



Improvement of the Oxidative Stability of Sesame Oil Using Spirulina as a Natural Antioxidant

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ABSTRACT

Background: Nowadays, natural antioxidants are used to replace synthetic antioxidants for delaying or preventing the oxidation of edible oils. In this study, phenolic compounds and antioxidant properties of methanol extracts of Spirulina microalgae (*Arthrospira platensis*) were measured. **Methods:** Phenolic content was measured by Folin–Ciocalteu method and antioxidant activity was measured by the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) method. Also, spirulina microalgae as natural antioxidant, was added to sesame oil for improving its oxidative stability. The effects of methanol extract of these microalgae in four concentrations (0, 500, 1000 and 2000 ppm) and synthetic antioxidants of butylated hydroxyl anyzole (BHA) and butylated hydroxytoluene (BHT) in two levels (100 and 200 ppm) in retarding the sesame oil oxidation were examined. **Results:** The results showed that spirulina extract had 50.54 mg gallic acid/g samples total phenols and antiradical activity (84.38%). By increasing the concentration of the extracts, the oxidation process decreased. Methanol extract at 1000 ppm concentration had the highest antiradical activity than other concentrations and its antiradical activity was alike the synthesis antioxidant of BHA. By Increasing of storage period, peroxide values in 500 and 1000 ppm of spirulina extracts first increased then decreased and in other treatment increased. During storage time thiobarbituric acid index increased. However, this increase was higher in control treatment than others. **Conclusion:** According to the stated content, it can be said that methanol extracts of spirulina have appropriate antioxidant properties and spirulina can be used as a natural antioxidant to improve the oxidative stability of sesame oil.

Keywords: Antioxidant; Methanol extract; Sesame oil; Spirulina

Introduction

Today, in order to remove or reduce chemical and synthetic compounds in foods, there is a lot of research to replace chemicals with their

natural varieties. In this regard, many efforts have been made to find natural antioxidants from plant and natural sources. The oxidation of lipids in food

not only leads to loss of nutritional and digestibility of food, but also oxidized products such as free radicals result in spontaneous oxidation and the production of undesirable chemical compounds, and as a result, it causes rancidity and off flavour in food. In addition, free radicals can lead to diseases like cancer (Sahsavari *et al.*, 2008). In order to maintain and increase the health of consumers as well as access to a new and inexpensive natural antioxidants research study is needed. For this reason, in recent years, much attention has been paid to natural herbal and natural substances that contain natural antioxidants. One of these sources is spirulina algae, which, in addition to countless properties, also contains antioxidant compounds. Spirulina is a dry biomass of *Arthrospira platensis* Cyanobacter (Belay, 2008). Spirulina is rich in protein, pigments (including chlorophyll, Xanthophyll, beta-carotene and Xanthine), carbohydrates, a relatively high amount of cyanocobalamin (vitamin B12), minerals and superoxide dismutase. Superoxide dismutase is an inhibitor of free radicals (Vonshak, 1997). For C-phycocyanin of Spirulina, anti-oxidant and anti-inflammatory properties (Romay *et al.*, 1998), antioxidant effects in vitro and in vivo, neuroprotective and liver protection effects have been reported (Romay *et al.*, 2003). The antioxidant properties of phycocyanin were attributed to radical inhibitory activity and metal chelating (Bermejo *et al.*, 2008). Flavonoids, beta-carotene, vitamin A, and alpha-tocopherol in spirulina are highly involved in the high antioxidant activity of this microalga (Wang *et al.*, 2007).

Accordingly, this study aimed to use Spirulina (*Arthrospira platensis*) microalgae as a natural compound for improving oxidative stability and increasing the shelf life of virgin sesame oil.

Materials and Methods

Plant material: Dried spirulina was bought from Sina Riz algae of Qeshm and was stored in vacuum at 4 °C until trial. The virgin sesame oil was purchased from an oil making workshop in Jiroft city.

Chemicals and reagents: 2, 2-diphenyl-1-picrylhydrazyl (DPPH), gallic acid, sodium

carbonate, Folin–Ciocalteu's phenol reagent, methanol, thiobarbituric acid, butylated hydroxytoluene (BHT), etc. were purchased from Merck (Darmstadt, Germany); all chemicals were from the reagent grade.

