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Spirulina (*Arthrospira platensis*) Extract as a Natural Antioxidant for Improving Oxidative Stability of Common Kilka (*Clupeonella cultriventris caspia*) Oil

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Abstract

The common kilka (*Clupeonella cultriventris caspia*), and the oil extracted from it, presented a good nutritional profile because of their rich chemical composition containing essential polyunsaturated fatty acids. The objective of this study was to evaluate how the extract of Spirulina (*Arthrospira platensis*) affected kilka oil oxidation in comparison with the effects of BHT and *β*-carotene. Kilka oil samples were stored at 60 °C for 15 days and chemical indicators of lipid oxidation were measured every three days. As a result, kilka oil samples treated with Spirulina extract exhibited a significantly lower Totox value and likewise lower amounts of peroxide and *p*-anisidine were observed when compared to the control group during storage. Spirulina extract and BHT increased the induction period of kilka oil by 38.94% and 35.54%, respectively. Comparatively, *β*-carotene was less effective in reducing kilka oil oxidation, with an induction period extension of 18.30%. The antioxidant powers of Spirulina extract, *β*-carotene, and BHT were 31.43, 19.47, and 29.71, respectively. Generally, the Spirulina extract was introduced as a powerful natural antioxidant that could effectively reduce the oxidation rate of kilka oil.

Keywords**:** Kilka oil, Natural antioxidant, Oxidative stability, Spirulina extract.

Introduction

Fish oil contains high amounts of long chain polyunsaturated fatty acids, especially eicosapentaenoic acid (C20:5 ω-3) and docosahexaenoic acid (C22:6 ω-3), which are importantly beneficial to the maintenance of health and can help prevent several human diseases. However, the long chain polyunsaturated fatty acids of fish oils are highly vulnerable to oxidative deterioration which consequently causes serious limitations on their pharmaceutical and edible consumptions (Sun, Wang, Chen, & Li, 2001). Oxidation of polyunsaturated fatty acids can generate a number of oxidation products that have negative impacts on sensory characteristics of fish oils. Oxidation also affects the amount of these fatty acids available to the body (Fhaner, Hwang, Winkler-Moser, Bakota, Liu, & 2016). In order to protect polyunsaturated fatty acids of fish oil from oxidative degradation, it would seem necessary to use efficient antioxidants. Furthermore, the intake of antioxidants along with a diet that is rich in polyunsaturated fatty acids can be recommended because the excessive intake of such fatty acids can increase the extent to which LDL lipids are prone to peroxidation (Dhavamani, Rao, & Lokesh, 2014).

Spirulina (*Arthrospira platensis*) is a cyanobacterium classified as blue green algae (Khan, Bhadouria, & Bisen, 2005). Spirulina is a high-quality healthy food with high levels of proteins, essential fatty acids, minerals, and vitamins. Spirulina contains protein-pigment complexes called phycobilisomes. Phycobilisomes are mainly

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composed of phycobiliproteins. Phycocyanin and allophycocyanin are also two other important phycobiliproteins that occur in Spirulina. Both Phycocyanin and allophycocyanin have the same chromophore group. Moreover, Spirulina contains phytopigments such as carotene and xanthophyll. Phycocyanin, carotene, and xanthophyll contribute to the antioxidant activity of Spirulina (Bhat & Madyastha, 2000). Furthermore, it has been proved that Spirulina is a useful source of superoxide dismutase – a scavenger of free radicals (Cohen, 1997).

Recently, several studies have explored the bioactive components and antioxidant activity of Spirulina. For instance, Wang, Pan, Sheng, Xu and Hu (2007) reported that the antioxidant activity of the Spirulina extract, exposed to supercritical carbon dioxide, was lower than BHT and Trolox under optimized conditions, but was higher than α-tocopherol (Wang, Pan, Sheng, Xu, & Hu, 2007). Furthermore, Estrada, Bescos, and Del Fresno (2001) showed that a protean extract of Spirulina potentially scavenged hydroxyl and peroxyl radicals and inhibited microsomal lipid peroxidation. On the other hand, Zhou *et al*. (2005) reported that phycocyanin shows pro-oxidant activity when exposed to light, but shows antioxidant activity in the dark. They also showed that the phycobilin moiety is the main part of phycocyanin which is involved in scavenging hydroxyl radicals. Also, trypsin hydrolysis showed that the antioxidant activity of phycocyanin is mainly attributed to the apoprotein portion of the molecule.

