

## Effects of Different Processing Methods on Phytochemical Compounds and Antioxidant Activity of *Spirulina platensis*

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### Abstract

**Background and Objective:** A *Spirulina platensis* is one of the major sources of functional food ingredients with nutraceutical properties. It is a very perishable and should be processed immediately after harvesting. Therefore, the main purpose of the present study was investigation the effect of different processing condition on the most important qualitative features of *Spirulina platensis*.

**Material and Methods:** Fresh *Spirulina platensis* was processed (shade, sun, oven, microwave, vacuum oven, freeze and spray-drying and freezing with and without blanching) and changes in its qualitative characteristics (minerals and fatty acids composition, total phenolic compounds and antioxidant activity) of samples were analyzed.

**Results and Conclusion:** Processing conditions significantly ( $p \leq 0.05$ ) affected the qualitative properties of the sample. The vacuum-oven dried sample had the highest level of total phenolic compounds and antioxidant activity because of the lower possibility of oxygen dependent degradation and enzymatic browning reactions. The mineral was not significantly different ( $p > 0.05$ ) in dried samples, while Na, K, Mg, Mn, Ca and P content of the frozen samples were reduced significantly. Various unsaturated essential fatty acids like  $\alpha$ -linolenic acid,  $\gamma$ -linolenic acid, arachidonic acid, eicosapentaenoic acid, and docosahexaenoic acid were detected in *Spirulina*. In this regards, spray and freeze-drying were the best processing methods in protecting UFA, and vacuum oven-drying was preferred in protecting total phenolic compounds and antioxidant activity of the *Spirulina platensis*.

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## 1. Introduction

*Spirulina (S.) platensis* is a multicellular and filamentous blue-green cyanobacterium with cylindrical cells arranged in unbranched helicoid trichomes. It is a suitable supplement in human, aquatic, livestock, and poultry diets. For centuries, native people from Chad Lake in Africa and Texcoco Lake in Mexico consume *S. platensis* as an important source of nutraceutical components [1,2].

Carotenoids, phycocyanin, chlorophyll, phenolics, super-oxide dismutase enzyme, and vitamins C and E with scavenging free radical in different methods are the main antioxidant components of *S. platensis*. This microalga is also an appropriate source of iron, calcium, phosphorus, magnesium, and potassium that are essential elements to regulate healthy functions in the human body. Furthermore, *S. platensis* is an appropriate source of

essential unsaturated fatty acids (USFA) with a high ratio of  $\gamma$ -linolenic acid (GLA) and noticeably physiological and biological functions. *S. platensis* also contains 50-60% proteins with high biological and nutritive value affiliated to its amino acids composition. The availability of different water and fat-soluble vitamins, especially vitamin B<sub>12</sub> and pro-vitamin A, in *S. platensis* was reported by Benelhadj et al. [1].

Fresh *S. platensis* usually contains 75-80% water. In order to extend its shelf life, water content of product must be reduced to 15%. Drying is the most widely applied treatment that inhibits microbial growth and prevents certain biochemical changes due to water activity reduction. At the same time, drying affects the qualitative properties of samples. Extended drying time or high drying temperature are effective parameters on antioxidant

activity reduction, changes in the volatile compounds and total phenolic content of product [3,4].

Freezing is also another method for processing raw products in order to protect qualitative properties of samples during storage [5]. This process also affects several quality-active properties of products. In this regard, it was reported the qualitative features, especially fatty acids contents of unbalanced stored beans in polyethylene bags at 22°C dropped appreciably in the first month of storage [6].

Due to high water content and perishable nature of the fresh *S. platensis*, the best processing method selection is necessary to protect quality and nutritional properties of this product. According to the available literature, there exist no sufficient data about the effect of processing methods on nutraceutical components of *S. platensis*. Therefore, the objective of this study was to determine the effects of different drying (shade, sun, oven, microwave, vacuum oven, freeze and spray-drying) and freezing (with or without blanching) methods on changes of total phenolic content, antioxidant activity, minerals and fatty acids composition as important parameters on quality of the processed *S. platensis*.

## 2. Materials and Methods

Folin-Ciocalteu reagent, butylated hydroxytoluene (BHT), gallic acid, 2, 2-diphenyl-1-picrylhydrazyl (DPPH), and n-hexane were purchased from Sigma (Sigma-Aldrich Corporation, St. Louis, MO, USA). Ethanol, methanol, hydrochloric acid, nitric acid, sodium carbonate, and potassium hydroxide were purchased from Merck (Merck Group, Darmstadt, Germany).

Fresh *S. platensis* was obtained from the Institution of Green Foundation, Research Center, Qeshm, Iran. It was chilled indirectly on an ice-cold pad and delivered to laboratory in 1 h.

The moisture, fat, protein, ash, crude fiber, and pH of *S. platensis* were measured immediately [7-12].

### Drying *Spirulina platensis*

Samples were spread uniformly on shallow trays at 1 cm thickness and dried using the following seven methods: (i) shade-drying; (ii) sun-drying; (iii) oven-drying; (iv) microwave-drying; (v) vacuum oven-drying; (vi) freeze-drying and (vii) spray-drying.

*S. platensis* was shade-dried according to the method described by Negi and Roy (2000) with some modifications [13]. The shade-drying process was conducted at 25±2°C and 70±2% relative humidity (RH); ventilation was provided with artificial airflow for 48 h. Sun-drying was carried out according to Sun et al. (2014) with minor modifications [3]. The sun-drying process was done in an open space under direct sunlight at 40±2°C and 46±2% (RH) for 24 h. The oven-drying process was

conducted at 80°C for 11 h in an oven (Memmert GmbH + Co.KG, Schwabach, Germany) equipped with an air circulator [3]. In the microwave-drying treatment, the wave power was set to 360 W (9 min) and 720 W (5 min) in the microwave (LG Electronics Inc., Yeouido-dong, SEL, South Korea) [14]. In the vacuum oven-drying treatment, the sample was dried at 65°C, 0.07 Mpa for 14 h in a vacuum oven (Memmert GmbH + Co.KG, Schwabach, Germany) [15]. In the freeze-drying treatment, the sample was dried at -76 to -80°C for 24 h in a freeze dryer (Telstar life sciences solutions, Spain) [3].

