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Effect of Different Processing Methods on Stability of Anthocyanin and Phycocyanin of *Spirulina platensis*

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ABSTRACT: A blue-green *Spirulina* microalgae is a *Cyanobacterium*, that is one of the most interesting functional sources of food ingredients with nutraceutical properties. It is a perishable product and should be processed immediately after harvesting. In the present study the stability and changes colorimetric properties and optical density of extracted phycocyanin and also anthocyanin content of *Spirulina platensis* after different processing condition (shade, sun, oven, microwave, vacuum oven, freeze- and spray-drying and freezing with and/or without blanching) were investigated. The results indicated the processing condition significantly affected the pigments content of sample. Non-blanched freezing was preferred in pigments conservation. In dehydrated samples, the freeze-dried sample had the least change in optical density of extracted phycocyanin than fresh sample. The levels of anthocyanin in frozen, spray-dried, freeze-dried and microwave-dried samples in comparison to fresh *Spirulina* were increased significantly ($P< 0.05$). In this regards, spray drying could be a practical drying method for processing *SP*, although, freezing is preferred.

Keywords: Bioactive Compounds, Blanching, Drying, Freezing, Optical Density, *Spirulina platensis.*

Introduction

Spirulina platensis (*sp*) is a *Cyanobacterium* in a member of *Oscillatoriaceae*. The origin of this blue-green microalga is dated back to 3500 million years (Desmorieux and Decaen, 2005). The natural growing of *Sp* is found in saline and highly alkaline lakes or shallow ponds of the tropics that it is very easy to harvest. *Sp* has 3.5–10 μm wide cells that are formed into long strands which look similar to a coiled spring (Chamorro-Cevallos *et al*., 2008; Lupatini *et al*., 2016). The cell-wall of *Sp* is composed of four layers (LI, LII, LIII and LIV). All layers are very weak, except LII which is made of peptidoglycan, and provides the rigidity of cell-wall. Protein and lipopolysaccharide nature of the cell-wall are favorable for easy digestion of *Sp* by humans (Eykelenburg, 1977).

Sp is a suitable source of food with different beneficial advantage for human health. It has pharmacological properties including antiviral, anti-bacterial, anti-platelet, anti-cardiotoxic, hypocholesterolemic, anti-nephrotoxic, and anti-hepatoxic effects and prevent cataracts, acute allergic rhinitis, cerebral ischemia, vascular reactivity cadmium, arsenic toxicities and experimental Parkinson's (Chamorro-Cevallos *et al*., 2008). In chemical components, *SP* has almost perfect balance of protein (50-60%), including the eight essential amino acids. It is easily digested, assimilated and

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satisfied starvation quickly. *SP* contains large quantities of carbohydrate, vitamins (particularly vitamin B_{12}), minerals, polyunsaturated fatty acids, particularly gamma linolenic acid (Casazza *et al*., 2015; Chamorro-Cevallos *et al*., 2008; Desmorieux *et al*., 2005). Other valuable nutrients, such as phycocyanin, phenolic compounds, sulfolipids, glycolipids, and antioxidant enzymes like superoxide dismutase, catalase and peroxidase also reported in *SP* (Antelo *et al*., 2008; Oliveira *et al*., 2009; Sami-Ismaiel *et al*., 2016). Phycocyanin is an accessory photosynthetic pigment from phycobiliprotein family. In cyanobacteria, different species of *Sp* are rich sources of this pigment. It is used in food products as a natural nutritious and coloring agent. It is a potential therapeutic agent in the oxidative treatment and as a fluorescent marker in research (Antelo *et al*., 2008). Anthocyanins are bioactive compounds in many fruits and vegetables. Anthocyanins are polyphenols components with antioxidant activity, plays an important role in the prevention of neuronal and cardiovascular diseases, cancer and diabetes (Patras *et al*., 2010). Phycocyanin and anthocyanins are two important pigments in *SP*.

Fresh *Sp* usually contains 75–80% water, and in order to assure microbiological stability and to provide longer shelf life, the humidity must be decreased to less than 15%. Dehydration is one of the oldest methods of preserving food. The qualitative properties of dried products are affected by drying method and their conditions (Naidu *et al*., 2016; Hossain *et al*., 2010). Freezing is another common preservative technique for perishable vegetables, which protects nutritional qualitative of foods better than any other methods. Despite the freezing has many advantages than other preservative methods, it is not completely preventing enzymatic reactions and microbial growth and usually accompanied with other processes such as blanching (Mazzeo *et al*., 2015).

In order to investigate the effect of preservative methods on important pigments of *Spirulina platensis*, different processing treatments such as shade-, sun-, oven-, vacuum oven-, microwave-, freeze- and spray-drying and also freezing were developed and effect of various methods on color properties and optical density (OD) of extracted phycocyanin of processed samples, and also anthocyanins content of them were identified.