Extraction: 10 g spirulina dried powder was mixed with 100 mL methanol–water (4:1 v/v) solvent. Extraction was carried out at ambient temperature (25 °C) for 24 h using a laboratory shaker. The ratio of methanol and water which lead to the highest yield of phenolic compounds and flavonoids during preliminary trials were selected as the best ratio. Similar ratio of methanol to water was used by (Shahdadi *et al.*, 2015). Each extract was filtered with whatman No. 1 filter paper. The obtained filtrate evaporated to dryness at 40 °C in a rotary evaporator (Buchi Laborator). Then all the extracts were stored at 4 °C until use.

Estimation of total phenolics: Total phenolic content of each extract was determined by the Folin–Ciocalteu micro method (Slinkard and Singleton, 1977). Briefly, 20 µL of extract solution were mixed with 300 µL of Na₂CO₃ solution (20%), then 1.16 mL of distilled water and 100 µL of Folin–Ciocalteu reagent added to mixture after 1 min and 8 min, respectively. Subsequently, the mixture was incubated in a shaking incubator at 40 °C for 30 min and its absorbance was measured at 760 nm. Gallic acid was used as a standard for calibration curve. The phenolic content was expressed as gallic acid equivalents by using the following linear equation obtained from calibration curve:

$$A (760 \text{ nm}) = 12.722C + 0.0034, R^2 = 0.9994$$

Where A is the absorbance and C is the concentration as gallic acid equivalents (µg/mL).

DPPH radical scavenging activity: The ability of extracts to scavenge DPPH radicals was determined according to the (Blois, 1958) method. Briefly, 1 mL of a 1 mM methanolic solution of DPPH was mixed with 3 mL of extract solution (with 50, 100, 250, 500, 800 and 1000 µg/mL concentrations). The mixture was then homogenized vigorously and left for 30 min in the dark place (at room temperature). Its absorbance

was measured at 517 nm and activity was expressed as percentage of DPPH scavenging relative to control using the following equation:

$$\text{DPPH scavenging activity (\%)} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

The results were compared with BHT and butylated hydroxyl anyzole (BHA) synthesized antioxidants. The quality of the radical scavenging property of extracts was determined by calculating the IC₅₀. The IC₅₀ value is the concentration of each extract required to scavenge the DPPH radical to 50% of the control.

Investigating the sesame oil sustainability: To evaluate the oxidative stability of sesame oil, the methanolic extracts of spirulina in four concentrations of 0, 500, 1000 and 2000 ppm and the synthetic antioxidants of BHA and BHT at 100 and 200 ppm to virgin sesame oil without antioxidant and citric acid were added and placed at 60 °C for 16 days. During this time, on days of 0, 4, 8, 12 and 16, peroxide value and thiobarbituric acid index were measured.

Peroxide value: Peroxide value of oils stored under accelerated oxidation conditions was determined by the iodometric determination method according to the (Association of official analytical chemists, 1990) methods.

Determination of thiobarbituric index in oil: 1 g of oil in 10 mL of carbon tetrachloride was dissolve and 10 mL of thiobarbituric acid solution (0. 67% thiobarbituric acid solution mixed with pure acetic acid) was added and 5 minutes in centrifugation at a speed 1000 g was placed. Then the supernatant (the upper part) was separated and placed in a boiling water bath for 30 minutes. The absorbance was measured at 532 nm. All of these steps were carried out with a control sample (no oil) (Association of official analytical chemists, 1990) .

Data analysis: The tests were conducted in a completely randomized design with factorial based

on three replications. Data were analyzed by SPSS 19 software. To compare the means, Duncan's test was used at the 5% confidence level.

Results

Evaluation of total phenolic compounds of methanolic extract of spirulina microalgae: Total phenolic compounds in the methanolic extract of Spirulina microalgae are 50.54 mg gallic acid/ g sample.

Antioxidant activity of methanolic extract of spirulina microalgae: **Figure 1** shows the percentage of DPPH free radical removal of methanolic extract of spirulina microalgae at various concentrations. In this chart, the absorption of free radicals of methanolic extract of spirulina with synthetic anti-oxidants BHA and BHT is also compared.