Spray-drying was performed using the method described by Shofinita et al. (2015) with slight modifications [16]. In the spray-drying treatment, aqueous *S. platensis* (at 1:1 w w<sup>-1</sup>) was dried in an industrial spray dryer (Maham Neyshabour Inc., Neyshabour, Khorasan, Iran). Drying conditions were defined as feed temperature at 25°C, inlet temperature of slurry at 170°C, outlet temperature of dry algae at 90°C and liquid feed pump rate of 25 m<sup>3</sup> h<sup>-1</sup>.

### Freezing of *Spirulina platensis*

*S. platensis* was frozen by the non-blanching freezing and blanching freezing methods. In non-blanching freezing, the oxygen content of the head space in the Falcon conical bottom centrifuge tubes was replaced by nitrogen gas, and the sample was frozen and stored at 20°C for 1 month in the freezer (Philver, Tehran, Iran). In blanching freezing, the sample was steam blanched at 95°C for 5 min and frozen according to the non-blanching freezing procedure [17].

### Total phenolic measurement

Total phenolic content of samples were measured by visible light spectrophotometric method. Different concentrations of *S. platensis* extract in ethanol/distilled water (30:70) was mixed with 10-fold dilution of Folin-Ciocalteu reagent and sodium carbonate solution (20% w v<sup>-1</sup>) and the absorbance was measured after 1 h at 725 nm with a UV-Vis spectrophotometer (UV 2100, Unico Inc., San Diego, CA, USA). The total phenolic content of the samples was expressed as gallic acid equivalent (GAE) in the dry matter of the extracts (mg g<sup>-1</sup>) [18].

### DPPH radical scavenging activity

An amount of 1 ml of *S. platensis* extract was added to 1 ml of DPPH solution (0.1 mmol in 1 l methanol) and the mixture was kept in darkness for 30 min. The UV absorbance was measured at 517 nm (UV 2100, UNICO, USA), [18].

The scavenging activity of the fresh and processed microalga was calculated using Eq. 1.

$$\text{Radical scavenging\%} = [(A_0 - A_1) / A_0] \times 100 \quad (1)$$

Where  $A_0$  is the absorbance of the control (distilled water) and  $A_1$  is the absorbance of the sample.

### Mineral composition analysis

Mineral content of samples were analyzed by inductively coupled plasma optical emission spectroscopy (Varian Inc., Palo Alto, CA, USA). In this order, prepared ash at 500-520°C was dissolved in HNO<sub>3</sub>: HCl (3:1) and mineral composition of diluted solution was determined. The instrumental conditions of ICP-OES were a radio frequency power of 1 kW, plasma flow rate of 15 l min<sup>-1</sup>, auxiliary flow rate of 1.5 l min<sup>-1</sup>, nebulizer flow rate of 0.75 l min<sup>-1</sup>, and pump flow rate of 15 rpm [19].

### Fatty acids composition analysis

Extracted oil from *S. platensis* was methylated with alcoholic potassium hydroxide. The methyl ester of fatty acids in hexane was used for analysis by gas chromatography (Agilent Technologies, Santa Clara, CA, USA). Gas chromatograph equipped with a capillary column (DB-23, J and W Scientific) in 30 m length × 0.25 mm internal diameter × 0.25 μm thickness. Helium (20 cm s<sup>-1</sup>) was carrier gas. The detector (FID) and injector temperatures were set at 250°C and 220°C, respectively. The initial oven temperature was 190°C and was increased to 220°C at a rate of 1°C per min. The peaks were identified according to the retention times of external standard of fatty acids methyl ester with a flame ionization detector [20].

### Statistical analysis

Statistical analysis of data was performed according to a completely randomized design followed by the least significant difference (LSD) test to compare the means at a significant probability level ( $\alpha=0.05$ ) using SAS software, Version 9 (IBM, New York, USA). All tests were conducted in triplicate, and the results are presented as the mean ± standard deviation (SD).

## 3. Results and Discussion

### Proximate composition

Proximate composition of the fresh *S. platensis* is presented in Table 1. The moisture content of product was 85.51% of the sample weight. Protein was the main macronutrient in the dry weight of sample. Other components such as ash, fat and crude fiber were also determined in relative high quantity. This product had an alkaline property. Similar components with different values are reported in other studies. For example, 74% protein, 7% ash and 8.3% lipid were reported in dry basis of fresh *Spirulina*. Differences in chemical composition of *Spirulina* are due to differences in genetic characteristics and algae production condition [21,22].

**Table 1.** Proximate composition of *Spirulina platensis* (g 100 g<sup>-1</sup> of dry matter)

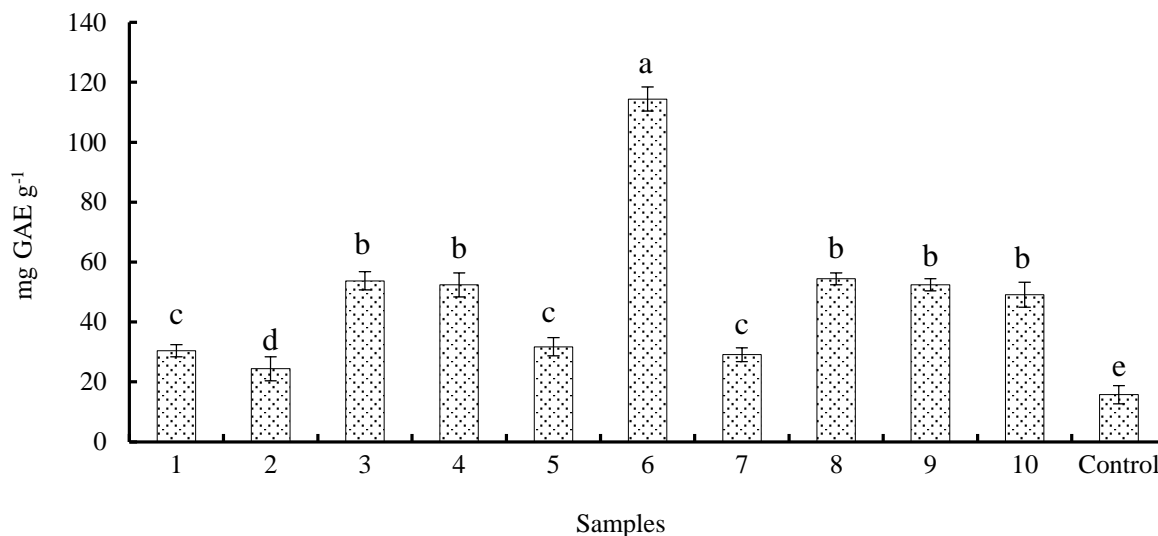
Components	Values (%)
Moisture (% wet weight)	85.51±0.1
Ash	14.22±0.3
Protein	55.52±0.57
Fat	11.94±0.57
Crude fiber	4.83±0.17
pH	8.53±0.02