Materials and Methods

–*Chemicals and materials*

Fresh *Sp* biomass was purchased from Institution of Green Foundation, Research Center, Qeshm, Iran. The sample was transported to the laboratory under cool condition. The quantities of moisture, ash, protein, fat, crude fiber and pH of the microalgae were immediately measured upon arrival (AOAC, 2007; AOAC, 2010). The results are presented in Table 1. Subsequently, microalgae were subjected to various processing methods.

Table 1. Chemical characteristics of *Spirulina platensis* microalgae (g $100g^{-1}$ 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)

Ethylene Diamine Tetra Acetic acid (EDTA), ethanol, potassium chloride buffer, sodium acetate buffer, *sodium* ***di***hydrogen *phosphate* and disodium hydrogen *phosphate* were from Merck (Darmstadt, Germany).

– *Drying methods*

Before drying, for all treatments, the *Sp* was spread uniformly on suitable container in 1cm thickness. After drying, it was kept in a polypropylene bag in refrigerator (4 °C).

In shade-drying (SHD): Sample was dried at 25±2°C and 70±2% relative humidity (RH) that was well-ventilated with the artificial airflow with a fan for 48 h. The final moisture content of the dried sample was 9% (wet basis).

In sun-drying (SD): Sample was dried under direct sunlight within 40±2°C and 46±2% RH for 24 h. The final moisture content of the dried sample was 3-4% (wet basis).

In oven-drying (OVD): The drying was carried out at 80°C for 10-11 h. The final moisture content of the dried sample was 5–6% (wet basis).

In microwave-drying (MD): Two microwave irradiation powers (720 W for 5 min and 360 W for 9 min) were applied for drying samples. The final moisture content of the dried sample was 6–7% (wet basis).

In vacuum oven-drying (VOD): Sample was dried in a vacuum oven (Memmert, Germany) at 65°C for 12-14 h in 0.07 MPa. The final moisture content of the dried sample was 6–7% (wet basis).

In freeze-drying (FD): Sample was frozen and dried in a freeze dryer (Telstar, Spain) at -80°C and dried in 10 Mbar for 22-24 h. The final moisture content of the dried sample was 7% (wet basis).

In spray-drying (SPD): Aqueous *Sp* (in a ratio of 1:1), was dried through an industrial plant spray dryer (Maham Co., Iran). Drying conditions were defined as follows: feed temperature at 25°C, inlet temperature of slurry at 170°C, outlet temperature of dry algae at 90°C, atomization airflow rate of 400 L/h, liquid feed pump rate of 25 m³/h. Spray drying duration was approximately 60 min. The final moisture content of the dried sample was 1-2% (wet basis).

– *Freezing methods*

In freezing without pretreatment blanching (NBF): Sample was placed in Falcon Conical-Bottom centrifuge tubes. Oxygen content of head space was replaced with nitrogen gas and was frozen in the freezer (Philver, Iran). Sample was stored at -20°C for 1 month.

In freezing with pretreatment blanching (BF): The *Sp* was steam blanched at 95°C for 5 min and was frozen according to the previous section.

– *Color properties and optical density of extracted C-phycocyanin*

Phycocyanin of *Sp* was extracted according to the method introduced by Baussiba and Richmond (1979). Approximately 7-10 g of microalgae sample was suspended in 200 mL of 0.1 M *sodium* phosphate buffer pH 7.0 containing 100 μg mL^{-1} lyzozyme and 10 mM EDTA. The enzymatic disintegration of the cell-wall was occurred in a shaking bath at 30 °C for 24 h. The slurry was centrifuged for 1 h at 4000 rpm to separate the cell debris. The color properties of the extracted phycocyanin from the fresh and processed *Sp* microalgae was measured through the colorimeter (FMS jansen Hunter Lab, Germany), in the reflectance mode at CIE L*, a*, b* color scale. The L* value represents lightness/darkness, a* refers to red/green color and the b* refers to blue/yellow color (Ciurzynska *et al*., 2014;

Sinthusamran and Benjakul, 2014). The Chroma was calculated through Eq. (1):

$$\text{Chroma} = \sqrt{(a^2 + b^2)} \quad \text{Eq. (1)}$$

The Hue angle was calculated through Eq. (2):

$$\text{Hue angle} = \tan^{-1}\frac{b*}{a*} \quad \text{Eq. (2)}$$

Furthermore, the absorbance of the clear blue supernatants at 615 nm through phosphate buffer as a blank was evaluated as a measure of phycocyanin concentration.