Microwave-assisted extraction (MAE) is a simple and economical technique for extracting many bioactive compounds. MAE can extract bioactive compounds in a shorter time and higher efficiency than conventional methods. Also, MAE method has several advantages such as lower consumption of solvents, higher selectivity of target molecules, rapid and volumetric heating of the absorbing medium. Furthermore, MAE has the potential for automation (Wang, Xi, Zheng, & Miao, 2010; Proestos & Komaitis, 2008).

This study was designed to examine microwave extraction on Spirulina, and to evaluate the effect of the Spirulina extract on the oxidation of common Kilka (*Clupeonella cultriventris caspia*) oil (KO) during accelerated storage. The antioxidant activity of Spirulina extract was compared with those of *β*-carotene and BHT.

Material and Method

Materials

Crude KO without antioxidants was supplied by a local fishery factory. Chemicals such as trichloroacetic acid, sodium thiosulphate, isooctane, anisidine, neocuproine ($C_{14}H_{12}N_2$) were obtained from Merck (Darmstadt, Germany). BHT, *β*-carotene, Folin-Ciocalteu reagent, Ethylenediaminetetraacetic acid (EDTA), 2, 2-diphenyl-1-picrylhydrazyl (DPPH), and iron (Fe^{2+}) chloride were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Spirulina Extract Preparation

A modified microwave oven (ME3410W; Samsung Electronics Co. Ltd., Malaysia) was used for extraction of Spirulina extract. Methanol (150 mL) was added to Spirulina powder (10 g) in a 500 mL flat bottom flask. The flask was placed in the microwave oven cavity and a condenser was used on the top (outside the oven). The mixture of Spirulina powder and methanol was irradiated with microwaves at 200 W for 5 min. Then, the extract was filtered using Whatman grade No. 1 filter paper and the solvent was evaporated to the extent that it became completely dry, using a vacuum rotary evaporator (T63AL model, Buchi Company, Switzerland) at 30 °C. The Spirulina extract powder was stored at -18 °C for further experiments.

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Antioxidant Properties of Spirulina Extract

Total phenolic content (TPC) was measured using the Folin–Ciocalteu method according to the procedure described by Pourashouri, Shabanpour, Abad and Zahiri (2016). The absorbance was measured at 725 nm. Results were expressed as mg of gallic acid equivalent per grams of sample (mg GAE/g).

Total flavonoid content (TFC) was measured using the aluminum chloride colorimetric method (Gülçin, Bursal, Şehitoğlu, Bilsel, & Gören 2010). The results are expressed as mg of quercetin equivalent per grams of sample (mg QUE/g).

Radical scavenging activity (RSA) of Spirulina extract was determined using DPPH, following the method of Choi, Rahman, Lee, Chang, and Lee (2016). The IC_{50} value was established by graph plotting, which took into account what percentage of the DPPH remains against Spirulina extract concentrations.

The reducing power of Spirulina extract was measured using ferrous ion reducing antioxidant power (FRAP) and cupric ion reducing antioxidant capacity (CUPRAC) assays. The FRAP of the Spirulina extract and that of the positive control (*L*-ascorbic acid) were determined by reducing the Fe^{3+} to Fe^{2+} following the method of Ardestani and Yazdanparast (2007). The CUPRAC of the Spirulina extract and the positive control (*L*-ascorbic acid) were measured, following the method of Apak, Güçlü, Özyürek, and Çelik (2008). Results were expressed as mg of ascorbic acid equivalent per mL of Spirulina extract (mg AAE/mL).

Ferrous ion chelating (FIC) of Spirulina extract and EDTA (positive control) was examined, following the method described by Farahmand, Golmakani, Mesbahi, and Farahnaky (2017). The EDTA was used as positive control. Results were expressed as mg of EDTA per g of sample.