All values are presented as mean ± SD (n=3)

### Total phenol contents (TPCs)

Differences in cell wall structure and components of various products are effective parameters on processing conditions. Electron microscopy of ultra-thin sections of *S. platensis* revealed that its cell wall is composed of four layers, identified from the innermost to outward pattern as LI, LII, LIII, and LIV. The LI layer contains β-glucan, LII is made of peptidoglycan which gives hardness and strength to this layer, Layer III is composed of protein fibrils, and Layer LIV is composed of material arranged in a linear manner, parallel with the trichome axis, considered analogous to that which is present in the cell wall of gram-negative bacteria [2]. Results show the TPCs of the processed samples were higher than it in the fresh one significantly ( $p \leq 0.05$ ) (Figure 1). It is due to destruction of the microalga cell wall by processing and improvement of phenolic components extraction. In this regard, another study on TPCs of mint had also indicated higher TPCs of dried sample than fresh one. Cell-wall destruction and breakage of ester and glycoside bonds of connected phenolic compounds are main reasons in accelerating extract and release of TPCs after drying [23]. Raja et al. [24] also confirmed the significant effects of drying processes (e.g., freeze, oven, and shade-drying) on increasing the measurable TPCs of *Carica papaya* leaves. In another study, significant effect of thermal processing on increasing the TPCs, individual phenolic acids, and antioxidant activities in four cultivars of purple-fleshed sweet potato was reported [25].

Comparison of processed samples demonstrates the highest TPCs was observed in the vacuum oven dried sample due to the low-pressure oxygen in processing chamber, which reduced oxidation of sensitive compounds and also heating effect on releasing bonded phenolic components and improving reaction of them with the Folin-Ciocalteu reagent. At high processing temperatures, changes in molecular structure and polymerization of sensitive phenolic compounds (due to thermal degradation) decrease TPCs of the product. The oxidation of phenolic compounds and a reduction in antioxidant activities of them (especially in caffeic acid, gallic acid, coumaric acid, and gentisic acid) accelerate at high temperatures and long process time.



**Figure 1.** Effects of different processing methods on total phenol content of *Spirulina platensis*

1 - shade-drying; 2 - sun-drying; 3 - oven-drying; 4 - microwave-drying (720 W); 5 - microwave-drying (360 W); 6 - vacuum oven-drying; 7 - freeze-drying; 8 - spray-drying; 9 - non-blanching freezing; 10 - blanching freezing; Control - fresh *Spirulina platensis*.

Conversely, other phenolic compounds (like quercetins, ferulic acid, vanillic acid, and sinapic acid) are stable to thermal degradation, and fewer changes occur in their antioxidant activities at high temperatures [26,27]. Therefore, the presence of oxygen and high temperature precipitated a greater reduction in the TPCs of the oven-dried sample than the vacuum oven-dried sample. In this regard, convective-drying at 70°C was reported as the worst method for drying jujube due to its adverse effects on reducing bioactive compounds [28]. A reduction in the TPCs during cooking is due to leaching into water, degradation during the heat processing, oxidation and/or isomerization by polyphenol oxidase, and/or a combination of these phenomena [25].

The slight destruction and low oxidation intensity of phenols and other antioxidants at low temperatures are major reasons for higher TPCs of frozen samples in comparison with sun, shade, and low-power microwave-dried samples. Enzymatic browning reaction in the presence of oxygen, phenolic compounds, and enzymes (especially polyphenol oxidase) is the most important oxidation reaction of phenolic compounds. Therefore, the reduced TPCs in the frozen samples were due to the oxidation of phenols resulting from the enzymatic browning reaction and/or their auto-oxidation during the freezing process. Blanching vegetables before freezing inactivates the oxidizing enzymes of phenols and can be an appropriate process for better protecting antioxidants such as flavonoid content [17]. However, due to the oxidation and destruction of phenolic compounds through heating at

95°C in the presence of oxygen, no significant difference ( $p > 0.05$ ) was observed between the blanching freezing and non-blanching freezing samples in terms of TPCs.

A lower reduction was observed in the TPCs of the spray-dried sample in comparison with the sun-, shade-, and low-power microwave-dried samples because of the short time of drying. However, due to the oxygen exposure under high temperature of spray drying which resulted in phenolic compounds destruction even in short processing time, the TPCs in this sample was lower in comparison with the vacuum oven-dried sample.

With respect to the sensitivity of phenolic compounds to oxidation when exposed to electromagnetic waves, microwave heating reduced the TPCs of the samples. Reducing the microwave power from 720 W to 360 W increased the drying time and a further reduction in the TPCs of the low-power microwave-dried sample was observed. Similar to the result of present study, drying citrus peel at higher microwave power levels and shorter drying times preserved higher phenolic compound content and reducing microwave power from 600 to 100 W diminished the TPCs of the sample [29].

Exposing *S. platensis* to very low temperatures for a long time in freeze-drying increased the molecular structure changes of phenolic compounds. The activity of degrading enzymes of phenols is another main reason for the reduction in TPCs of the lyophilized sample. Furthermore, phenolic compounds are often found in the external areas of the vacuoles. Therefore, if the cellular structure deteriorates in the drying process, these

compounds are more sensitive to degradation. It has been the other main reason for reducing antioxidant compounds in lyophilized samples [30]. The loss of TPCs in freeze-dried tropical fruit (-50°C for 24 h) due to the decomposition of polyphenols by degrading enzymes was reported by Shofian et al. [31], corresponded with the results of the present study. The higher reduction in TPCs in the sun and shade-dried samples compared with the other samples was a result of the oxidation and degradation of phenolic compounds due to the presence of oxygen and light (solar radiation and ultraviolet) and the enzymatic reaction. Furthermore, a higher reduction in TPCs was observed in the sun-dried sample than in the shade-dried sample. Increasing the loss of TPCs in the shade-dried sample compared with the oven-dried sample was due to the oxidation and destruction of phenolic compounds during longer drying time in the presence of oxygen and the existence of active enzymes. However, the insufficient release of phenols because of the non-appropriate cell wall-polyphenol destruction in this method is another reason of present finding. In this regard, Periche et al. [32], reported an increased loss of flavonoids and phenolic acids in stevia leaves that were shade-dried at 20°C for 30 days compared with hot air-dried at 180°C for 3 min.

