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Original article

Efficiency of spearmint (*Mentha spicata* L.) and liquorice (*Glycyrrhiza glabra* L.) extracts in oxidative stability of fish oil under accelerated conditions

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Summary. Nowadays, innumerable synthetic and naturally sourced antioxidants are used to overcome the quality problems of fish and fish products. When considering the rejection of synthetic additives by consumers, demand for the natural sources food supplements has increased. This study focuses on the potential use of spearmint (*Mentha spicata* L.) and liquorice (*Glycyrrhiza glabra* L.) extracts as natural antioxidant in fish oil during accelerated storage. Ethanolic (70%) extracts of spearmint and liquorice were prepared and synthetic antioxidant butylated hydroxytoluene (BHT) added into the fish oil in different concentrations (100, 500 and 1,000 ppm). The peroxide value (PV), thiobarbituric acid value (TBARS), para-anisidine value (p-Av) and UV-spectrum analyses of fish oil were evaluated during storage at 55oC. Throughout the the storage period the PV, TBARS value, p-Av, conjugated dienes (UV232) and conjugated trienes (UV270) values of spearmint and liquorice extracts added samples were significantly lower than the control and BHT added samples. According to results of the study, 500 and 1,000 ppm concentrations of spearmint and liquorice extracts were more effective in prevention of lipid oxidation in fish oil. The results revail that spearmint and liquorice extracts could be a natural antioxidant source for oxidative stability of fish oil. These findings can help to evaluation of liqurice and spearmint as a natural antioxidant source in fish oil industry.

Keywords: Fish oil, liquorice extract, spearmint extract, lipid oxidation, accelerated storage

Introduction

Fish oil is one of the richest source of long-chain polyunsaturated fatty acids (PUFAs) of omega-3 type. PUFAs are conditionally essential nutrients for adequate growth, development and function in humans (1). Among PUFAs, omega-3 PUFAs (EPA, eicosapentaenoic acid (C20:5n3) and DHA, docosahexaenoic acid (C22:6n3)) have gained popularity and include the prevention of a number of diseases, such as cardiovascular diseases, inflammation, hypotriglyceridemic effect, allergies, hypertension, arthritis, autoimmune disorders and cancer (2). However, due to the high degree of unsaturation, omega-3 PUFA are extremely vulnerable to lipid oxidation which is responsible for undesirable colours, off odours, off flavours and loss in nutritive value. The autoxidation of omega-3 PUFAs can occur due to free radicals generated by light, heat, metal ions and enzymes (3). Therefore, synthetic and naturally sourced antioxidants are used to overcome the stability problems of PUFAs. However, some of the commercial antioxidants, such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), tert-butylhydroquinone (TBHQ) and alpha-tocopherol may promote DNA damage by binding to nucleic acids, therefore, exert mutagenic, cancerous and cytotoxic effects (4).

In recent years, there has been an increasing interest in extraction of antioxidants from agricultural and industrial by-products (5). *Mentha spicata* L. (spearmint) is an aromatic plant belonging to the Lamiaceae family. This family is a rich source of polyphenolic

compounds and hence possesses strong antioxidant properties (6). Spearmint extract has been found to have antioxidant and antiperoxidant properties due to the presence of eugenol, caffeic acid, rosmarinic acid and α-tocopherol (7). The most abundant polyphenols in the *Mentha* were found as rosmarinic acid, eriocitrin and salvianolic acid (8). Additionally, the phenolic content of *M. spicata* was determined as caffeic acid and rosmarinic acid (9), gallic acid, cinnamic acid and p-coumaric acid (10). Various studies conducted on the antioxidant property of spearmint (6, 11-14), however its application in fish oil has never been studied before. *Glycyrrhiza glabra* L. belongs to the Leguminosae family and native to the Mediterranean and certain areas of Asia. The roots and rhizomes of *Glycyrrhiza* species named as liquorice. Recently, the flavonoids in liquorice have attained a considerable interest for their structural diversity and important pharmacological activities of the isolated flavonoids, such as chalcones, isoflavones, isoflavans, flavonones, flavanonols, isoflavenes and arylcoumarins (15, 16). More than 400 compounds have been isolated from *Glycyrrhiza* species untill now. Among these constituents, triterpenoid saponins and flavonoids are reported to be the main chemical composition of the ingredients in liquorice (17). Although literature is available on the antioxidant properties of liquorice and spearmint extracts, but no report describing efficiency of liquorice and spearmint extracts for the stabilization of fish oil has been presented so far. Therefore, the principal aims of this study were to investigate the antioxidant potential of *Mentha spicata* L. (spearmint) and *Glycyrrhiza glabra* L. (liquorice) extracts and to evaluate their efficiency in minimizing oxidative rancidity of fish oil under heat accelerated conditions.