Initial Quality and Oxidation Indices of KO

Iodine value was measured using the Wijs method, following the AOCS Official Method Cd1-25 (AOCS, 2000). Free fatty acid content was measured using the AOCS Official Method Cd 3d–63 (AOCS, 2000). The peroxide value (PV) was measured using iodometric titration according to AOCS Official Method Cd 8-53. The PV results were expressed as milliequivalents of active oxygen per kg of KO. The anisidine value (AV) was quantified, following the AOCS Official Method Cd 8-53. The AV results were expressed as mg per kg of KO (AOCS, 2000). The Totox value (TV) was quantified by eq. (1) (Frankel, 2012).

$$TV = 2\,(PV) + AV \qquad \text{eq. (1)}$$

To determine the fatty acid profile of KO, fatty acid methyl esters (FAMEs) were prepared following the method of Golmakani, Rezaei, Mazidi, and Razavi (2012). Fatty acid profile of KO was analysed using a GC system (SP-3420A, Beijing Beifen-Ruili Analytical Instrument, Beijing, China) equipped with a split/splitless injector, BPX70 capillary column (30 m × 0.25 mm id; 0.25 μm film thickness, SGE Analytical Science, Melbourne, Australia), and a flame ionization detector (FID) according to the method of Keramat and Golmakani (2016).

Oxidative Stability of KO Incorporated with Spirulina Extract during Accelerated Storage

Spirulina extract was added to the KO at concentration of 1000 ppm and was shaken until the extract completely dissolved. *β*-Carotene and BHT were added to the KO at concentration of 100 ppm and were shaken until the extract completely dissolved. For the control sample, no antioxidant was added. The KO samples (50 mL) were kept in open amber bottles in an incubator at 60 °C for 15 days. The oxidation of KO samples was monitored by measuring PV and AV every 3 days.

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Kinetic Measurements

Induction period (IP) was defined as the number of days taken to reach a PV of 20 meq O_2/kg (Keramat & Golmakani (2016). The protection factor (PF) was measured using eq. (2).

$$PF=\frac{IP_a}{IP_c} \quad \text{eq. (2)}$$

Where IP_a was the IP of the KO sample containing antioxidant (Spirulina extract, β-carotene, and BHT) and the IP_c was the IP of the control sample (Hraš, Hadolin, Knez, & Bauman, 2000).

Antioxidant activity (AA) correlated with the antioxidant concentration. AA was calculated using eq. (3).

$$AA=\frac{IP_a-IP_c}{[AH]\times IP_c} \quad \text{eq. (3)}$$

[AH] was antioxidant concentration in ppm unit (Antolovich, Prenzler, Patsalides, McDonald, & Robards, 2002). Furthermore, antioxidant power (AOP) was calculated using eq. (4) (Silva, Borges, & Ferreira, 2001).

$$AOP=100-\left(\frac{IP_c}{IP_a}\times 100\right) \quad \text{eq. (4)}$$

Statistical Analysis

Results are reported as mean ± standard deviation of three measurements. A general linear model (GLM) procedure was applied for determining significant differences among mean values. Results were analysed using SAS (Statistical Analysis Software, version 9.1; SAS Institute Inc., Cary, NC).

Results

Antioxidant Activity of Spirulina Extract

TPC and TFC levels of Spirulina extract were 38.04 ± 3.07 mg GAE/g and 1.86 ± 0.33 mg QUE/g, respectively. Spirulina extract significantly reduced the DPPH radical with IC_{50} value of 0.78 ± 0.09 mg/mL.

Reducing power of Spirulina extract in FRAP and CUPRAC assays were 12.16 ± 0.04 and 78.32 ± 0.13 mg AAE/mL, respectively. The FIC of Spirulina extract was 0.066 ± 0.001 mg EDTA/g.

Initial Quality and oxidation indices of KO

The acid value, PV, AV, TV, and IV of KO at the beginning of the experiment were 1.13 ± 0.02%, 3.16 ± 0.01 meq O_2/Kg, 4.77 ± 0.04 mg/Kg, 11.09 ± 0.01, and 178.70 g I_2/100 g oil respectively.

Fatty acid profile of KO is presented in Table 1. The crude KO was mainly comprised of saturated fatty acids, followed by monounsaturated fatty acids and polyunsaturated fatty acids. Palmitic acid was the most abundant saturated fatty acid. However, oleic acid was the major monounsaturated fatty acid. Docosahexaenoic acid and eicosapentaenoic acid were the dominant polyunsaturated fatty acids.