According to **Figure 1**, with increasing concentrations of the extracts, the percentage of DPPH free radical scavenging in all samples increases. The highest and lowest amount of DPPH free radical absorption was related to the concentration of 1000 ppm of synthetic antioxidant BHT and the concentration of 50 ppm spirulina extrat, respectively. At 50 ppm concentration, the percentage of free radical of the spirulina methanolic extract was greater than that of the BHA. At concentrations of 800 and 1000 ppm, there was no significant difference between the percentage of DPPH-free radicals in Spirulina and BHA.

As shown in **Figure 2**, the lowest and highest IC₅₀ values were observed in BHT and methanolic extracts of spirulina, respectively. Significant differences were observed between IC₅₀ of the studied extracts ($P < 0.05$).

Antioxidant activity of spirulina methanolic extracts in sesame oil:

- *Evaluation of peroxide numbers of sesame oil containing methanolic extract of spirulina microalgae on different days*

Peroxide is the primary product of oxidation of fatty substances. The higher the degree of oils unsaturation, oil or fatty matter is more likely to be

oxidized. When peroxide levels reach a certain level, various changes occur and aldehyde and ketone volatile substances make that contribute to the unpleasant odors and flavors in fatty substances (Prabhasankar *et al.*, 2009). The peroxide value was measured on the days 0, 4, 8, 12 and 16 in the conventional AOAC method and expressed in meq/1000 g oil.

According to the results of **Table 1**, the control treatment had the highest peroxide value until the eighth day. From the eighth to the twelfth day, the amount of peroxide value decreased and then increased. At 500 and 1000 ppm of methanolic extract of spirulina, the amount of peroxide value increased to the 12th day and then decreased. In the other treatments, the process showed an increasing trend during the storage period.

Data from **Table 1** is observed that in the first day, the peroxide value of all sample were zero. On the fourth day, the highest amount of peroxide value was related to control (22.62 meq / kg oil). The lowest amount of peroxide was related to 200 and 100 BHT concentration, which did not show a significant difference with 200 ppm BHA ($P > 0.05$). In the 8th storage day, the control treatment had the highest peroxide value and BHT treatment at 200 ppm had the lowest peroxide number (7.36 meq / kg oil), which had no significant difference with the concentration of 100 ppm of the same antioxidant (8.3 meq / kg oil).

On the 12th day, the concentration of 500 ppm of spirulina extract had the highest peroxide value. The lowest amount of peroxide value was related to 200 ppm BHT. On the 16th day of the experiment, the highest amount of peroxide values was related to 100 ppm BHA concentration (82.88 meq / kg oil). Concentrations of 500 and 2000 ppm

of spirulina extracts showed no significant difference in terms of peroxide values ($P > 0.05$).

Evaluation of the thiobarbituric acid index of sesame oil containing spirulina Eextracts in different days: According to **Table 2**, it is observed that with increasing the maintenance period, the index of thiobarbituric acid increased. The control treatment had the highest and the 200 ppm BHT had the lowest index of thiobarbituric acid. thiobarbituric acid trends 2000 ppm of spirulina extract and 100 ppm BHT were very close in different storage days.

According to **Table 2**, at the first day of the experiment, all of the treatments studied showed a number of acid (0). On the fourth day, the BHT 100 and 200 ppm treatments showed the lowest amount of thiobarbituric acid index. There was no significant difference between the thiobarbituric acid indexes of other treatments ($P > 0.05$). On the eighth day, the control treatment showed the highest levels of thiobarbituric acid (0.122 mg malonaldehyde / kg oil) and 200 ppm BHT had the lowest index of thiobarbituric acid (0.1 mg malondialdehyde / kg oil). It showed that there was no significant difference with 100 ppm BHT, 200 ppm BHA treatments and 1000 and 2000 ppm concentrations of spirulina methanolic extracts ($P > 0.05$). At the twelfth day, maintenance of the lowest amount of thiobarbituric acid index was related to 200 ppm BHT treatments, which showed no significant difference with the 100 ppm concentration of the same antioxidant ($P > 0.05$).