Material and Methods

Materials

Fresh spearmint leaves were collected from the district of Pozantı in Adana (Turkey) in the summer 2017. Liquorice was collected from Hatay (Turkey) in summer of 2017. Fish (anchovy) oil was commercially purhased from a fish oil factory (Sürsan Su Ürünleri San. Tic. A.) located in Samsun Turkey.

Methods

Extraction procedure

The plant materials were dried at 40°C for 48 h in oven. Dried plants were ground into powder using laboratory blender. Ultrasound-assisted extraction of plants were conducted in an ultrasonic bath (Kudos-HP series, China) according to method of Tabaraki et al. (18). Ultrasonic bath frequency was adjusted to 250 W of power, 40 kHz. Plants powder and solvent (ethanol 70%) were blended (1:10, g:ml) in conical flash and sonicated for 60 min at ambient temperature in ultrasonic bath. After the extraction, the extracts were filtered through Whatman no.1 filter paper and concentrated by using rotary evaporator (IKA, HB-10 digital, Germany) at 40˚C under vacuum.

Sample preperation

Plant extracts were added to fish oil at concentrations of 100, 500 and 1.000 ppm and denoted as LE1, LE5, LE10 for liquorice and SE1, SE5, SE10 for spearmint. Synthetic antioxidant (BHT) was employed at its legal limit of 100 ppm (19) to compare the efficacy of natural antioxidants and was denoted as BHT. All fish oil samples were sonicated with ultrasonic vibrations for 30 min at room temperature in order to perform dispersion of spearmint extract, liquorice extract and BHT in fish oil. Aliquots of fish oil (2 ml) containing different concentrations of extracts (LE and SE), BHT and fish oil (control) were placed in brown colored glass vials and stored in an laboratory oven (Binder ED 53) at fixed temperature of 55˚C for 21days.

Total phenolic content analysis

The total phenolic content of plant extracts (liquorice and spearmint) were analyzed with using method of Singleton et al. (20). Aliquots of 100 µl of the extract were mixed with 500 µl of the Folin-C and pure water (6 ml) for 2 min. After the solution was mixed, 2 ml of Na_2CO_3 (15 %,w/v) was added into the solution while mixing it for 0.5 min and was filling up to 10 ml with water. Eventually the solution containing plant extract was stored for 2 h at room temperature and then spectrometric analysis was performed at 750 nm absorbance. The total phenolic contents of plant ex-

tracts were given as GAE (mg gallic acid/g dry weigh of plant extract).

Antioxidant activity analysis

Antioxidant activity of plant extracts and BHT were determined using the ABTS.+ (2,2′-azinobis 3-ethylbenzothiazoline-6-sulfonic acid, diammonium salt) radical scavenging decolourization assay by measuring the absorbance at 734 nm using spectrophotometer (Evolution 160 UV–vis, Thermo Scientific) (21). 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 and allowing the mixture to stand in the dark at room temperature for 12–16 h before use. The antioxidant activities of BHT and plant extracts were given as Trolox equivalent antioxidant capacity (TEAC, mmol trolox eq./100 g dry weight of plant extract).

Peroxide value

Peroxide value (PV) analyses of samples were performed using method of AOAC (22). Approximately 2 g fish oil sample was blended with 30 ml of chloroform-glacial acetic acid solution (3 cloroform:2 glacial acetic acid, v/v) and 1 ml of saturated KI solution was added. The mixture was stirred up and stored in dark for 5 min. Distilled water (75 ml) added to mixtures and mixture was titrated with $Na_2S_2O_3$ (0.1M) in the presence of starch solution as an indicator. The results were calculated as; PV (meq/kg) = K x (V-V_0) x 12.69 x 78.8 / w where K is the concentration of $Na_2S_2O_3$ consumed (mol/lt), V is the volume of $Na_2S_2O_3$ consumed during titration (ml), V_0 is volume of the blank consumed during titration (ml), w is the weight of the fish oil (g).