Effect of Spirulina Extract on KO Oxidation

PV, AV, and TV

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The PVs of KO samples during accelerated storage period are illustrated in Fig. 1a. The PV of the control increased gradually until the 6th day. However, the hydroperoxide formation rate of the control group increased sharply after reaching a PV of 21.22 meq O_2/kg on 6th day, indicating an accelerated degradation process. Furthermore, similar to the control sample, all KO samples exhibited almost identical trends of increase in their PVs after 6 days of storage. Thereafter, the PV of the control was significantly higher than the PVs of samples containing antioxidants. The Spirulina extract, BHT, and β-carotene appeared to be equally effective in reducing the PV of KO after 9 days of storage, but the Spirulina extract and BHT were found to be more effective than β-carotene after 15 days of storage. At the end of the storage period, the corresponding percentage of inhibition by the β-carotene was 36.79%, while inhibition percentages of Spirulina extract and BHT were 56.23% and 58.20%, respectively in comparison with the control.

The AVs of KO samples during the storage period are presented in Fig. 1b. The AVs of KO samples containing Spirulina extract, BHT, and β-carotene were significantly lower than the control group during the storage period. After 15 days of storage, AVs of samples containing Spirulina extract and BHT reached 30.49 and 19.49 mg/kg, respectively. Therefore, BHT was more effective than the Spirulina extract in lowering the AV of KO. The AVs of β-carotene sample was similar to that of BHT after 6 days of storage. However, the AV of β-carotene samples increased faster than that of BHT after 9 days of storage.

According to Fig. 1c, TV results were very similar to those of PV. Spirulina extract and BHT significantly reduced the TV of KO, in comparison with the control, during storage. After 15 days of storage, the corresponding percentages of inhibition by Spirulina extract and BHT were 54.43% and 58.31%, respectively, compared to the control. Comparatively, β-carotene was less effective in reducing KO oxidation and reduced the TV of KO by 37.68%, in comparison with the control.

Kinetic Parameters

IP, PF, AA, and AOP of KO samples are shown in Table 2. The samples added with Spirulina extract and BHT exhibited the greatest stability and extended the IP of KO by 38.94% and 35.54%, respectively. The presence of β-carotene in the samples was less effective and extended the IP of KO by 18.30%.

AA of Spirulina extract was lower than that of BHT and β-carotene. This may be due to the fact that AA depends on antioxidant concentration.

The highest PF and AOP were observed in samples containing Spirulina extract. Spirulina extract and BHT were more effective than β-carotene in increasing the PF and AOP of KO.

Discussion

Antioxidant Activity of Spirulina Extract

Spirulina extract showed high levels of TPC and TFC. The TPC of Spirulina extract in this study (38.04 ± 3.07 mg GAE/g) was significantly higher than that of methanolic extract of *Spirulina maxima* (15.4 mg total phenolics/g alga dry matter) as described by Miranda, Cintra, Barros, and Mancini-Filho, (1998). Furthermore, the conventional extraction method caused the TPC of methanolic extract of the Spirulina to become 2.83 mg GAE/g (Shalaby & Shanab, 2013). Therefore, the TPC of Spirulina extract, as extracted by the MAE method in this study, was 13.44 times higher than that of conventional extraction method.

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The DPPH radical is commonly used in preliminary stages of screening for compounds that could scavenge activated oxygen species. Handling the DPPH radical is much more stable and easier than oxygen free radicals (Tominaga, Kobayashi, Goto, Kasemura, & Nomura, 2005). Spirulina extract exhibited a high level of RSA. Also, El-Baky, El Baz, and El-Baroty (2009) reported that *Spirulina maxima* phenolic compounds showed antioxidant effects on DPPH radical scavenging while IC_{50} values ranged from 23.22 to 35.62 mg/mL. Shalaby and Shanab (2013) reported that methanolic extract of Spirulina contained phycobilin pigments (C-phycocyanin, allophycocyanin, and C-phycoerytherin) and phenolic (pyrogallol) and flavonoid (catechin) compounds which may exhibit great antiradical activity. According to Romay et al. (1998), C-phycocyanin donates a hydrogen atom which is attached to C-10 bridge of the tetrapyrrole molecule to form a carbon-centered radical with resonance stabilization. This resonance stabilization extends over the entire bilirubin molecule and is responsible for the antiradical activity of C-phycocyanin. It has been shown in previous studies that pyrogallol and catechin exhibit strong radical scavenging activities (Furuno, Akasako, & Sugihara, 2002; Nanjo, Mori, Goto, & HARA, 1999).