#### **DPPH radical scavenging activity**

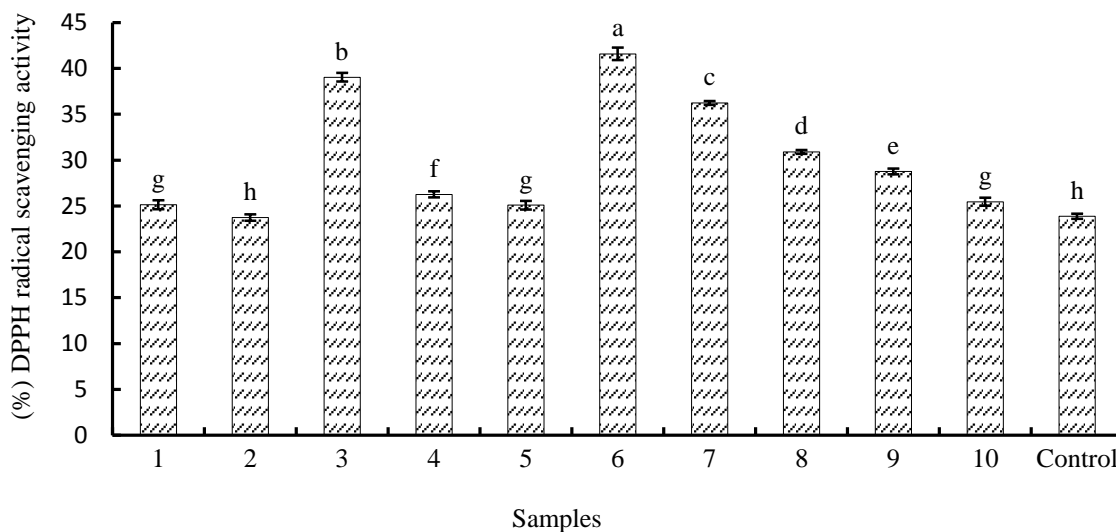
The antioxidant activities of both fresh and processed samples are shown in Figure 2. Significant differences ( $p \leq 0.05$ ) in antioxidant activity were observed between fresh and processed *S. platensis*, except for sun-dried samples. The major antioxidant compounds in *S. platensis* consist of  $\beta$ -Carotene and  $\alpha$ -tocopherol with free radicals scavenging ability, trace elements of selenium and zinc with reduction potential, catalase, peroxidase and superoxide dismutase enzymes through the neutralization of active electrons of free radicals, and other components like phycocyanin pigment and ascorbic acid [33].

The vacuum oven-dried sample had the highest level of antioxidant activity. The TPCs of the vacuum oven dried sample had important impact on its antioxidant activity, and as observed in the previous part, the TPCs of this sample were higher than that of other samples. The most important reactions occurring during hot-air drying are caramelization, the Maillard reaction, the Strecker degradation of dicarbonyls and amino acids, the oxidation of phenolic compounds, and the hydrolysis of glycosides and ester bonds, all of which have significant effects on the antioxidant activity of processed samples. Imines (Schiff base) from the Maillard reaction, Amadori and Heyns products, and dihydrocyclic derivatives, by reducing

hydroperoxides, increase the stability and antioxidant activity of the products. Moreover, melanoidins with chelating metals and redoxes with scavenging free radicals has positive effect on antioxidant activity of the samples [34]. Therefore, various by-products of non-enzymatic browning reactions increased the antioxidant activity of the processed samples in comparison with the fresh sample. Similar to the result of the present research, the antioxidant activities of different varieties of heated onion at high temperatures were also increased compared with unheated onions [27].

On the other hand, chemical changes in the structure of food antioxidants, due to oxidation reactions in thermal processes like cooking, drying, and storing reduce activity of them. In this regard, it was reported that 50% of tocopherols in rye flour destroys during baking [34]. Furthermore, in the natural structure of food, fat globules are protected against oxygen by hydrated protein layers. In dehydrated food, this protective layer is damaged, and fat is exposed to oxygen. Therefore, lipid oxidation in dried foods is accelerated and also free radicals reduce the antioxidant activity of products [34]. Due to the oxidation and structural changes in some antioxidants at high temperatures in the presence of oxygen, the oven-dried sample had lower antioxidant activity than the vacuum oven-dried sample. Convective-heating is considered as the worst method for drying some products due to the adverse effects on reducing antioxidant properties [28].

The antioxidant activity of freeze-dried *S. platensis* was less than that of the oven-dried samples due to a reduction in non-enzymatic browning reactions in freeze-drying. Rupturing and release of oxidative and hydrolytic enzymes from the cell walls, due to the formation of ice crystals, has important effect on deterioration of phenolic and antioxidant compounds of the freeze-dried samples [24]. Nevertheless, the freeze-dried *S. platensis* had higher level of antioxidant activity than the sun, shade-microwave, spray-dried and frozen samples, which consequently were attributed to lower oxidation and structural changes of antioxidants under reduced temperatures and oxygen pressure. Freezing conditions have also significant effects on quality of processed products. Increasing the freeze processing rate in freeze dried sample reduces the size of the ice crystals and the cell wall damage caused by them than that of conventional freezing method. Therefore, the loss of antioxidant compounds in frozen samples due to release of them from damaged cell was more than it in the freeze-dried sample.



**Figure 2.** Effects of different processing methods on antioxidant activity of *Spirulina platensis*

1 - shade-drying; 2 - sun-drying; 3 - oven-drying; 4 - microwave-drying (720 W); 5 - microwave-drying (360 W); 6 - vacuum oven-drying; 7 - freeze-drying; 8 - spray-drying; 9 - non-blanching freezing; 10 - blanching freezing; Control - fresh *Spirulina platensis*.