Thiobarbituric acid-reactive substances analysis (TBARS)

Thiobarbituric acid content determination was conducted according to method of AOCS (23). The spectrophotometric determinations of thiobarbituric acid content were performed according to the principle of colorization of malondaldehyde (MDA) present in the lipids with TBA reagent. Lipid dissolved in n-buthanol was mixed with the same amount of TBA reagent. The absorbance of the samples were recorded at 530 nm using a spectrophotometer 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)

Conjugated dienes (UV_{232}) and conjugated trienes (UV_{270})

UV spectrum was determined by the method of IUPAC (International Union of Pure and Applied Chemistry) (24). Oil samples were dissolved in iso-octane and the value of absorptivity at 232 nm (UV_{232}-conjugated dienes) and 270 nm (UV_{270}-conjugated trienes) was measured by spectrophotometer (Evolution 160 UV–vis, Thermo Scientific).

P-anisidine value analysis

Analysis of p-anisidine (*p-Av*) was performed by method of IUPAC (25). Samples (0.5 g) were dissolved in n-hexane (25 ml) and the absorbances of the mixtures were determined at 350 nm (X_1). P-anisidine reagent was mixed with the solution (5 ml) and kept for 10 min in the dark, before the absorbance measuring (X_2) at the 350 nm was done. *p-Av* values were determined for this equation:

p-Av = 25 (1.2 x [*X2* - *X1*]) / sample weight (g)

Statistical analysis

Experiments and analyses were conducted triplicate. Data were evaluated using the analysis of variance (ANOVA) and differences between means of parameters were compared using the Duncan's test at the 5% significance level. Statistical analysis was performed using SAS program (Statistical Analytical Systems, Cary, NC)

Results and Discussion

Total phenolic content and antioxidant activity

Mentha species are rich in phenolic acids and flavonoids, including eriocitrin, luteolin glucoside, rosmarinic acid and caffeic acid (12). Phenolic compounds are the major class of natural antioxidants present in plants and are usually quantified using the Folin–Ciocalteu method (26). In this study, the total phenolic content of speamint extract was found 87.52

mg of gallic acid equivalents per gram of dry extract (mg GAE/g) which is higher than methanolic extract of *M. spicata* determined as 76.3 mg GAE/g by Scherer et al. (26). Dorman et al. (12) reported the total phenolic content in different varieties of *Mentha* changed between 128–230 mg GAE/g. In another study, Benabdallah et al. (27) reported that the total phenolic content of five *Mentha* spp. ranged from 14.7 to 43.2 mg GAE/g. *Glycyrrhiza glabra* root extract contains saponin triterpenes (glycyrrhizin, glycyrrhetinic acid and liquoiric acid) and flavonoids (liquirtin, isoflavonoids and formononetin). The total phenolic content of liquorice extract in this study was determined as 224.16 mg GAE/g which was lower than those reported by Aday et al. (28) in liquorice root sherbet (379.72 mg GAE/g).

Antioxidant capacity is an important health-related parameter and it is highly correlated with phenolic bioactives of foods (29). The antioxidant activities (ABTS.+ scavenging ability) of SE, LE and BHT were found to be 26.13, 34.81 and 14.62 (TEAC; mmol trolox eq./100 g dw) respectively. The antioxidant activities of spearmint and liquorice extracts in this study were higher than those (6.45 μmol trolox/mL and 8.99 μmol trolox/mL for spearmint and liquorice root sherbet, respectively) reported by Aday et al. (28) and Xylia et al. (30). According to Seram et al. (31), the synergism between the antioxidants in the extract makes the antioxidant activity not only dependent on the concentration, but also on the structure and the interaction between the antioxidants.

Peroxide value

Peroxide value (PV) is the measure of degree of initial oxidation of oils and fats (32). The PVs of fish oil containing both BHT and different concentrations of spearmint (SE1, SE5 and SE10) and liquorice (LE1, LE5 and LE10) extracts during the storage are presented in Fig. 1. The initial PV of fish oil was found to be 3.49 meq/kg. During the storage period a continuous increase in PV was observed for all samples. This increase in PV is attributed to the formation of hydroperoxides, i.e. primary oxidation products. The PV of the control samples increased faster and reached 16.32 meq/kg on 15th day of the storage, followed by a decrease on the 18th day of storage. This decrease in PV