The reducing power assay determines the hydrogen-donating ability of Spirulina extract (Apak *et al*., 2008). The reducing power of Spirulina extract in this study (FRAP and CUPRAC values of 12.16 ± 0.04 and 78.32 ± 0.13 mg AAE/mL of sample, respectively) was significantly higher than those found in the methanolic extract of *Chlorella marina* (0.73 ± 0.026 mg AAE/g) and *Dunaliella salina* (0.70 ± 0.031 mg AAE/g) as described by Hemalatha, Girija, Parthiban, Saranya, and Anantharaman (2013).

The CUPRAC value of Spirulina extract was significantly higher than that of FRAP value. This could be explained by the quickness of the CUPRAC reagent in oxidizing thiol-type antioxidants, whereas the FRAP assay was incapable of measuring certain thiol-type antioxidants (Apak *et al*., 2008).

The chelating effect is an indicator of the extent to which antioxidant properties are exhibited by pure compounds and plant extracts (Ebrahimzadeh, Pourmorad, & Bekhradnia, 2008). Spirulina extract showed relevant degree of chelating effect (FIC of 0.066 ± 0.001 mg of EDTA per g of sample), but its capacity was, as expected, less than that of EDTA. Bermejo, Piñero, and Villar, (2008) reported that phycocyanin exhibited a significant metal-chelating activity when it was isolated from a water extract of Spirulina.

Initial Quality and Oxidation Indices of KO

The acid value and PV of KO were within the limits established by the codex standard for edible fats and oils. Therefore, the initial oxidation status was low (Codex Alimentaius, 2015). The IV indicates the unsaturation degree of KO. In this study, the IV of KO (IV of 178.70 g I_2/100 g oil) was higher than those of salmon, tuna, sardine, squid liver, and cod liver oils (IVs of 166, 162, 155, 169, and 143 g I_2/100 g oil, respectively) as described by Endo, Tagiri-Endo, and Kimura (2005). Higher IV of KO in this study could be explained by the prominent presence of eicosapentaenoic acid and docosahexaenoic acid.

The fatty acid profile of KO was in good agreement with the fatty acid profile of the same fish species in previous reports (Pazhouhanmehr, Farhoosh, Esmaeilzadeh Kenari, & Sharif, 2015). Furthermore, polyunsaturated fatty acid/saturated fatty acid ratio (0.54) was higher than the minimum level established by the UK Department of Health (0.45) (HMSO, 1994).

The polyene index of KO in this study (0.86) was higher than the most important fish species of the southern coasts of the Caspian Sea such as pike perch, common carp, and golden grey mullet (0.57, 0.52, and 0.41, respectively). In spite of those indices, the KO was susceptible to oxidation which makes the addition of

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antioxidants necessary (Pirestani, Sahari, Barzegar, & Nikoopour, 2010; Prato & Biandolino, 2012; Osman, Suriah, & Law 2001).

Effect of Spirulina Extract on KO Oxidation

PV, AV, and TV

The PV is an indicator that measures the total hydroperoxide contents of KO. During the storage period, PVs of the Spirulina extract, BHT, and *β*-carotene samples were significantly lower than that of the control. *β*-Carotene inhibited the KO oxidation in a manner somewhat similar to the action of Spirulina extract and BHT, after 6 days of storage. However, at the end of the storage period, Spirulina extract and BHT were found to be more effective than *β*-carotene. Therefore, the differences between antioxidant activity of the Spirulina extract, BHT, and *β*-carotene became clearly evident at the advanced stages of oxidation. Spirulina extract reduced the KO oxidation to the same extent as BHT did. In agreement with this, Athukorala *et al*. (2003) indicated that *Crateloupia filicina* (edible red alga extract) was better able to inhibit oxidation of linoleic acid and fish oil, compared with the inhibitory effect of α-tocopherol, BHT, and BHA. Also, Lima Araújo, Domingues, Sabaa Srur, and Da Silva (2006) reported that the PVs of Anabaena PCC7119 (1000 ppm) extracts were comparable to that of BHT (100 ppm).

AV indicates the amount of carbonyl compounds, principally, 2-alkenals (Frankel, 2012). Although Spirulina extract significantly lowered the AV of KO, but its effectiveness was slightly lower than that of BHT. Similar to PV results, the differences between inhibitory effects of *β*-carotene and BHT were more obvious at the latter stages of oxidation. Similarly, Keramat, Golmakani, Aminlari, & Shekarforoush (2016) reported that β-carotene reduced the oxidation of virgin olive oil to the same extent as BHT did after 21 days of storage, while BHT was found to be more effective than β-carotene after 42 days.

