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Effects of processing on stability of water- and fat-soluble vitamins, pigments (C-phycocyanin, carotenoids, chlorophylls) and colour characteristics of *Spirulina platensis*

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RESEARCH ARTICLE

Abstract

Spirulina platensis is an important aquatic source of functional components in foods and pharmaceutical products. The main purpose of present research was investigating the effect of processing methods (shade-, sun-, oven-, microwave-, vacuum oven-, freeze- and spray-drying and freezing with and without blanching) on colour properties and important photochemical components (vitamins and pigments) of *Spirulina*. In dehydrated samples, the spray-dried *Spirulina* had the highest level of carotenoids and chlorophylls. Due to the protein structure of C-phycocyanin, the lyophilised sample contained the highest C-phycocyanin level. There were significant correlations in pigments content and colour properties (L^* a^* b^* ΔE) of processed samples. Thiamine, niacin, pyridoxine, cyanocobalamin and retinol were important water- and fat-soluble vitamins of *Spirulina*. Generally, spray drying was a suitable method for retarding chlorophylls degradation, carotenoids oxidation and isomerisation, and preserving the highest level of vitamins in *Spirulina platensis* compared to other drying processes, although, non-blanched freezing was preferred.

Keywords: C-phycocyanin, cyanocobalamin, retinol, spray-drying, non-blanched freezing

1. Introduction

In food industry, there is an increased demand for various natural-driven components like pigments, proteins and vitamins in order to replace the synthetic counterparts and increase the nutritional value of food products (Fahmy Shabana *et al.*, 2017). Microalgae have been used as important biochemical sources, which may be rare in plants and animals. *Spirulina platensis (Sp)* is a *Cyanobacterium*, or more commonly the blue-green microalgae, that is a member of *Oscillatoriaceae*. *Sp* consists of cells in 3.5-10 μm wide, lined up side-by-side (Desmorieux and Decaen, 2005). The cell wall is composed of four layers (LI, LII, LIII and LIV). Peptidoglycan LII layer provides the rigidity of cell wall. The absence of cellulose and the existence of protein and lipopolysaccharide are the most important reasons for easy digestion of *Sp* by humans (Eykelenburg, 1977). *Sp* grows particularly in alkaline, brackish and saline waters. Its annual production worldwide in 2000 was estimated to be approximately 2000 ton (Chamorro-Cevallos *et al.*, 2008). The *Sp* contains 50-60% proteins, vitamins (particularly vitamin B12), minerals, polyunsaturated fatty acids, particularly gamma linolenic acid and polysaccharides with anti-tumour and anti-viral effects (Oliveira *et al.*, 2009). *Sp* is a valuable source of natural antioxidants like water-soluble phycocyanin, carotenoids, phenolic compounds, superoxide dismutase, catalase and peroxidase. *Sp* is an effective product for treating certain allergies, anaemia, cancer, hepatotoxicity, viral and cardiovascular diseases, hyperglycaemia, hyperlipidaemia, immunodeficiency and inflammatory processes (Dartsch, 2008; Wan *et al.*, 2016).

Pigments such as chlorophylls, carotenoids, xanthophylls and phycobiliproteins are one of the most important phytochemical components of *Cyanobacteria* (Antelo *et al.*, 2008; Benelhadj *et al.*, 2016). Phycocyanin is the

main pigment produced by *Cyanobacterium* and acts as a photoreceptor of photosynthesis in *Sp*. It is consumed as a natural nutritious, colouring agent in foods and cosmetics, potential therapeutic ingredient in oxidative disease treatment and fluorescent marker in research (Antelo *et al.*, 2008). The quantity of phycocyanin is 19.12-19.28 mg per 100 mg of dry weight of *Sp* (Sarada *et al.*, 1999). The yield of phycocyanin extraction was reported 45.7% in the crude *Sp* (Boussiba and Richmond, 1979). Carotenoids are the organic pigments naturally occurring in the chromoplasts of plants and some other photosynthetic organisms like algae, some types of fungi and bacteria. Chlorophylls are important pigments in photosynthetic algae, bacteria, and plants, which have a major contribution to photosynthesis (Roca *et al.*, 2016). Carotenoids and chlorophylls have different protective influence against atherosclerosis, cancer, osteoporosis, cataracts, neurodegenerative diseases, mutagenesis, and oxidative stress (Cervantes-Paz *et al.*, 2014).