– *Anthocyanin content*

The total anthocyanin content was determined using pH differential method. The pH differential method has been widely used to determine total monomeric anthocyanins of fruits and vegetables. Theoretically, anthocyanins appear in the oxonium form at pH 1.0 and the hemiketal form at pH 4.5. The structural transformation of anthocyanins under different pH values will exhibit different UV–Vis absorptions, and this process is reversible. Therefore, the difference in UV–Vis absorbance is proportional of anthocyanins concentration. The extract was prepared by mixing sample (2 g) with 20 ml ethanol and centrifuge (sigma, Germany) at 3500 rpm for 10 minute. The supernatant was separated and all the remaining pigments were washed with another 20 ml of ethanol. The supernatant was collected in 50 ml volumetric flask with ethanol. The total anthocyanin content was determined spectrophotometrically at pH 1.0 (0.025 M potassium chloride buffer) and at pH 4.5 (0.4 M sodium acetate buffer). The absorbance of the sample was calculated according to Eq. (3): (Leong and Oey, 2012)

$$A = (A\lambda \text{ vis-max} - A700)\ \text{pH } 1.0 - (A\lambda \text{ vis-max} - A700)\ \text{pH } 4.5 \quad \text{Eq. (3)}$$

where A is the sample absorbance, Aλ vis-max is the maximum sample absorbance reading at specific wavelength for pH 1.0 and pH 4.5 (510 nm), while A700 is the absorbance reading at 700 nm for pH 1.0 and pH 4.5.

The concentrations were expressed as milligrams per gram of dried samples and calculated using appropriate extinction coefficients of the major anthocyanin compounds of interest using Eq (4) (Jiang *et al*., 2017; Monica Giusti and Wrolstad, 2001):

$$\text{Anthocyanin (mg/g)} = \frac{A \times MW \times DF \times V \times 10^3}{1 \times M \times \varepsilon} \quad (4)$$

MW is the molecular weight (449.2 g/mol) of cyanidin-3-glucoside, DF is the dilution factor, V is the distilling volume (L), ε is the molar extinction coefficient (29,600) of cyanidin-3-glucoside, l is the path length and M is the weight of sample (g).

– *Statistical analysis*

Statistical analysis of the data was performed through (ANOVA) according to completely randomized design followed by LSD's test to compare the means of the dependent variables at significant probability level using a statistical analysis system (SAS 9.0 Institute, Inc, Cary, NC, USA). All measurements were carried in triplicate order, and the results are presented as mean ± standard deviation (SD). Correlation confidents of pigments and color parameters were also investigated.

Results and Discussion

– *Optical density of C-phycocyanin in fresh and processed Spirulina microalgae*

Phycocyanin is a bluish pigment in *Sp* microalgae with molecular weight of 28000-30000 Da. Monomeric aggregation of phycocyanin changes its spectroscopic

properties like the long-wavelength absorption maxima (λ_{max}), absorption coefficient, optical rotatory dispersion and circular dichroism spectra and light isomer (left or right turns). Reactions between chromophore of a monomer with other monomers depend on the structural properties, particle size and concentration of proteins. The phycocyanin particle size depends on the type of organism and extraction conditions, including pH, iononic strength, temperature, protein concentration and the presence or absence of nonionic detergents. In the pH range of 5-6, the sub-units of the phycocyanin protein structure is an equilibrium of monomer ($(\alpha\beta)_6$) and hexamer ($6(\alpha\beta)$) and at pH of 7 is an equilibrium of monomer ($(\alpha\beta)_3$) and trimer ($3(\alpha\beta)$). The non-protein fraction of phycocyanin, with an open-chain tetrapyrrole strucrure (bilin), is attached to the apoprotein by one or two thioether bonds in a covalent manner. Phycocyanin consists of two (α *and* β) sub-units, where α subunit has one point and β has two points for connecting phycocynobilin to apoprotein. Phycocyanin is a completely water soluble pigment with high proportional resistance against light and sensitive to temperature. Separation of chromophore from protein in different processes changes phycocyanin structure (Glazer, 1976).

The OD of extracted phycocyanin from fresh and processed *Sp* is shown in Figure 1, where significant differences ($P<0.05$) were observed in the fresh and processed sample. Change percentage of OD in processed samples with different methods than fresh one is also indicated in Table 2.