Table 2 shows that at the 16th day, the lowest amount of thiobarbituric acid index was related to the 200 ppm BHT (0.194 mg malonaldehyde/kg oil), followed by 100 ppm of this antioxidant (0.222 mg malonaldehyde / kg oil).

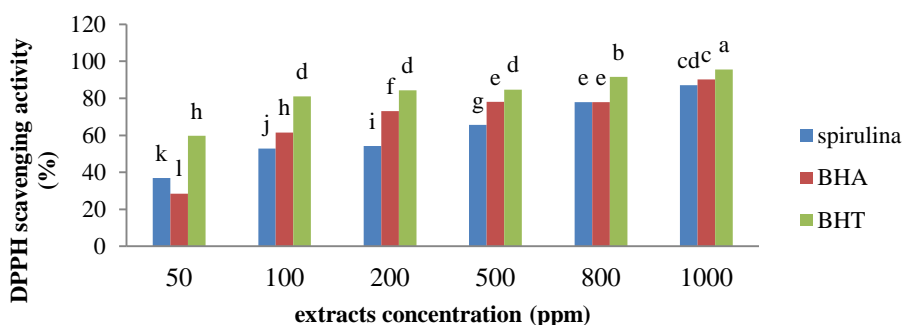


Figure 1. DPPH radical scavenging activity of methanolic extracts of spirulina

Each observation is a mean ± SD of 3 replications. In each figures, means with same superscripts had no significant difference with each other (P >0.05)

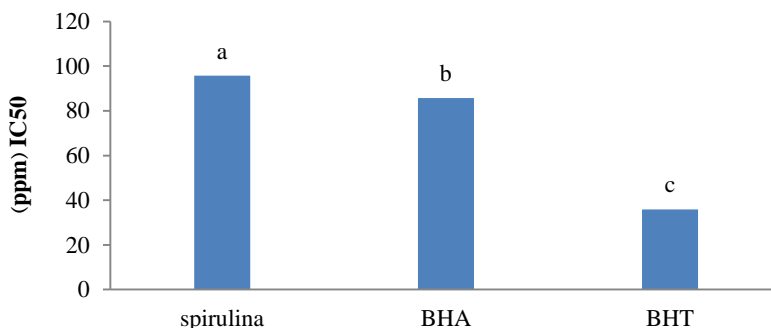


Figure 2. Comparison of IC50 values (ppm) in different extracts

Each observation is a mean ± SD of 3 replications. In each figures, means with same superscripts had no significant difference with each other (P > 0.05)

Table 1. Peroxide values of all treatments on different days

Treatments	Storage days				
	0	4	8	12	16
Control	0 ^q	22.65 ^l	42.46 ^{hi}	40.23 ⁱ	60.88 ^c
Spirulina 500 ppm	0 ^q	14.75 ^m	27.77 ^k	49.01 ^f	44.20 ^{gh}
Spirulina 1000 ppm	0 ^q	10.61 ⁿ	25.70 ^k	44.70 ^{gh}	44.20 ⁱ
Spirulina 2000 ppm	0 ^q	6.62 ^o	16.93 ^m	37.73 ⁱ	44.50 ^{gh}
BHA 100 ppm	0 ^q	6.77 ^o	15.20 ^m	46.38 ^g	84.82 ^a
BHA200 ppm	0 ^q	0 ^q	10.50 ⁿ	39.08 ⁱ	81.57 ^b
BHT 100 ppm	0 ^q	0 ^q	8.30 ^{no}	35.88 ^j	75.27 ^c
BHT 200 ppm	0 ^q	0 ^q	7.36 ^o	28.60 ^o	72.88 ^d

In each row and column, numbers with same superscripts are not statistically significant (P > 0.05)

Table 2. Thiobarbituric acid index (mg of malondialdehyde per kg of oil) of all treatments in different days