The TV indicates the total amounts of primary and secondary oxidation products. The TVs of KOs supplemented with Spirulina extract and BHT were lower than that of the control group over the 15 days of storage. Accordingly, Spirulina extract contained phytochemicals that efficiently retarded the TV of KO. Shalaby and Shanab (2013) reported that the methanolic extract of Spirulina contained polar antioxidants such as phycobilin pigments (C-phycocyanin, allophycocyanin, and C-phycoerytherin) and phenolic (pyrogallol) and flavonoid (catechin) compounds. According to the polar paradox hypothesis, polar antioxidants are able to protect bulk oils against oxidation better than nonpolar antioxidants can. Unlike nonpolar antioxidants, polar antioxidants are mainly accumulated at air-oil interface where oxidation is supposed to occur (Frankel, Huang, Kanner, & German, 1994). Thus, compounds of methanolic extract of Spirulina can migrate and concentrate at the air-oil interface where oxidation is initiated. *β*-Carotene showed less effectiveness compared to Spirulina extract and BHT during the storage period. This may be due to the fact that *β*-carotene had a high electron reduction potential and could not easily donate the hydrogen atom to alkyl or peroxy radicals of polyunsaturated fatty acid to quench these free radicals (Lee, Ozcelik, & Min 2003). Also, Keramat *et al*. (2016) reported that *β*-carotene was less effective than the essential oils of different plants. *β*-carotene was also less effective compared to BHT in preventing the oxidation of virgin olive oil.

Kinetic Parameters

Antioxidant indices of KO samples were diverse. By adding Spirulina extract and BHT, the IPs of KO samples were dramatically increased. Accordingly, here, Spirulina extract was proved to be a natural antioxidant which can replace its synthetic counterpart, BHT. Also, Chakraborty, Joseph, and Joseph (2016) reported that by combining seaweeds (*Kappaphycus alvarezii*, *Hypnea musciformis*, and *Jania rubens*) extracts and by adding them to sardine oil samples, the IPs of the samples significantly increased. In addition, Xiaojun, Xiancui, Chengxu, and Xiao (1996) showed that IP of fish oil containing polyphenol extracts of macroalga *Sargassum kjellmanianum* were higher than that of control.

AA was a function of the antioxidant concentration. In this study, the AA of Spirulina extract was lower than those of *β*-carotene and BHT, which was due to the higher concentration of Spirulina extract. However, the Spirulina extract could be used at higher concentrations compared to BHT because the Spirulina extract is a natural product. Similarly, Keramat *et al*. (2016) reported that the AA of BHT and *β*-carotene (both at 100 ppm) in virgin olive oil were higher than different plant essential oils (1000 ppm), whereas the IP of the essential oils were similar to that of the BHT but higher than that of *β*-carotene.

Evaluation of the effectiveness exhibited by antioxidants frequently corresponded to an extension of the IP because the antioxidant compound was added. This delay was often expressed as PF or AOP (Hraš, Hadolin, Knez, & Bauman 2000; Silva, Borges, & Ferreira, 2001). The PF and AOP of KO samples supplemented Spirulina extract and BHT were higher than *β*-carotene. Also, Keramat *et al*. (2016) showed that the AOP of virgin olive oil samples supplemented with BHT and different plant essential oils were higher than that of *β*-carotene.

In this study, the inhibitory effects of microwave extracted Spirulina extract on KO oxidation was evaluated in comparison with *β*-carotene and BHT. The results indicated that Spirulina extract significantly retarded the oxidation of KO. This can be attributed to the existence of several antioxidant components in the Spirulina extract such as phycobiliproteins, carotene, xanthophyll pigments and superoxide dismutase which reduce or even prevent KO oxidation. The function of Spirulina extract in retarding KO oxidation was as effective as BHT; however, the Spirulina extract was more effective than *β*-carotene in the same sense. In conclusion, Spirulina extract can be used as a potential natural antioxidant for protecting KO against oxidation. Further researches will be required to determine the antioxidant activity of Spirulina extract and its components in affecting the KO at low and high temperatures. The optimum concentration of Spirulina extract in stabilizing the KO deserves to be quantified in future research.