Vitamins, the natural micronutrients constituents of food, are relatively unstable components affected by food processing factors like moisture, heat, light, oxygen, pro-oxidants, reducing agents, pH, time and condition of storage and also other food components (Lebiedzinska *et al.*, 2007; Zafra-Gomez *et al.*, 2006). They are classified to two main groups: water- and fat-soluble vitamins (Jin *et al.*, 2012). Fat-soluble vitamins are absorbed with fat content of diet and stored in the fatty tissues. They are provided from plant and animal foods or dietary supplements (Ottaway, 1993). Vitamin A (retinol), vitamin E (α-tocopherol), vitamin D (calciferol) and vitamin K1 (phylloquinone) are fat-soluble vitamins that are less heat-labile than water-soluble vitamins like vitamin B groups, while in the presence of oxygen they can be degraded at high temperatures (Hosseini *et al.*, 2014). Vitamin A is an alcohol diterpene in predominantly available form of fatty acid esters in nature. The unsaturated *trans* isomer of vitamin A is the most biologically active type of it (Ottaway, 1993). Vitamin A is necessary for the visual system of humans. Vitamins D are a group of cyclic alcohols that derived from sterols. They have important roles in the normal levels of blood calcium and phosphate maintenance, bone formation and restoration of higher vertebrates (Hosseini *et al.*, 2014). The tocopherols are derivatives of tocol. The tocotrienols have three unsaturated isoprenoid in the side chain. α, β, γ and δ tocopherols differences depend on the number and position of methyl groups on the ring. Antioxidant activity is the most important biological properties of tocopherols. Vitamin K has various forms in different sources. Vitamin K1 (phytomenadione or phylloquinone) is found in green plants and vegetables, potatoes and fruits, whereas vitamin K2 (menaquinone) can be found in animal and microbial products (Deman, 1999). In water-soluble vitamins, vitamins B are essential micronutrient and must be consumed in food sources. They are suitable cofactors for enzymatic reactions with important catalytic functionality to completion of drive metabolic processes. The shortage of thiamin (B1) and pyridoxine (B6) in diet with different vital functions in the metabolism underlie specific diseases (Kadakal *et al.*, 2007). Cyanocobalamin (B12) is an important component of several enzymes and is involved in the metabolism of certain amino acids. It is an essential nutrient for human growth and cell development. The deficiency of vitamin B12 in humans is manifested by anaemia and neuropathy (Heudi *et al.*, 2006). Due to the critical role of vitamins in nutritional aspects of products and relative instability of them, qualitative and quantitative analyses of them are important issues, a challenging task for food manufacturers. High-performance liquid chromatography (HPLC) is the preferred technique for vitamin separation because of its high selectivity (Ekinci, 2005).

Sp has high water content (75-80%) and short shelf life (3-4 days at 4 °C). Therefore, it is necessary to processed immediately after harvesting. Drying is one of the oldest known methods to preserve vegetables due to reduction water activity and prevention of microorganisms development in products (Michalska *et al.*, 2017). Freezing is another effective technique for extending shelf life of products. Although, various level of quality deterioration occurs during processing and storage of frozen foods (Ban *et al.*, 2016). Heat processing affects significantly micronutrients of food products. In this regard, there was reported significant effect of different heating methods on decreasing water- and fat-soluble vitamins of vegetables like broccoli and red pepper. In boiling, stewing, steaming, pressure steaming and microwave heating, boiling and microwave heating had the most deleterious effects on vitamins (Bernhardt and Schlich, 2006). Nevertheless, in available literature, there exist no sufficient data about the photochemical components and effect of processing on quantitative and nutraceutical properties of *Sp*. Therefore, the object of present study was to determine the effects of different drying and freezing methods (e.g. shade-, sun-, oven-, microwave-, vacuum oven-, freeze- and spray-drying and freezing with and/or without pre-treatment blanching) on changes colour properties, C-phycocyanin, carotenoids, chlorophylls and water- and fat-soluble vitamins contents of *Sp*. Results are valuable in determining the appropriate processing condition of *Sp* to preserve qualitative properties of the fresh product.