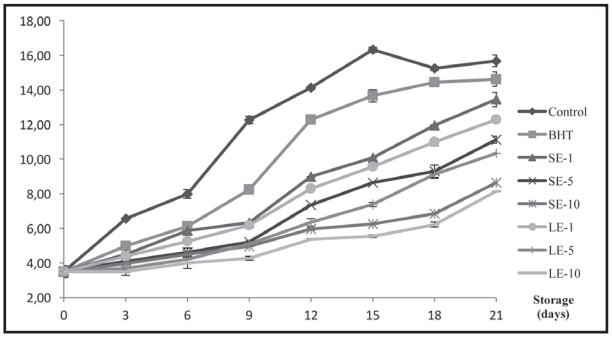

Figure 1. Changes in peroxide value (PV) (meq/kg) of fish oil supplemented with different concentrations of liquorice extract (LE), spearmint extract (SE) and butylated hydroxytoluene (BHT) during storage.

may be related to the observation of Shahidi et al. (33), who suggested that a decrease in PV after long heating times may be due to volatilization of some breakdown products of lipid hydroperoxides, formed in the primary stages of oxidation. The results of this study consistent with the results of Iqbal et al. (32, 34) who observed a sharp decrease in sun flower oil under heat accelerated conditions after 20th day of the storage. It was reported that peroxide value of good quality fish lipid should be less than 5 meq/kg (35). The control samples and BHT added samples exceeded this value on the 3th and 6th day of the storage, respectively. Significant differences (P<0.05) were observed among the control, spearmint (SE1, SE5, SE10), liquorice (LE1, LE5, LE10) and BHT samples at the end of the storage period. Similar results were reported by Topuz et al. (36) in anchovy oil treated with pomegranate peel extract. Marinova and Yanishlieva (14), observed lower PV in sunflower oil treated with different contreations of spearmint compare with contol and BHT added samples under heat accelerated conditions. If the PVs of all samples were compared, PVs of the control sample were observed significantly (P<0.05) higher than those of samples contained different concentrations of spearmint, liquorice extract and BHT during the storage period. The peroxide value evaluation reveals that the addition of spearmint and liquorice extracts in fish oil has a great effect in retarding primary lipid oxidation compared to contol and BHT added samples, particularly 500 and 1.000 ppm concentrations comparable to 100 ppm of BHT at its legal limit (19).

Conjugated dienes (UV_{232}) and conjugated trienes (UV_{270})

The determination of UV_{232} and UV_{270} values is a good parameter for the measurement of oxidative deterioration of oils, and thus a good indicator of effectiveness of antioxidants. (32). Fig 2. and Fig 3. show the formation of conjugated diens and triens in the control, BHT, spearmint and liquorice extracts added fish oil under heat accelerated storage. At the beginning the UV_{232} absorbance value of fish oil was found 2.16 and a regular increase was observed for all samples. However, the increase rate of UV_{232} absorbance value in spearmint and liquorice extracts added samples was very slow compared to the control and BHT added samples. Throughout the the storage period the UV_{232} values of spearmint and liquorice extracts added samples were significantly (P<0.05) lower than the control and BHT added samples, thus indicating good antioxidant activity of the extracts under investigation. The highest UV_{232} absorbance value was observed in the control (3.65) followed by BHT (3,57), SE1 (3.24), SE5 (3.18), LE1 (3.12), LE5 (2.98), SE10 (2.92) and LE10 (2.86) at the end of the storage.

The initial UV_{270} absorbance value of the control sample was 0.16, which was considerably lower than the initial value of UV_{270} (0.28) determined by Topuz et al. (36). During the storage period, the UV_{270} absorbance value of the control and BHT added samples were significantly (P<0.05) higher than the other fish oil samples contained different concentrations of spearmint and liquorice extracts (Fig. 3). The UV_{270} absorbance values of all samples exhibited increasing trends from the beginning of the storage and reached the highest value in the control at the end of the storage (0.89). This increase in UV_{270} absorbance values of samples might be stemmed from the formation of conjugated triens from primary oxidation products i.e. hydroperoxide and conjugated diens. 500 and 1.000 ppm extract added samples showed the significant antioxidant effect, due to the lowest UV_{270} absorbance values were observed in these samples during the storage period. Among the all concentrations, 500 and 1.000 ppm LE and SE showed higher antioxidant activity, while the addition of 100 ppm extracts was less effective in preventing oxidation.