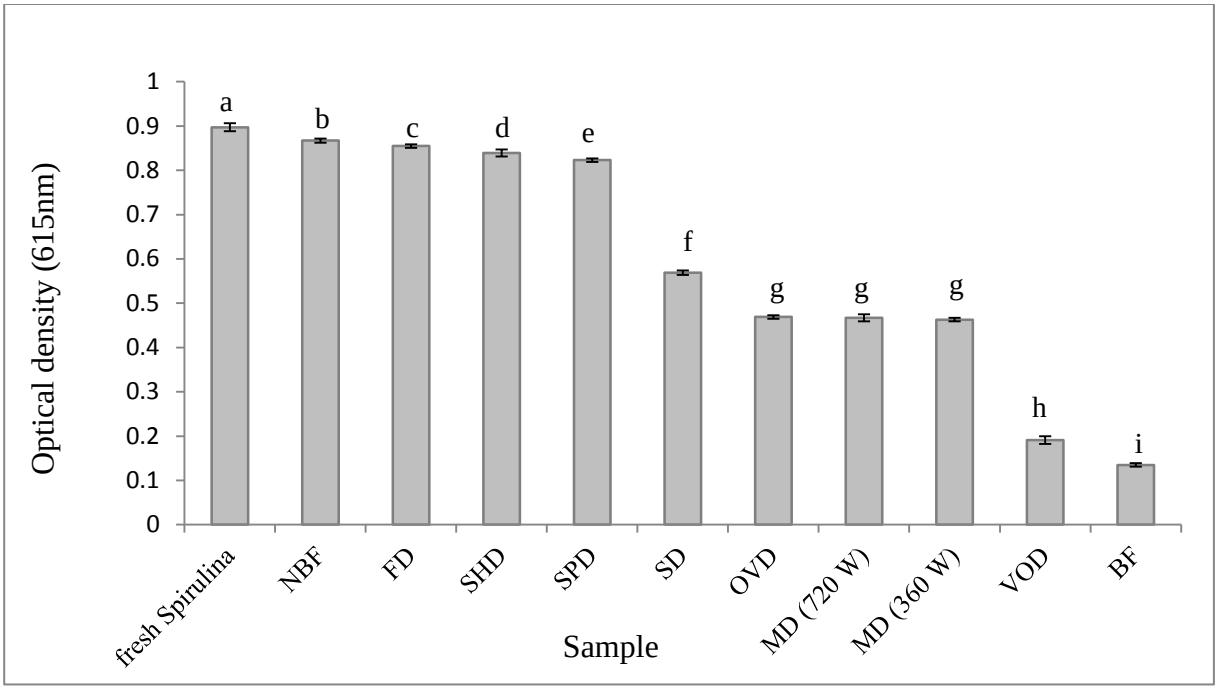

Fig. 1. Effects of different processing methods on OD of extracted phycocyanin in *Spirulina* microalgae SHD: shade-dried, SD: sun-dried, OD: oven- dried, MD: microwave-dried, VOD: vacuum oven-dried, FD: freeze- dried, SPD: spray- dried, NBF: non-blanched frozen, BF: blanched frozen. Values are the mean±SD (n=3); Means with different letter within columns are significantly different ($P<0.05$) as LSD's test.

The highest level of OD the highest concentration of phycocyanin was observed in the fresh. In processed sample, non-blanched frozen sample had the highest

level of OD with the least quantitative change than fresh sample (3%). Little effect of freezing process on phycocyanin was due to the imposedphysical damages on cellular material by ice crystal with higher volume and high difference in density of water and ice. Concentration of intercellular material induced denaturation of protein fraction of pigment structure because of changing pH. The highest change percentage of OD than fresh sample (76.2%) was observed in the blanched frozen sample. Blanching of *Sp* before freezing reduced phycocyanin content of sample significantly ($P<0.05$). It was due to the denaturation of the protein fraction and deformation of this pigment at 95ºC.

In drying methods, FD was the best process in protecting phycocyanin pigment of *Sp* microalgae with the least change of OD than fresh sample (4.2%). However, increasing water volume due to ice crystallization and extended time of drying, resulted physical damages in cell-wall and changed slightly phycocyanin structure. Due to the absence of destructive factors for denaturation of proteins such as heat or microwave radiation, the reduction in OD of shade-dried sample was lower in comparison with oven-, microwave- and spray-dried samples. Results of another study on *Sp* phycocyanin indicated that the phycocyanin of dried *Sp* in shade at 25°C under air circulation was better preserved in comparison with sun-dried and oven-dried samples at 50°C (Doke, 2005). It is obvious that the OD of extracted phycocyanin from oven- and microwave-dried samples was reduced significantly ($P<0.05$), which attributed to the denaturation of the phycocyanin protein fraction and bonds breakage of pigment structure. However, there were no significant ($P>0.05$) difference between oven- and microwave-dried samples and also effect of increasing microwave power from 360 W to 720 W was not significant ($P>0.05$). This pigment can resist heating temperature until 60°C. While rapid denaturation takes place at 65°C and an increasing above 65°C, accelerate deterioration and denaturation of pigment structure and reduce its concentration (Antelo *et al*., 2008). In addition to heating temperature, increasing processing time intensified denaturation and degradation of phycocyanin structure. Therefore, despite the lower temperature in VOD, the high sensitivity of pigment to treatment condition, especially long processing period in VOD enhanced OD reduction of its extracted phycocyanin than OVD method. In dehydreated samples, dried sample through VOD had the highest reduction of OD than fresh sample (70.6%).

According to the findings, phycocyanin had a relatively high resistance to ultraviolet radiation of sun. Heat and electromagnetic waves in OVD and MD methods had more destructive effects on this pigment than the presence of sun radiation. Short drying period in SPD decreased the denaturation of phycocyanin protein fraction and its structural deterioration; therefore, the spray-dried sample had the higher OD than oven-, microwave- and sun-dried samples. Consequently, due to the sensitivity of phycocyanin pigment to temperature and time of process, electromagnetic wave and ultraviolet radiation, different methods of dehydration with significant ($P<0.05$) effects on denaturation of protein fraction, bonds breakage and separation of chromophore from protein changes OD of extracted phycocyanin and its structure.