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Table 1. Fatty acid profile of Kilka oil

Fatty acid	Lipid number	Relative peak area (%)
Myristic acid	C14:0	8.20
Palmitic acid	C16:0	26.68
Stearic acid	C18:0	5.66
Oleic acid	C18:1 (9)	30.45
Linoleic acid	C18:2 (9, 12)	2.56
Arachidic acid	C20:0	1.56

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Gadoleic acid	C20:1 (11)	3.50
Heneicosanoic acid	C 21:0	1.04
Arachidonic acid	C 20:4 (5, 8, 11, 14)	1.76
Eicosapentaenoic acid (EPA)	C 20:5 (5,8,11,14,17)	7.87
Docosahexaenoic acid (DHA)	C 22:6 (4,7,10,13,16,19)	10.68
Saturated fatty acid (SFA)		42.10
Unsaturated fatty acid (UFA)		56.82
Monounsaturated fatty acid (MUFA)		33.95
Polyunsaturated fatty acid (PUFA)		22.87
Polyene index (EPA+DHA /Palmitic acid)		0.69

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Table 2. Kinetic measurements of Kilka oil containing Spirulina extract, β-carotene, and BHT

Oxidation index	Control	Spirulina extract	BHT	β Carotene
Induction period (h)	$84.42^{d*} \pm 0.02$	$117.29^{a} \pm 0.12$	$114.43^{b} \pm 0.03$	$99.87^{c} \pm 0.04$
Antioxidant activity		$3.89^{c} \pm 0.01$	$35.55^{a} \pm 0.01$	$18.30^{b} \pm 0.03$
Protection factor		$1.39^{a} \pm 0.01$	$1.36^{b} \pm 0.01$	$1.18^{c} \pm 0.00$
Antioxidant power		$28.03^{a} \pm 0.08$	$26.23^{b} \pm 0.01$	$15.49^{c} \pm 0.04$

*Values are presented as mean ± standard deviation. In each row, means with different letters are significantly different (P<0.05).

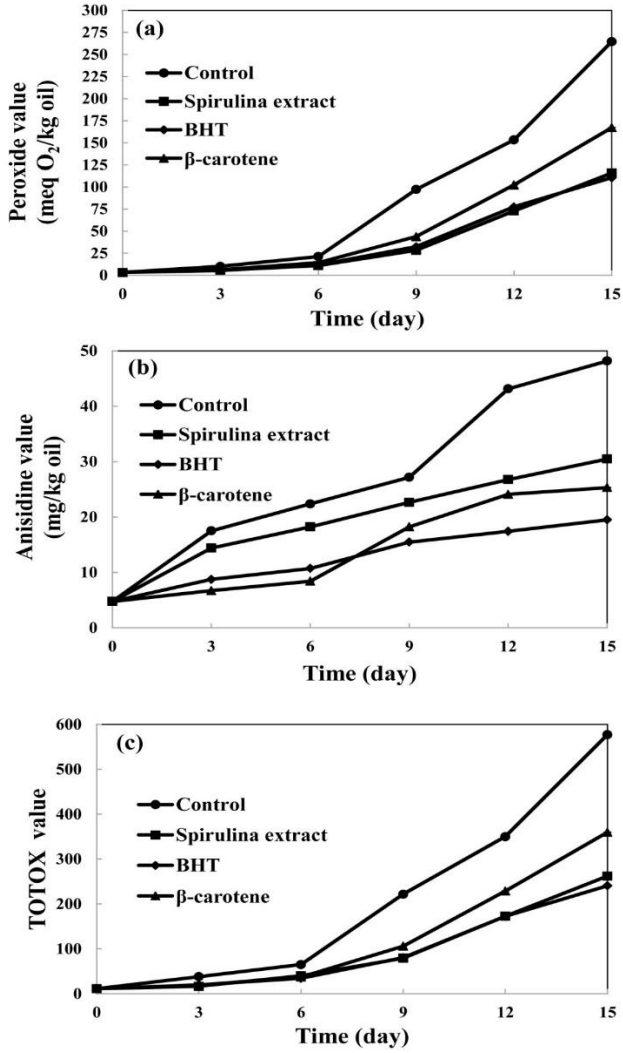

Figure 1. Changes in (a) peroxide value, (b) anisidine value, and (c) Totox value of Kilka oil samples containing Spirulina extract, β-carotene, and BHT during accelerated storage.