Similar to the effect of drying on *S. platensis*, dried *Cacao pod husks* by freeze-drying had greater phenolic content and antioxidant activity compared with the hot-air dried and fresh samples [35]. Wojdylo et al. [28] also reported freeze-drying as a convenient method for preparing high quality dried jujube in terms of antioxidant activity. The antioxidant activity of the spray-dried sample, due to a reduction in heat exposure time was higher than that of the shade, sun, and microwave-dried samples. Due to short drying periods in spray-drying, the non-enzymatic browning reaction and the formation of by-products with antioxidant activity were decreased. Consequently, there was less antioxidant activity in this sample than in the oven-dried sample. In microwave-drying, electromagnetic waves penetrates in the food and, by stimulating friction in the polar molecules (specifically, water), heat is produced. In microwave heating, about 10% of antioxidants and especially  $\alpha$ -tocopherols are destroyed in 6 minutes (critical time), after which, the destruction increases up to 40%. In microwave heating, acceleration of fat oxidation and production of hydroperoxides and other secondary products induce degradation of antioxidant components [34]. Therefore, the vacuum oven, oven, spray, and freeze-dried samples, and also non-blanching frozen sample had more antioxidant activity than the microwave-dried sample. Oxidation at atmospheric pressure reduces antioxidant activity of the samples. Furthermore, increasing processing time by reducing the microwave power from 720 W to 360 W was intensified molecular structure changes and antioxidant components polymerization. Therefore, drying at higher microwave

power and shorter drying times preserves higher content of antioxidant components [29].

The oxidation of antioxidants in sun and shade-dried samples, due to the presence of oxidizing agents, reduced the antioxidant activities of the samples more than processed ones with other methods. In the freezing, due to the physical damage to cellular material by the ice crystals formation, antioxidants are exposed to oxidizing agents. In this regard, more oxidative change in the antioxidants of frozen food than in the refrigerated food was reported by Pokorny et al. [34]. In addition, the lower antioxidant activity in the frozen samples compared with the oven, spray, and freeze-dried samples was due to the absence of appropriate conditions for non-enzymatic browning reaction and not producing by-products with antioxidant activities in freezing. A reduction in the antioxidant activity of the blanching freezing sample compared to the non-blanching freezing sample was attributed to oxidation and destruction of the antioxidants structure in the presence of oxidizing agents like oxygen and high temperature process and also leaching them in blanching process.

Therefore, due to special structural properties and components in *S. platensis*, such as significant amount of polyunsaturated fatty acids (PUFA) and high water activity, the intensity of oxidation and destruction of antioxidants structure and activity reduction of them in processed *S. platensis* were significantly affected with respect to different conditions in various drying methods (e.g., heating method, temperature, processing time, presence or absence of oxygen and light).

### Mineral content

The mineral contents of the fresh and processed *S. platensis* are shown in Table 2. Due to the stable nature of minerals, different drying methods had no significant effect ( $p > 0.05$ ) on their content in dried *S. platensis*. Other researchers also reported thermal processing, like drying, did not significantly change the mineral content of the heated vegetables, like *Aloe vera* [36].

Among the different treatments, only blanched and non-blanched freezing reduced significantly ( $p \leq 0.05$ ) the sodium, potassium, magnesium, manganese, calcium, and phosphorus contents of samples. A reduction in the minerals of frozen samples was due to leaching them in blanching water or drip loss of defrosted samples. In this regard, blanching and cooking leafy vegetables like kale and spinach before freezing significantly reduced the phosphorus, potassium, calcium, magnesium, and manganese contents. Only the sodium content of vegetables cooked in 2% brine increased significantly [5].

According to the literature review, the slight changes in the mineral content of processed samples are due to leaching in physical and mechanical treatments or transferring through the processing instruments or food containers. For instances, cooking in galvanized containers or storing in zinc oxide-coated packages increase the zinc content of food. Furthermore, minerals are mostly affected by milling, soaking, cooking, germinating, and fermenting processes. Loss of mineral occurs through leaching into the cooking water, and this loss is reduced in steamed foods compared with boiled foods [37]. In addition to the effects of processing conditions on mineral content, bioavailability of minerals and their solubility is affected by processing due to the destruction of the cell wall, denaturation of proteins, release of organic acids, and activity of inhibitor chelating agents like phytic acid or tannins. The by-products of the Maillard reaction during dehydration have opposite effects on the solubility of minerals such as zinc through connection with them. However, the intensity of the connection depends on the rate of non-enzymatic browning reactions and the nature of food proteins [37].

### Fatty acids composition

The compositions of fatty acids in the fresh and processed *S. platensis* are presented in Table 3. USFA was a significant portion of the fatty acids profile, where the PUFA level was higher compared with the monounsaturated fatty acids (MUFA). In the fresh and processed microalga, PUFA consisted of linoleic acid, GLA, docosahexaenoic acid,  $\alpha$ -linolenic acid, arachidonic acid, and eicosapent-aenoic acid; MUFA consisted of oleic

acid, palmitoleic acid, vaccenic acid, and 9-eicosanoic acid; and the saturated fatty acids (SFA) consisted of palmitic acid, stearic acid, and myristic acid with the highest levels, respectively. More changes were observed in the content and composition of USFA in comparison with SFA in processed *S. platensis*. It is attributed to the greater sensitivity of the unsaturated bonds of fatty acids to thermal processes in comparison with saturated bonds. Oxidation is one of the most important effective factors on changes the fatty acids profile which occur in the two forms of auto-oxidation and photo-oxidation [38]. Various factors like the degree of USFA, the presence of Fe and Cu, oxygen, light, high temperatures, long process time, and high water activity of food accelerate the oxidation of fatty acids. Other important reactions are isomerization and polymerization. USFA has a great affinity for isomerization and formation of geometric or positional isomers. The isomerisation of double bonds in different places of the fatty acid carbon chain changes the position of fatty acid carbons configuration [38]. In terms of total SFA content, oven-dried (41.13%), low power microwave-dried (41.48%), sun-dried (41.89%), and high power microwave-dried (41.95%) samples had maximum differences with the fresh *S. platensis* (42.14%), respectively. Among the different treatments, freeze and spray-dried samples had the least difference with fresh *S. platensis* in PUFA and MUFA content. Freezing had a slight effect on the fatty acids profile of the sample. Investigation the effects of freezing at  $-15^{\circ}\text{C}$  and  $-25^{\circ}\text{C}$  on SFA and USFA (18 carbons) in milk and cheese confirmed present result [39]. The USFA content of dried soybean with convention oven ( $40^{\circ}\text{C}$ ,  $60^{\circ}\text{C}$ , and  $100^{\circ}\text{C}$ ) and microwave heating ( $40^{\circ}\text{C}$  and  $60^{\circ}\text{C}$ ) methods decreased significantly ( $p \leq 0.05$ ) [40]. In another study, a significant reduction in MUFA (oleic, eicosaenoic and erucic acid) and PUFA (linoleic and linolenic acid) content of processed cress seed on roasting ( $150^{\circ}\text{C}$  for 3 min) and boiling ( $95^{\circ}\text{C}$  for 15 min) was reported due to oxidative reactions and decomposition of triglycerides. As well as, a slight increase in palmitic, stearic, and arachidic acid of roasted sample was reported [41]. Therefore, changes in the fatty acids profile of processed *S. platensis* is attributed to oxidation (auto-oxidation and photo-oxidation), isomerization, and the polymerization of fatty acids under different conditions. An increase in temperature and drying process time accelerated the degradation and destruction of USFA [41]. Furthermore, the presence of Fe and Cu and the high water activity of microalga accelerated the oxidation of fatty acids.