– ***Color properties of extracted phycocyanin from fresh and processed microalgae***

The results of colorimetry from extracted phycocyanin are shown in Table 3. There were significant differences (P<0.05) in color indexes (L*, a*, b*, chroma, hue angle) of extracted phycocyanin from fresh and processed microalgae. The highest and the lowest L* was observed in fresh *Sp* and sun-, oven- and vacuum oven-dried samples, respectively. Dried samples through sun, oven and vacuum oven was not different significantly (P>0.05).

The highest and the lowest +a* was observed in shade- and vacuum oven - dried samples, respectively. In processed samples, high-power microwave-dried sample had the highest b* and non-blanched frozen sample had the lowest b*, respectively. Vaccum oven- and spray dried samples had no significant difference (P>0.05) in terms of b*. Moreover, no significant difference (P>0.05) was observed between shade- and freeze-dried samples. Differences in color properties of extracted phycocyanin were due to theeffectiveness of different conditions on pigment changes. Denaturation of the protein, separation of α and β subunits, chromophore separation from protein and structural changes under different conditions are the most important reasons

Table 2. Change percentage of OD of phycocyanin and anthocyanin in the processed *Spirulina*

Sample	Percentage changes	
	OD of phycocyanin	Anthocyanin
Fresh spirulina	0	0
NBF	3	70.19
FD	4.2	56.29
SHD	5.8	-22.51
SPD	7.4	59.93
SD	32.8	-61.92
OVD	42.8	-30.79
MD (720 W)	43	13.90
MD (360 W)	43.4	3.64
VOD	70.6	0
BF	76.2	40.39

SHD: shade-dried, SD: sun-dried, OD: oven- dried, MD: microwave-dried, VOD: vacuum oven-dried, FD: freeze- dried, SPD: spray- dried, NBF: non-blanched frozen, BF: blanched frozen.

Table 3. Effects of different processing methods on color indexes of phycocyanin pigment

Sample	Color parameters				
	L*	a*	b*	Chroma	Hue angle
SHD	$6.42^{g}\pm0.02$	$14.26^{a}\pm0.09$	$1.28^{g}\pm0.03$	$14.32^{a}\pm0.09$	$5.13^{i}\pm0.08$
SD	$6.19^{i}\pm0.04$	$13.47^{b}\pm0.08$	$1.98^{e}\pm0.05$	$13.62^{b}\pm0.09$	$8.36^{h}\pm0.15$
OVD	$6.15^{i}\pm0.04$	$-0.56^{i}\pm0.11$	$3.10^{b}\pm0.08$	$3.15^{h}\pm0.10$	$100.28^{d}\pm1.72$
MD (720 W)	$6.97^{e}\pm0.05$	$2.82^{g}\pm0.04$	$3.57^{a}\pm0.06$	$4.55^{g}\pm0.05$	$51.72^{f}\pm0.69$
MD (360 W)	$6.34^{h}\pm0.02$	$0.42^{h}\pm0.01$	$2.77^{c}\pm0.06$	$2.80^{i}\pm0.06$	$81.32^{e}\pm0.28$
VOD	$6.13^{i}\pm0.04$	$-1.46^{k}\pm0.12$	$2.46^{d}\pm0.03$	$2.86^{i}\pm0.05$	$120.58^{c}\pm2.15$
FD	$6.75^{f}\pm0.01$	$13.24^{c}\pm0.07$	$1.32^{g}\pm0.08$	$13.31^{c}\pm0.08$	$5.67^{i}\pm0.32$
SPD	$7.69^{d}\pm0.01$	$8.86^{d}\pm0.08$	$2.57^{d}\pm0.07$	$9.23^{d}\pm0.06$	$16.17^{g}\pm0.56$
NBF	$8.70^{b}\pm0.03$	$6.90^{e}\pm0.08$	$0.29^{h}\pm0.03$	$6.91^{f}\pm0.08$	$2.43^{j}\pm0.29$
BF	$8.29^{c}\pm0.07$	$-1.29^{j}\pm0.02$	$1.84^{f}\pm0.03$	$2.25^{j}\pm0.02$	$125.16^{b}\pm0.58$
Fresh Spirulina	$12.34^{a}\pm0.03$	$3.76^{f}\pm0.02$	$-6.55^{i}\pm0.05$	$7.55^{e}\pm0.04$	$299.90^{a}\pm0.25$

SHD: shade-dried, SD: sun-dried, OD: oven- dried, MD: microwave-dried, VOD: vacuum oven-dried, FD: freeze- dried, SPD: spray- dried, NBF: non-blanched frozen, BF: blanched frozen. 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 LSD's test.

of color differences in extracted pigment from various treatments. Since the spectroscopy and light scattering properties in colorimetery are dependent on monomeric proteins status and particle size of phycocyanin, structural changes of phycocyanin affected these properties significantly.