Treatments	Storage days				
	0	4	8	12	16
Control	0 ^k	0.079 ^{ij}	0.221 ^{fh}	0.336 ⁱ	0.551 ^a
Spirulina 500 ppm	0 ^k	0.07 ^{ij}	0.161 ^h	0.236 ^f	0.375 ^b
Spirulina 1000 ppm	0 ^k	0.063 ^{ij}	0.137 ⁱ	0.200 ^{fg}	0.311 ^e
Spirulina 2000 ppm	0 ^k	0.058 ⁱ	0.131 ⁱ	0.170 ^h	0.276 ^c
BHA 100 ppm	0 ^k	0.061 ^{ij}	0.165 ^h	0.217 ^{fg}	0.325 ^{de}
BHA200 ppm	0 ^k	0.052 ^j	0.144 ^{hi}	0.176 ^h	0.286 ^e
BHT 100 ppm	0 ^k	0.047 ^{jk}	0.120 ⁱ	0.142 ^{hi}	0.222 ^{fg}
BHT 200 ppm	0 ^k	0.041 ^k	0.100 ⁱ	0.113 ⁱ	0.194 ^{gh}

In each row and column, numbers with same superscripts are not statistically significant ($P > 0.05$)

Discussion

Measuring phenolic compounds and anti-catalytic activity of extracts and essential oils is an important tool for understanding the importance of plant species from a health perspective (Chang *et al.*, 2007). Phenolic or polyphenol compounds are secondary metabolites with a circular structure that can be based on the main component of their molecules in 15 different groups, such as phenolic acids, flavonoids, anthocyanins, quinones, catechins, tannins, and others (Đilas *et al.*, 2002). It has been shown that phenolic compounds have antioxidant activity. These compounds prevent the oxidation of lipids and affect the formation and modification of proxyl free radicals (Ćetković, 2008).

According to (Alavi *et al.*, 2016), the total phenol present in spirulina was about 68.72 mg/g of gallic acid per gram of spirulina. The measured amount of pure spirulina phenol in the study of (Liu *et al.*, 2011) was 19.47 mg, equivalent to the gallic acid per gram of sample. The most common phenolic compounds in cyanobacteria are amino acids similar to mycopurine and phenolic pigments. It has been reported that these molecules have antioxidant activity (Stengel *et al.*, 2011). Initial evaluation of total phenol content on spirulina for using in pasta was carried out by (De Marco *et al.*, 2014). From the three spirullain extracts (water, hexane and ethyl acetate), only the

aqueous extract showed more phenolic compounds and antioxidant activity, which was 40.08 mg, equivalent to the gallic acid per gram of sample. Also, spirulina-containing pasta had a higher phenolic content and an antioxidant activity than the control pasta.

(Shalaby and Shanab, 2013) attributed the antioxidant activity of spirulina extracts (methanol, methanol 50%, and aqueous) to the presence of phenols due to their high levels of active biological phytochemicals (sterols, flavonoids, reducing sugars, tannins and anthraquinones).

(Jaime *et al.*, 2005) isolated and analyzed the antioxidants of spirulina by the fluid extraction method. They introduced carotenoids, phenolic compounds and decomposed products of chlorophyll as the most important antioxidant compounds of spirulina extracts. The most common phenolic component in cyanobacteria is amino acids similar to mycopurine and phenolic pigments. It has been reported that these molecules have antioxidant activity (Stengel *et al.*, 2011).

(Romay *et al.*, 1998) showed that phycocyanin (spirulina pigment) has inhibitory properties of various harmful free radicals, such as alkoxy, hydroxy, and peroxide.

The results of this study confirm the study of (Prabhasankar *et al.*, 2009) in increasing activity of DPPH radical scavenging and metals chelating using *Sargassum marginatum* seaweed. Figure 2

shows the amount of IC₅₀ of the extracts and synthesized antioxidants.

Combination of natural antioxidants in *Spirulina platensis*, such as alpha and beta-carotene, xanthophyll, cryptoxanthin, zeaxanthin, vitamin C, tocopherol, polyphenolic polar compounds, and especially phycocyanin pigment, through synergistic interactions, scavenging free radicals and metals chelating mechanisms prevent lipid peroxidation and delay this process (Samad luy, 2007).

The reason for the decline of the peroxide values of control and 500 and 1000 ppm spirulina treatments in the final days of the storage period converts peroxide into secondary oxidation compounds, such as aldehydes and ketones. The rest of the treatments were able to prevent the formation of secondary oxidation products, thus showed a higher peroxide value.

