}	4.80 ±0.20^{A}
+GTE 200	2.60 ± 0.24^{B}	3.20 ±0.20 $^{ab, AB}$	3.40 ±0.24^{AB}	4.40 ±0.24$^{ab, A}$
+GTE 500	1.60 ± 0.24 ^{B}	2.40 ±0.24$^{ab, AB}$	3.40 ±0.24^{A}	3.00 ±0.31$^{bc, AB}$
+GTE 700	1.40 ± 0.24	2.40 ±0.24^{ab}	2.60 ± 0.24	3.00 ± 0.00^{bc}
+WPS 200	1.40 ±0.37^{C}	2.40 ±0.31$^{ab, BC}$	3.20 ±0.24^{AB}	4.20 ±0.24$^{ab, A}$
+WPS 500	1.60 ±0.20 ^{B}	2.40 ±0.20$^{ab, AB}$	3.60 ±0.37^{A}	3.00 ±0.24$^{bc, AB}$
+WPS 700	2.00 ± 0.24	2.40 ±0.37^{ab}	2.20 ±0.48	3.00 ± 0.24^{bc}
+ROS 200	1.80 ± 0.24 ^{B}	3.00 ±0.24 $^{ab, AB}$	3.60 ±0.24^{A}	4.60 ±0.20$^{ab, A}$
+ROS 500	1.80 ± 0.24	2.20 ±0.60^{ab}	3.20 ± 0.24	3.40 ±0.31^{bc}
+ROS 700	1.40 ± ^{B}0.31	1.80 ± 0.40$^{b, B}$	3.80 ±0.20^{A}	2.40 ±0.31$^{c, AB}$

Values are given as means ± SD (n = 5). Control, no antioxidants; GTE, green tea extract; WPS, wild pistachio; ROS, rosemary
A-C Different uppercase letters in the same row indicate significant differences (P < 0.05).
a-f Different lowercase letters in the same column indicate significant differences (P < 0.05).

emulsion decreased the oxidative stability (Nielsen and Jacobsen. 2009).

In conclusion, emulsified fish oil with fish gelatin although offered some protection against oxidation, but the protection was only for a few days. So, further protection is still necessary to obtain more oxidative stability. Slight increases in oxidation products were found in samples with the WPS and GTE extract. Thus, the use of plant extracts in combination with emulsion could retard the lipid oxidation in the fish oil-in-water emulsion more effectively. The addition of high levels of the plant leaves extracts to emulsion was more effective in protection. The results of the present study confirmed that the type and concentration of plant extract had a great impact antioxidant activity. It was found that the wild pistachio extract had better antioxidant activity than rosemary and same as green tea. For the future work, the isolation, characterization and antioxidant activity measurement of major compounds and different extraction method from wild pistachio is recommended.

Acknowledgements

This work was financially supported by the Vice Presidency of Research and Technology, Gorgan University of Agricultural Sciences and Natural Resources (grant number: 92-314-85).

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