Regarding the effect of different processing conditions on color properties of extracted phycocyanin from *Sp*, the highest and lowest chroma was observed in shade-dreid and blanched frozen samples, respectively. Low-power microwave- and vacuum oven-dried samples was not different significantly in term of chroma ($P>0.05$). Its noteworthy that chroma increased with increasing the absolute value of a* and b*.

Hue angle of extracted phycocyanin in various samples varied in vide range from 2.43° (red) to 299.90° (violet). Hue angle of extracted phycocyanin from fresh sample was 299.90° and was located in blue area. Hue angle differences between non-blanched frozen sample and shade-, sun-, freeze- and spray-dried samples with fresh *Sp* were about 283-298° and they are located in red area. No significant differences ($P>0.05$) were observed in hue angel of shade- and freeze-dried samples. Hue angle differences in blanched frozen sample, oven- and microwave-dried samples with fresh sample were 173-250° and they are located in yellow to green area. The highest and the lowest Hue angle was observed in fresh and non-blanched frozen samples, respectively. Significant ($P<0.05$) differences in hue angle of extracted phcocyanin from fresh and processed samples were indicated the effectiveness of different processing conditions on pigment changes.

Therefore, color differences in extracted phycocyanin from different treatments were due to chemical reactions in pigment structure. In intensified heat processing, phycocyanin concentration was reduced and its color changes from blue to yellow area.

– ***Anthocyanin content in fresh and processed microalgae Spirulina platensis***

Anthocyanins are a major unstable class of polyphenolics that confer the visual quality of fruits and vegetables, contributing to the red, blue and purple pigments in plant tissues. Their color is pH dependent, and also their stability is influenced by temperature, pH, presence of oxygen, light, ascorbic acid, sugars and metal ions. Polyphenoloxidase, anthocyanase, peroxidase and b-glucosidase are the most important degrading enzymes of anthocyanin (Hager *et al*., 2008; Patras *et al*., 2010).

Total content of anthocyanin in fresh and processed *Sp* microalgae are shown in Figure 2. Percentage changes of anthocyanin indicated the difference between anthocyanin content in fresh *Sp* and processed samples (Table 2). Positive and negative changes in various processed samples are indicative the reduction and increment of their anthocyanin content than fresh sample, respectively. Non-blanched frozen sample had the highest anthocyanin (Cyanidin-3-glucoside) content. Change of anthocyanin content in this sample was -70.19% in comparison with fresh *Sp*. The anthocyanine content of blanched frozen sample was lower than the non-blanched frozen sample. Enzymatic reactions of active enzymes such as polyphenol oxidase during freezing process and preservation decompose and reduce anthocyanin content of the frozen sample. Despite the positive effect of blanching process on protecting anthocyanin and preventing their more losses during preservation with destroying

the degrading anthocyanin enzymes, heat treatment at 95℃ for 5 min and the presence of oxygen during blanching reduced anthocyanin content of blanched frozen sample significantly. Various results had been reported from the effect of the blanching in different conditions on total content of anthocyanins. The effect of microwave blanching on anthocyanin content of strawberry puree was demonstrated the blanched sample had higher content of anthocyanin than non-blanched sample and the rate of its browning reaction was lower during preservation. It is due to deactivation of enzymes that decompose anthocyanin (Monica Giusti and Wrolstad, 2001). Patras *et al*. (2010) was reported 43% reduction of Cyanidin-3-glucoside during blanching of blueberry at 95℃ for 3 min. Therefore, effect of the blanching on anthocyanin content of samples is depended significantly on the processing conditions and the nature of samples.

The level of anthocyanin in frozen, spray-dried, freeze-dried and microwave-dried samples are significantly ($P<0.05$) higher than the control sample. Significant growth in anthocyanins content of processed samples is attributed to the performance of the processing conditions on them. Thermal treatment and freezing increase the dispersion and release of bonded anthocyanins from cellular membrane (Leong and Oey, 2012). Therefore, measurable anthocyanins content of samples after processing compared with the fresh sample were increased significantly. In this respect, higher content of anthocyanin (Cyanidin-3-glucoside) was reported in processed summer fruits like peach and cherry and