**Table 2.** Effects of different processing methods on mineral contents of *Spirulina platensis*

Samples	minerals (mg 100 g <sup>-1</sup> )								
	Na	K	Mg	Mn	Ca	P	Fe	Zn	Cu
SHD	1266.65 <sup>a</sup> ±0.03	2204.72 <sup>a</sup> ±0.02	562.92 <sup>a</sup> ±1.03	16.71 <sup>a</sup> ±0.26	642.33 <sup>a</sup> ±2.08	1566.67 <sup>a</sup> ±0.02	231.33 <sup>a</sup> ±0.58	8.55 <sup>a</sup> ±0.09	0.68 <sup>a</sup> ±0.02
SD	1266.65 <sup>a</sup> ±0.02	2204.72 <sup>a</sup> ±0.03	562.90 <sup>a</sup> ±0.01	16.71 <sup>a</sup> ±0.26	642.00 <sup>a</sup> ±1.73	1566.67 <sup>a</sup> ±0.03	231.67 <sup>a</sup> ±1.15	8.55 <sup>a</sup> ±0.10	0.68 <sup>a</sup> ±0.01
OD	1266.66 <sup>a</sup> ±0.02	2204.73 <sup>a</sup> ±0.01	562.92 <sup>a</sup> ±1.01	16.72 <sup>a</sup> ±0.26	642.00 <sup>a</sup> ±1.00	1566.68 <sup>a</sup> ±0.03	231.33 <sup>a</sup> ±0.58	8.56 <sup>a</sup> ±0.04	0.68 <sup>a</sup> ±0.02
MD (720 W)	1266.66 <sup>a</sup> ±0.01	2204.72 <sup>a</sup> ±0.01	562.90 <sup>a</sup> ±0.01	16.71 <sup>a</sup> ±0.31	642.00 <sup>a</sup> ±1.73	1566.67 <sup>a</sup> ±0.14	231.33 <sup>a</sup> ±0.58	8.55 <sup>a</sup> ±0.05	0.69 <sup>a</sup> ±0.01
MD (360 W)	1266.67 <sup>a</sup> ±0.02	2204.72 <sup>a</sup> ±0.01	562.91 <sup>a</sup> ±0.00	16.72 <sup>a</sup> ±0.23	642.00 <sup>a</sup> ±0.00	1566.67 <sup>a</sup> ±0.03	231.67 <sup>a</sup> ±0.58	8.56 <sup>a</sup> ±0.04	0.68 <sup>a</sup> ±0.00
VOD	1266.65 <sup>a</sup> ±0.02	2204.73 <sup>a</sup> ±0.00	562.92 <sup>a</sup> ±1.01	16.71 <sup>a</sup> ±0.25	642.00 <sup>a</sup> ±1.00	1566.66 <sup>a</sup> ±0.17	231.33 <sup>a</sup> ±0.58	8.56 <sup>a</sup> ±0.03	0.68 <sup>a</sup> ±0.01
FD	1266.66 <sup>a</sup> ±0.01	2204.74 <sup>a</sup> ±0.02	562.92 <sup>a</sup> ±1.01	16.72 <sup>a</sup> ±0.26	642.33 <sup>a</sup> ±0.58	1566.67 <sup>a</sup> ±0.02	231.67 <sup>a</sup> ±1.53	8.57 <sup>a</sup> ±0.05	0.69 <sup>a</sup> ±0.02
SPD	1266.65 <sup>a</sup> ±0.14	2204.74 <sup>a</sup> ±0.01	562.92 <sup>a</sup> ±1.42	16.72 <sup>a</sup> ±0.26	642.33 <sup>a</sup> ±2.52	1566.68 <sup>a</sup> ±0.15	231.33 <sup>a</sup> ±0.58	8.57 <sup>a</sup> ±0.06	0.69 <sup>a</sup> ±0.02
NBF	957.50 <sup>b</sup> ±2.03	1820.48 <sup>b</sup> ±0.03	333.58 <sup>b</sup> ±0.58	14.37 <sup>b</sup> ±0.05	322.67 <sup>b</sup> ±2.52	1347.00 <sup>b</sup> ±1.48	231.33 <sup>a</sup> ±0.58	8.55 <sup>a</sup> ±0.05	0.68 <sup>a</sup> ±0.02
BF	957.37 <sup>b</sup> ±2.19	1820.46 <sup>b</sup> ±0.04	333.62 <sup>b</sup> ±1.49	14.36 <sup>b</sup> ±0.06	323.00 <sup>b</sup> ±2.00	1346.33 <sup>b</sup> ±1.28	231.33 <sup>a</sup> ±1.15	8.56 <sup>a</sup> ±0.04	0.68 <sup>a</sup> ±0.02
Fresh <i>S. platensis</i>	1266.67 <sup>a</sup> ±0.00	2204.74 <sup>a</sup> ±0.00	562.92 <sup>a</sup> ±1.37	16.72 <sup>a</sup> ±0.25	642.33 <sup>a</sup> ±0.58	1566.68 <sup>a</sup> ±0.02	231.67 <sup>a</sup> ±0.58	8.56 <sup>a</sup> ±0.04	0.69 <sup>a</sup> ±0.01

Values are presented as mean±SD (n=3).

Values followed by the same letter, within the same column, were significantly different (p≤0.05) according to the Least Significant Difference (LSD) test.

SHD - shade-drying; SD - sun-drying; OD - oven-drying; MD - microwave-drying; VOD - vacuum oven-drying; FD - freeze-drying; SPD - spray-drying; NBF - non-blanched freezing; BF - blanched freezing.