Thiobarbituric acid reactive substances (TBARS)

Thiobarbituric acid is the most widely used method in order to determine secondary oxidation products i.e. aldehydes or carbonyls in oil and oily foods (33). The effects of BHT, spearmint and liquorice extracts on thiobarbituric acid reactive substance (TBARS) formation in fish oil under heat accelerated conditions are presented in Fig. 4. Initially, TBARS value of fish oil was observed as 2.87 mg malondialdehyde/kg. A continuous increase in TBARS value was observed for all the samples during the storage period. The TBARS values of the control (7.82 mgMA/kg) and BHT samples (7.02 mgMA/kg) were significantly (P<0.05) higher than those of treated with spearmint and liquorice extracts at the end of the storage. The TBARS value in freshly caught fish is typically between 3 and 5, but levels of 5–8 mgMA/kg flesh are generally regarded as the limit of acceptability for fish stored in ice (Nunes et al., 1992). The lowest TBARS values were observed in SE10

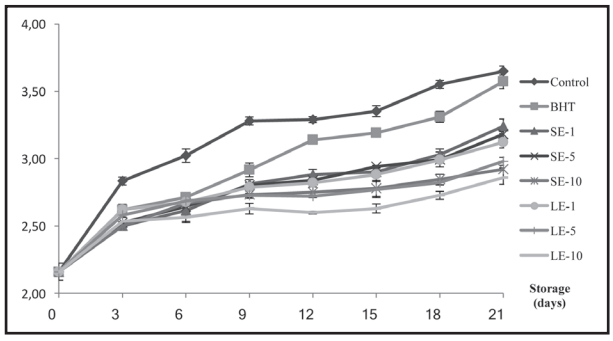

Figure 2. Changes in UV232 nm absorbance values of fish oil supplemented with different concentrations of liquorice extract (LE), spearmint extract (SE) and butylated hydroxytoluene (BHT) during storage.

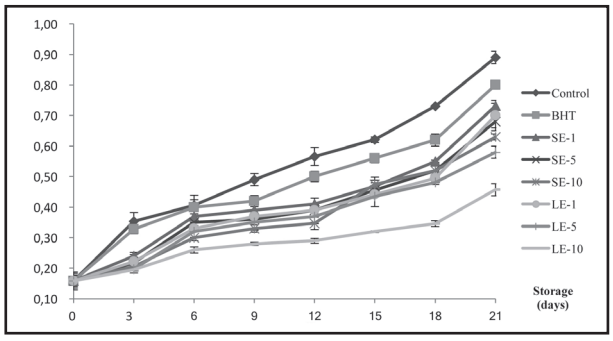

Figure 3. Changes in UV270 nm absorbance values of fish oil supplemented with different concentrations of liquorice extract (LE), spearmint extract (SE) and butylated hydroxytoluene (BHT) during storage.

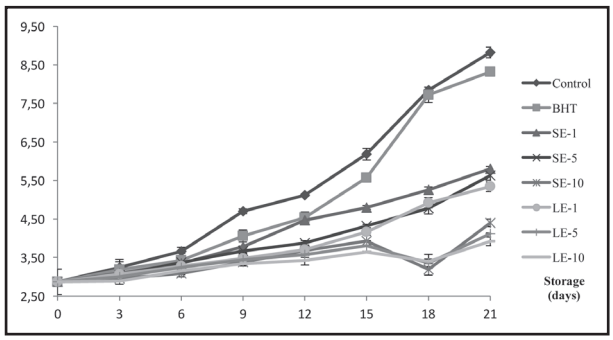

Figure 4. Changes in thiobarbituric acid reactive substances value (TBARS) (mg malonaldehyde/kg) of fish oil supplemented with different concentrations of liquorice extract (LE), spearmint extract (SE) and butylated hydroxytoluene (BHT) during storage.

(4.40 mgMA/kg) and LE10 (3.92 mgMA/kg) samples throughout the accelerated storage and remained under acceptable limit at the end of the storage. Topuz et al. (36) reported that the supplementation of 1.000 ppm pomegranate peel extract in anchovy oil showed the highest antioxidative activiy in suppresion of lipid oxidation. Among the all concentrations, addition of 1.000 ppm of spearmint and liquorice extracts into fish oil was the most effective concentration in prevention of lipid oxidation. There are various studies that support the oxidation retarding effect of spearmint and liquorice extracts in this study. Zhang et al. (38), investigated he potential of liquorice extract (LE) as a dietary supplement for sheep to improve antioxidant capacity of meat. Their results revealed that supplementation with LE in animal diet increased antioxidant content and radical scavenging activity while it decreased reactive oxygen species and TBARS levels of meat. Dietary LE supplementation can improve antioxidant capacity of meat. Kanatt et al. (6), evaluated the effectiveness of mint extract (ME) (*Mentha spicata* L.) as a natural antioxidant for lamb meat. It showed a high superoxide- and hydroxyl-scavenging activity and retarded lipid oxidation, monitored as TBARS lamb meat. TBARS results of present study indicate that the addition of 1.000 ppm spearmint and liquorice extracts in fish oil has shown great effect on TBARS formation compared to other samples.