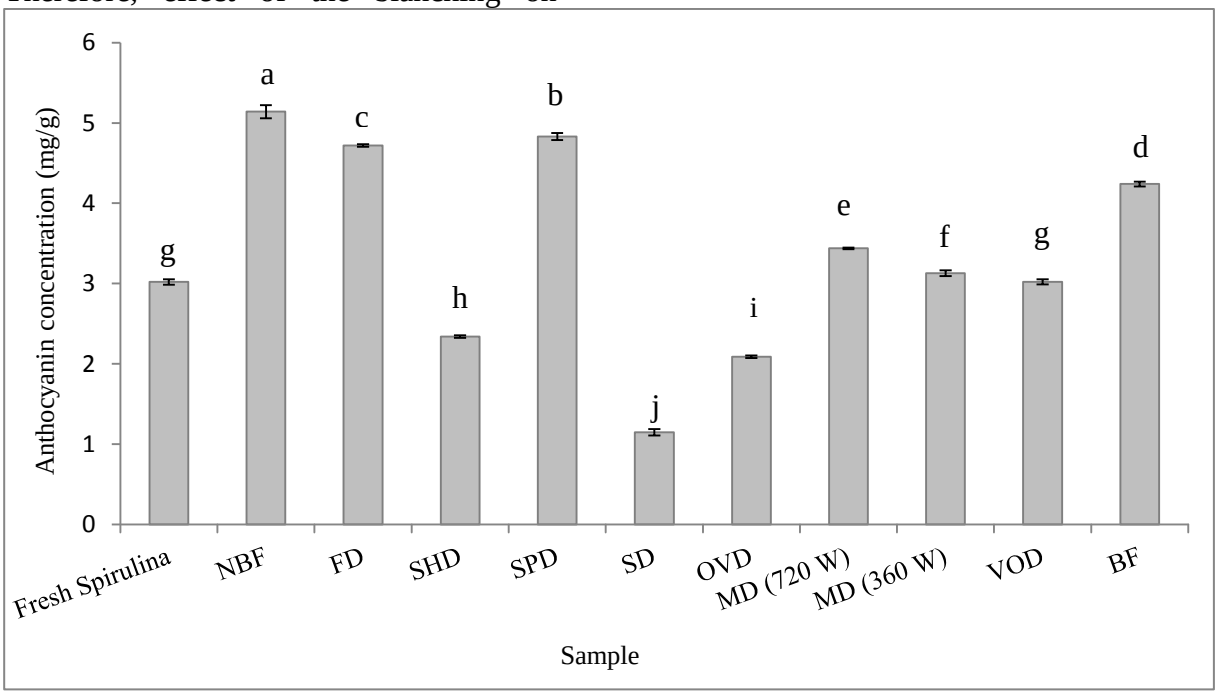

Fig. 2. Effects of different processing methods on anthocyanin content of *Spirulina* microalgae SHD: shade-dried, SD: sun-dried, OD: oven- dried, MD: microwave-dried, VOD: vacuum oven-dried, FD: freeze- dried, SPD: spray- dried, NBF: non-blanched frozen, BF: blanched frozen. Values are the mean±SD (n=3). Means with different letter within columns are significantly different ($P<0.05$) as LSD's test.

vegetables by freezing, boiling (98℃ for 10 min) and freeze-drying as compared to fresh sample. This is due to disruption of cells membrane and enhancement the

release of membrane-bond anthocyanins (Leong and Oey, 2012).

The anthocyanin decomposition intensity in spray-dried sample was lower than others dried samples due to the short exposure to heat and absence of oxygen in comparison to other drying methods. Although, alkaline conditions, high water activity, presence of Fe and Cu in *Sp* microalgae can induce breakage of covalent bonds decomposition and loss of anthocyanin in spray-dried sample than non-blanched frozen sample. Furthermore, SPD condition affects qualitative properties of dried sample. In this regard, simpson *et al*. (1985) reported the high outlet temperatures intensified the anthocyanine's decomposition of food, that temperatures below 90°C resulted minimal degradation.

Although, *Sp* microalgae dried in FD at low pressure oxygen and temperature without appropriate conditions for non-enzymatic reactions of anthocyanin, extended time of drying process increased the deficiency of anthocyanin content of freeze-dried than spray-dried sample. Destructive effect of the electromagnetic waves in short time of heat exposure in MD on anthocyanin was lower than that of the heat exposure to OVD method. Low-power in comparison to high-power increased deterioration of anthocyanin since the sample was exposed to electromagnetic waves for a longer time. High temperature and long-time of process in OVD decomposed and reduced anthocyanin content of sample. However, low pressure oxygen and low-temperature in VOD reduced the non-enzymatic reactions intensity and anthocyanin deficiency in comparison with OVD. In this regards, there was reported anthocyanin in black berry and strawberry puree (Cyanidin-3-glucoside and plargonidin-3-glucoside) were highly affected by thermal processing and a significant reduction was observed at 70℃ for 2 min. High temperature and high pH accelerated decomposition of anthocyanins into benzonic acid derivatives (Patras *et al*., 2010). Cooking also changes total anthocyanins content significantly. Wet heating processes such as boiling and steaming usually decrease anthocyanins contents of vegetables, while dry heating processes such as microwave and baking increase anthocyanin level. Steaming at 100C for 20 min reduced 8–10% of the total anthocyanin content in Purple-fleshed sweet potato (PSP). Leaching to cooking water, time and temperature of process, sample surface area, and their thermal decomposition have affected the total contents of anthocyanins in the cooked PSP (Hong and Koh ,2016). Hydrolysis of the 3-glycosidic linkage to produce more labile aglucone and hydrolytic opening of the pyrylium ring to form a substituted chalcone which degrades into a brown insoluble compound of a polyphenolic nature are the most important mechanisms for the thermal degradation of anthocyanin (Simpson *et al*., 1985).