**Table 3.** Fatty acids composition in fresh and processed samples

Samples	Fatty acids profile (%)												
	C14:0	C16:0	C16:1	C18:0	C18:1, n9	C18:1, n11	C18:2	C18:3, n6	C18:3, n3	C20:1	C20:4	C20:5	C22:6
SHD	0.98 <sup>a</sup> ±0.03	37.80 <sup>ab</sup> ±0.03	2.89 <sup>cd</sup> ±0.03	3.34 <sup>b</sup> ±0.02	8.03 <sup>d</sup> ±0.03	0.75 <sup>cd</sup> ±0.02	24.81 <sup>e</sup> ±0.00	13.71 <sup>abc</sup> ±0.04	1.79 <sup>b</sup> ±0.00	0.54 <sup>bcd</sup> ±0.03	0.30 <sup>c</sup> ±0.00	0.35 <sup>h</sup> ±0.04	3.92 <sup>c</sup> ±0.03
SD	0.97 <sup>a</sup> ±0.06	37.61 <sup>b</sup> ±0.01	1.99 <sup>f</sup> ±0.02	3.31 <sup>bc</sup> ±0.02	7.71 <sup>f</sup> ±0.02	0.80 <sup>a</sup> ±0.01	29.91 <sup>b</sup> ±0.02	12.39 <sup>d</sup> ±0.04	1.02 <sup>h</sup> ±0.03	0.51 <sup>d</sup> ±0.01	0.17 <sup>e</sup> ±0.01	0.44 <sup>g</sup> ±0.01	3.12 <sup>c</sup> ±0.00
OD	0.83 <sup>b</sup> ±0.03	36.92 <sup>d</sup> ±0.26	2.71 <sup>e</sup> ±0.04	3.39 <sup>a</sup> ±0.02	8.19 <sup>a</sup> ±0.02	0.73 <sup>d</sup> ±0.00	31.70 <sup>a</sup> ±0.05	13.40 <sup>c</sup> ±0.02	1.09 <sup>g</sup> ±0.01	0.55 <sup>abc</sup> ±0.00	0.30 <sup>c</sup> ±0.01	0.00 <sup>j</sup> ±0.00	0.00 <sup>a</sup> ±0.00
MD (720 W)	0.92 <sup>ab</sup> ±0.07	37.79 <sup>ab</sup> ±0.01	2.94 <sup>abc</sup> ±0.01	3.24 <sup>d</sup> ±0.00	8.18 <sup>ab</sup> ±0.00	0.79 <sup>ab</sup> ±0.00	25.00 <sup>ef</sup> ±0.00	13.79 <sup>abc</sup> ±0.01	1.43 <sup>e</sup> ±0.00	0.54 <sup>bcd</sup> ±0.00	0.33 <sup>bc</sup> ±0.00	2.24 <sup>b</sup> ±0.00	2.82 <sup>f</sup> ±0.03
MD (360 W)	1.00 <sup>a</sup> ±0.02	37.18 <sup>c</sup> ±0.14	2.92 <sup>bc</sup> ±0.03	3.30 <sup>c</sup> ±0.00	6.30 <sup>g</sup> ±0.00	0.76 <sup>c</sup> ±0.00	25.00 <sup>ef</sup> ±0.00	13.57 <sup>bc</sup> ±0.00	1.30 <sup>f</sup> ±0.00	0.43 <sup>f</sup> ±0.03	0.23 <sup>d</sup> ±0.00	3.74 <sup>a</sup> ±0.05	4.26 <sup>a</sup> ±0.01
VOD	0.98 <sup>a</sup> ±0.03	37.82 <sup>ab</sup> ±0.06	2.98 <sup>a</sup> ±0.03	3.38 <sup>a</sup> ±0.02	7.80 <sup>e</sup> ±0.01	0.76 <sup>c</sup> ±0.00	25.95 <sup>c</sup> ±0.05	13.88 <sup>ab</sup> ±0.59	1.61 <sup>d</sup> ±0.00	0.57 <sup>ab</sup> ±0.02	0.17 <sup>e</sup> ±0.03	0.00 <sup>i</sup> ±0.00	3.65 <sup>d</sup> ±0.04
FD	0.99 <sup>a</sup> ±0.01	37.80 <sup>ab</sup> ±0.01	2.95 <sup>ab</sup> ±0.00	3.32 <sup>bc</sup> ±0.00	8.15 <sup>bc</sup> ±0.02	0.79 <sup>a</sup> ±0.01	24.94 <sup>f</sup> ±0.06	13.63 <sup>abc</sup> ±0.00	1.58 <sup>d</sup> ±0.02	0.55 <sup>abc</sup> ±0.01	0.33 <sup>bc</sup> ±0.01	0.91 <sup>e</sup> ±0.00	4.06 <sup>b</sup> ±0.06
SPD	0.99 <sup>a</sup> ±0.02	37.85 <sup>a</sup> ±0.03	2.93 <sup>abc</sup> ±0.02	3.38 <sup>a</sup> ±0.02	8.19 <sup>ab</sup> ±0.01	0.74 <sup>cd</sup> ±0.03	25.07 <sup>d</sup> ±0.00	13.83 <sup>ab</sup> ±0.04	1.79 <sup>b</sup> ±0.01	0.56 <sup>ab</sup> ±0.04	0.00 <sup>f</sup> ±0.00	0.82 <sup>f</sup> ±0.03	3.88 <sup>a</sup> ±0.06
NBF	0.99 <sup>a</sup> ±0.01	37.88 <sup>ab</sup> ±0.02	2.94 <sup>abc</sup> ±0.01	3.30 <sup>c</sup> ±0.00	8.14 <sup>c</sup> ±0.00	0.76 <sup>c</sup> ±0.02	24.78 <sup>e</sup> ±0.02	13.68 <sup>abc</sup> ±0.02	1.66 <sup>c</sup> ±0.02	0.52 <sup>cd</sup> ±0.00	0.37 <sup>a</sup> ±0.00	1.18 <sup>c</sup> ±0.04	3.91 <sup>c</sup> ±0.02
BF	0.99 <sup>a</sup> ±0.13	37.79 <sup>ab</sup> ±0.01	2.85 <sup>d</sup> ±0.05	3.29 <sup>c</sup> ±0.02	8.03 <sup>d</sup> ±0.04	0.76 <sup>c</sup> ±0.01	24.70 <sup>h</sup> ±0.02	13.56 <sup>bc</sup> ±0.04	1.58 <sup>d</sup> ±0.04	0.47 <sup>e</sup> ±0.00	0.34 <sup>ab</sup> ±0.04	1.00 <sup>d</sup> ±0.01	3.89 <sup>a</sup> ±0.01
Fresh <i>S. platensis</i>	0.99 <sup>a</sup> ±0.02	37.83 <sup>a</sup> ±0.00	2.95 <sup>ab</sup> ±0.00	3.32 <sup>bc</sup> ±0.00	8.17 <sup>ab</sup> ±0.00	0.79 <sup>ab</sup> ±0.00	25.00 <sup>de</sup> ±0.00	13.99 <sup>a</sup> ±0.00	1.84 <sup>a</sup> ±0.00	0.58 <sup>a</sup> ±0.00	0.32 <sup>bc</sup> ±0.00	0.00 <sup>j</sup> ±0.00	4.29 <sup>a</sup> ±0.00

Values are presented as mean±SD (n=3).