The difference in the oxidation progression process for oil samples containing spirulina extracts and the control sample can be attributed to the participation of compounds with anti-oxidant activity of spirulina in sesame oil. The presence of flavonoids in spirulina (9.15 mg quercetin per gram of spirulina) and total antioxidant capacity indicate that these compounds contributed to the antioxidant activity of sesame oil and caused a difference in the process evolution diagram of oxidation. In the study of (Alavi *et al.*, 2016) the average antioxidant activity of Spirulina-containing oil was calculated +0.42, indicating the antioxidant activity potential of this microalga in virgin olive oil.

The difference in the protective effect of spirulina extracts against the synthetic antioxidant BHT can be explained by the fact that BHT is an antioxidant compound (pure); however, spirulina is a cell mass that only some of its components have antioxidant properties. It should also be noted that the addition of food additives (synthetic antioxidants) to many oils is not permitted (Ho and Shahidi, 2005).

Thiobarbituric acid is widely used as an indicator of secondary oxidation of fat, due to the presence of reactive substances from the second

phase of autoxidation, in which peroxides are oxidized to aldehyde and ketone and these substances react with thiobarbituric acid.

The decrease of thiobarbituric acid Index in oils containing spirulina is due to the antioxidant properties of its pigments, which led to a reduction in the oxidation of the fatty matter. From the natural antioxidant compounds, spirulina are alpha and beta-carotene, xanthophyll, cryptoxanthin, zeaxanthin, vitamin C, tocopherol, polyphenolic compounds, etc. (Deng and Chow, 2010).

The effectiveness of spirulina microalgae on the improvement of the oxidation stability of the sesame oil relative to the control treatment can be attributed to the participation of phenols, flavonoids, and pigments, such as carotenoids, decomposed chlorophylls, and other compounds in this microalga. In the study of antioxidant activity of two spirulina microalgae biomass including (green and orange) in different levels (0, 0.75 and 1.25% w/w) in oily food, increased oxidation resistance in emulsions has been proven (Goli *et al.*, 2005). Similarly, (Colla *et al.*, 2017) also demonstrated that the *S. platensis* powder was able to delay the oxidation process and reduce the formation of peroxides, effectively acting as an antioxidant in the soybean oil.

Santoso *et al.* investigated the antioxidant capacity of seven species of Indonesian seaweeds in an emulsion system of fish oil (Santoso *et al.*, 2004). They observed that seaweed extracts had lower peroxide values than that of control. In this study, the spirulina sample had a significantly lower peroxide value than the peroxide values of beta-carotene and alpha-tocopherol samples at the end of storage period. (Alavi and Golmakani, 2017) investigated improving oxidative stability of olive oil and incorporation of Spirulina. Their results showed that the presence of carotenoid and chlorophyll compounds from Spirulina in the medium can retard the oxidation of olive oil.

Conclusions

In this study, spirulina (*Arthrospira platensis*) was used as a natural compound to improve oxidative stability and increase the shelf life of

sesame oil. The results showed that methanolic extract of spirulina microalgae had 50.54 mg of gallic acid per gram of sample.

The antioxidant activity of the extracts was determined by DPPH assay that the antioxidant activity in the methanolic extract of this microalgae and BHT and BHA was concentration-dependent and free radical scavenging DPPH activity increased with increasing of concentration. Using methanolic extracts of spirulina in sesame oil by examining the oxidative stability factors proved that spirulina improves the oxidative stability of sesame oil compared to control. The results of this study showed that there was no significant difference in peroxide and thiobarbituric acid index of the treatments in the early days; however, by passing time, the difference was more significant. Especially the amount of thiobarbituric acids obtained from hydro peroxides oxidizing during the final days. By

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- increasing the concentration of the extracts, oxidation was delayed better. In general, methanolic extract of spirulina had a good effect on the delay of sesame oil oxidation, which was compatible with concentrations of 100 and 200 ppm of BHA synthetic antioxidant.
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- All authors contributed with study design, data collection, data handling and manuscript preparation. All authors read and approved the final manuscript.
- Conflict of interest**
- The authors declare that they have no conflict of interests.
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