Due to the existence of deteriorative factors for anthocyanin decomposition and long-time of process in the presence of oxygen and light (Solar radiation and ultraviolet) in traditional methods, the least level of anthocyanin was observed in the sun-dried *Sp*. The decline in anthocyanin content of shade-dried sample was lower in comparison with sun- and oven-dried samples, and anthocyanins content of this sample was lower than the spray-dried, lyophilize-, microwave- and vacuum oven-dried samples, due to higher enzymatic degradation of anthocyanin with active degrading enzymes in this method. Oxygen also plays a vital role in anthocyanin degradation processes. Oxygen accelerates degradation of

anthocyanins either through a direct oxidative mechanism or through the activation of oxidizing enzymes (Patras *et al*., 2010). Negative change of anthocyanin levels in sun-, shade- and oven-dried samples in Table 2 was indicative of the reduction of anthocyanin in these processed samples than fresh sample. Reduction of anthocyanin in sun-dried sample was more than the shade- and oven-dried samples.

Therefore, anthocyanin's stability in processing is highly affected with process conditions such as presence of oxygen, light, temperature, time of process, enzyme activities and pH (Patras *et al*., 2010). It can be deduced that the differences in drying methods and conditions like heating method, temperature, processing time, presence or absence of oxygen and light, in addition to high water activity, alkaline condition, metals existence such as Fe and Cu and unsaturated fatty acids in *Sp* microalgae are the most important parameters in reducing anthocyanin content of processed *Sp* with different methods. Reducing anthocyanin content of processed *Sp* is due to decomposing anthocynin at high pH, glucoside bonds breakage with thermal degradation, pirilium ring hydrolization, oxidation and enzyme degradation.

Correlation coefficients of dependent variables in present research are shown in Table 4. No significant correlation ($P>0.05$) was observed among the anthocyanin with OD of phycocyanin and color indexes of extracted phycocyanin from fresh and processed *Sp*. Significant ($P<0.05$) positive correlation coefficients were existed among OD of phycocyanin with (L^*), (a^*) and Chroma. In addition, significant ($P<0.05$) negative correlation coefficients were observed between OD of phycocyanin and (b^*) index. Therefore, with increasing OD, blue and red colors of extracted phycoyanin were intensified significantly. Furthermore, there exist negative significant ($P<0.05$) correlation coefficients among hue angle with (a^*) and (b^*). Therefore, colorimetic properties of extracted phycocyanin are suitable criteria for estimating OD of phycocyanin.

Conclusion

The components sustainability of processes *Sp* microalgae are dependent to processing conditions including: light, oxygen, heat, enzymes, process time, pH and the feature of high water activity, presence of Fe and Cu and unsaturated fatty acids in this sample.

Table 4. Correlation coefficients of pigments and color properties of extracted phycocyanin in *Spirulina* microalgae

	Phycocyanin	**L***	**a***	**b***	**Hue angle**	**Chroma**	**Anthocyanin**
Phycocyanin	1						
L*	0.48**	1					
a*	0.66**	-0.11	1				
b*	-0.59**	-0.90**	-0.08	1			
Hue angle	-0.06	0.70**	-0.57**	-0.70**	1		
Chroma	0.66**	-0.06	0.98**	-0.18	-0.45**	1	
Anthocyanin	0.26	0.29	0.00	-0.03	-0.19	-0.10	1

Significant at * ($P<0.05$)
Significant at ** ($P<0.01$)

In dehydrated samples, due to the high sensitivity of protein segment in phycocyanin structure, FD had the highest levels of OD in extracted phycocyanin from microalgae. Low-power of microwave irradiation in comparison to high-power had more detrimental effect on pigments. High temperature and long-time of process in OVD had a significant ($P<0.05$) effect on destruction of *Sp* pigments. SPD method was superior to preserve the anthocyanin pigment. According to the results, non-blanched freezing is considered as the best method to protect the phycocyanin and anthocyanin pigments of *Sp* microalgae; this finding is due to the slow rate of pigment destructive processes such as degradation, oxidation, isomerization and enzymatic and or non-enzymatic browning reactions in freezing conditions. Blanching process significantly ($P<0.05$) increased pigment deterioration in the frozen sample. The color indexes are appropriate measure for evaluating the extent of phycocyanin to the process in *Sp*. Optical density was increased with enhanced color indexes (+a* and -b*) of extracted phycocyanin.

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