P-anisidine values (p-Av)

The p-anisidine values (p-Av) of fish oil containing BHT and different concentrations of spearmint (SE1, SE5 and SE10) and liquorice (LE1, LE5 and LE10) extracts during the storage period are presented in Fig. 5. The p-anisidine value (p-Av) provides useful information on non-volatile carbonyl compounds formed in oils during processing and is often used to determine the secondary oxidation product such as hydroperoxide decomposition products. Para-anisidine value essentially reflects how the lipid has been handled and stored versus peroxide value, which measures current oxidation (39). At the beginning of the storage, p-Av of fish oil was observed as 3.62 which was lower than 15.82 of fish oil reported by Yerlikaya et al. (39). Throughout the storage period, p-Av of all samples showed significant (P<0.05) increase. The control sample value was significantly higher (P<0.05) than the samples comprised different concentration of LE, SE and BHT during the storage. The p-Av of the control reached 21.85 at the end of the storage, which is slightly higher than the acceptable limit (20) denoted by Gokoglu et al. (40). The addition of spearmint and liquorice extracts significantly retarded the oxidation level since the lowest p-anisidine values were observed in these groups during the storage period, especially in 500 and 1.000 ppm concentrations. The p-Av values of SE5 and SE10 were 5.47 and 5.06 on 3th day of the storage, while reached 12.29 and 11.88, respectively at the end of the storage. Fish oil supplemented with 500 and 1.000 ppm liquorice extract showed the lowest p-Av during the storage and significantly (P<0.05) remained lower (11.05 and 9.71, respectively) at the end of the storage period. Zhang et al. (41) denoted that the liquorice flavonoids depending on hydroxyl groups

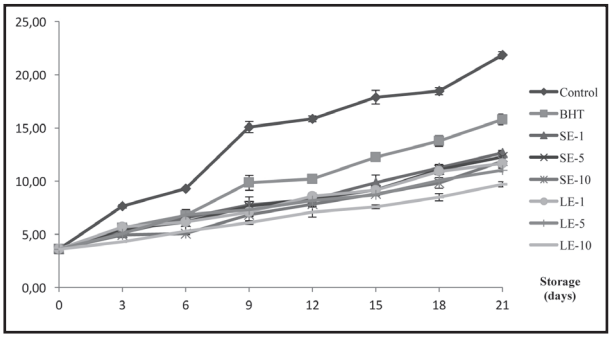

Figure 5. Changes in para-anisidine values (p-Av) of fish oil supplemented with different concentrations of liquorice extract (LE), spearmint extract (SE) and butylated hydroxytoluene (BHT) during storage.

can scavenge free radials and chelate metal ions, which help to improve oxidation resistance. Taken together, the lowest p-Av values were observed in fish oil treated with 500 and 1.000 ppm liquorice extract following 500 and 1.000 ppm spearmint extract at the end of the storage.

Conclusion

Fish oil is very vulnerable to lipid oxidation which has restricted the shelf life. The present study showed that spearmint and liquorice extracts can be recommended as a natural source of antioxidants in order to stabilization of fish oil. Ethanolic extracts of spearmint and liquorice at concentration of 500 and 1.000 ppm have retarding effect on lipid oxidation comparable to synthetic antioxidants, i.e. BHT at its legal limit. It is necessary to conduct additional further studies in order to evaluate the other parameters (color, sensory attributes etc.) of fish oil.

Highlights

The study is based on the effect of natural antioxidants on the oxidative stability of fish oil.

The high antioxidant activity and total phenolic content of natural extracts were evaluated.

Addition of spearmint and liquorice extracts to fish oil retarded the lipid oxidation.

Findings revail that spearmint and liquorice extracts can be recommended as a potent source of natural antioxidant.

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