Values followed by the same letter, within the same column, were significantly different (p≤0.05) according to the LSD Test.

SHD - shade-drying; SD - sun-drying; OD - oven-drying; MD - microwave-drying; VOD - vacuum oven-drying; FD - freeze-drying; SPD - spray-drying; NBF - non-blanched freezing; BF - blanched freezing.



## 4. Conclusion

Processing conditions are effective parameters on qualitative properties of products such as antioxidant activity, total phenolic content and changes in fatty acid composition or mineral status of product. Due to perishable and nutritious nature of the fresh *S. platensis* and lack of sufficient information about effect of processing on nutraceutical components of *S. platensis*, in the present research, effect of different methods of drying and freezing on quality of this product was considered.

Total phenolic content and antioxidant activity of the processed samples were increased in comparison to the fresh one significantly ( $p \leq 0.05$ ). It was due to the destruction of the microalga cell wall in processing, conversion of bonded phenolic compounds to free form and improvement of their extraction. Furthermore, production of new compound such as melanoidin affected the TPCs and antioxidant activity of dried samples. The lower antioxidant activity of frozen samples compared with the oven, spray, and freeze-dried samples was due to the absence of appropriate conditions for production of non-enzymatic browning reaction by-products with antioxidant activity. Oxidation and destruction of components in the presence of oxygen and high temperature and also leaching them to water were reduced antioxidant activity of blanched product. The highest TPCs and antioxidant activities were recorded in *S. platensis* treated with the vacuum oven-drying method because of the absence of oxygen.

The concentrations of minerals remained reasonably constant in the dehydrated samples; only in frozen ones, significant reductions in sodium, potassium, magnesium, manganese, calcium, and phosphorus contents of *S. platensis* were observed due to leaching them in blanching water or drip loss of defrosted samples. The results of this study indicate that *S. platensis* has an excellent fatty acids profile and is a rich source of essential fatty acids, specifically, GLA. PUFA level was higher compared with the MUFA. More changes were observed in the content and composition of USFA in comparison with SFA of processed *S. platensis*. Changes in the fatty acids profile of processed *S. platensis* were attributed to oxidation (auto-oxidation and photo-oxidation), isomerization, and the polymerization of fatty acids under different conditions. An increase in temperature and drying process time accelerated the degradation and destruction of USFA. Furthermore, special nature of the fresh product and the presence of components with pro-oxidant activity such as minerals accelerated the oxidation of fatty acids. In general, spray and freeze-drying were introduced as the best methods in protecting UFA, and vacuum oven-drying was preferred in preserving total phenol and antioxidant activity of the *S. platensis*.

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## 6. Conflict of Interest

The authors declare no conflict of interest.

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## تأثیر روش‌های مختلف فرآوری بر ترکیبات شیمیایی و فعالیت ضداکسایشی ریز جلبک *Spirulina platensis*

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### چکیده

**سابقه و هدف:** اسپیرولینا پلاتنسیس یکی از منابع عمده ترکیبات فراسودمند با خواص غذا دارویی است. این محصول بسیار فسادپذیر است و لازم است بلافاصله پس از برداشت فرآوری شود. بنابراین، هدف اصلی مطالعه حاضر بررسی اثر شرایط متفاوت فرآیند بر مهمترین ویژگی‌های کیفی اسپیرولینا پلاتنسیس بود.

**مواد و روش‌ها:** اسپیرولینا پلاتنسیس تازه با روش‌های گوناگون (خشک کردن در سایه، خشک کردن در آفتاب، خشک کردن در گرمخانه، خشک کردن در ماکروویو، خشک کردن در گرمخانه تحت خلا، خشک کردن انجمادی، خشک کردن پاششی و انجماد با و بدون آنزیم‌بری) تحت فرآوری قرار گرفت و تغییرات ویژگی‌های کیفی آن (مواد معدنی، ترکیب اسیدهای چرب، میزان کل ترکیبات فنولیک و فعالیت ضداکسایشی نمونه‌ها بررسی شد.

**یافته‌ها و نتیجه‌گیری:** شرایط فرآوری اثر معنی‌داری بر ویژگی‌های کیفی نمونه‌ها داشت ( $p \leq 0.05$ ). نمونه خشک شده در گرمخانه تحت خلا بیشترین فعالیت ضداکسایشی و بالاترین محتوی ترکیبات فنولیک را داشت، چراکه انجام فرایندهای شیمیایی تخریبی وابسته به اکسیژن و واکنش‌های قهوه‌ای شدن آنزیمی در این روش کمتر است. میزان مواد معدنی در نمونه‌های خشک شده تفاوت معنی‌دار آماری باهم نداشتند ( $p \leq 0.05$ ), درحالی که میزان سدیم، پتاسیم، منگنز، منیزیم، کلسیم و فسفر نمونه‌های منجمد به‌طور معنی‌داری کاهش یافته بود. اسیدهای چرب ضروری غیراشباع گوناگون مانند آلفا لینولنیک اسید، گاما لینولنیک اسید، آراشیدونیک اسید، ایکوزاپنتانویک اسید و دوکوزاهگزانویک اسید در اسپیرولینا شناسایی شدند. براین اساس، خشک کردن پاششی و خشک کردن انجمادی، بهترین روش‌های فرآوری برای حفظ اسیدهای چرب غیر اشباع بودند، و از نظر حفظ ترکیبات فنولیک کل و ویژگی‌های ضد اکسایشی اسپیرولینا پلاتنسیس، روش خشک کردن در گرمخانه تحت خلا ارجح بود.

**تعارض منافع:** نویسندگان اعلام می‌کنند که هیچ نوع تعارض منافی مرتبط با انتشار این مقاله ندارند.