2. Materials and methods

Materials

Fresh *Sp* was prepared from Institution of Green Foundation, Research Center, Qeshm, Iran. The sample was transported to the laboratory in cool condition (4±2 °C). The quantities of moisture, ash, protein, fat, crude fibre and pH of the microalgae were measured immediately upon arrival (AOAC, 2007). The results are presented in Table 1.

Table 1. Chemical characteristics of *Spirulina platensis* microalgae (g/100 g 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 fibre	4.83±0.17
pH	8.53±0.02

All values are presented as mean ± st. dev. (n=3).

Lysozyme (L6876), papain enzyme (P3250), α-amylase enzyme (10065), butylated hydroxytoluene (BHT) (≥99%, FG), thiamine hydrochloride (47858), riboflavin (PHR1054), nicotinic acid (PHR1276), D-pantothenic acid (47867), pyridoxal hydrochloride (P9130), folic acid (F8758), cyanocobalamin (V2876), retinol (R7632), α-tocopherol (T3251), phylloquinone (47773), n-hexane (HPLC grade) and celite were purchased from Sigma-Aldrich (Steinheim, Germany). Ethylenediaminetetraacetic acid (EDTA) (purified grade, ≥98.5%), Ethyl acetate (HPLC grade), Ethanol 99.5% (HPLC grade), Methanol (for analysis EMSURE® ACS, ISO, Reag. Ph Eur), Acetone (for analysis EMSURE® ACS, ISO, Reag. Ph Eur), Petroleum ether (purified grade, ≥98.5%), Ascorbic acid (for analysis EMSURE® ACS, ISO, Reag. Ph Eur), Phosphate buffer (purified grade, ≥98.5%), Sodium acetate buffer (purified grade, ≥98.5%), Hydrochloric acid (Reag. Ph Eur, Reag. USP), Potassium hydroxide (purified grade, ≥98.5%), Sodium cyanide (purified grade, ≥98.5%) and Sodium sulfate (purified grade, ≥98.5%) were from Merck (Darmstadt, Germany).

Drying treatments

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), well-ventilated through 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 in a open space under direct sunlight at 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 (OD), drying was done in an oven at 80 °C equipped with air circulator. The drying time was 10-11 h. The final moisture content of the dried sample was 5-6% (wet basis).
- In microwave-drying (MD), the microwave power was set to 360 W for 9 min, and 720 W for 5 min. The final moisture content of the dried sample was 6-7% (wet basis).
- In vacuum oven-drying (VOD) drying was conducted at 65 °C and a pressure of 0.07 Mpa, for 12-14 h. The final moisture content of the dried sample was 6-7% (wet basis).
- In freeze-drying (FD), the pressure was reduced to 10 Mbar. The temperature in the drying chamber was -76 to -80 °C, the samples were kept in the drying chamber for 22-24 h. The final moisture content of the dried sample was 7% (wet basis).
- In spray-drying (SPD), aqueous *Sp* (at 1:1 ratio), was dried through an industrial plant spray dryer (Maham Neyshabour Inc., Khorasan, 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, atomisation airflow rate of 400 l/h and liquid feed pump rate of 25 m^3/h. Spray drying duration was approximately 60 min. The final moisture content of the dried sample was 1-2% (wet basis).

Freezing treatments

In freezing without blanching pre-treatment (NBF), sample was placed in Falcon Conical-bottom Centrifuge Tubes. Oxygen of headspace was replaced with nitrogen and *SP* was frozen and stored at -20 °C for 1 month.

In freezing with pre-treatment blanching (BF), the *Sp* was steam blanched at 95 °C for 5 min and was frozen according to NBF procedure.

Determination of C-phycocyanin

Approximately 7-10 g of *Sp* was suspended in 200 ml of 0.1 M sodium phosphate buffer pH 7.0 containing 100 µg/ml lysozyme 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 4,000 rpm to separate the cell debris. The absorbance of the supernatants was measured at 615 nm through phosphate buffer as a blank. The C-phycocyanin concentration was calculated as follows Equation (1):

$$\text{C-phycocyanin (\%)} = \frac{A_{615} \times na \times 100}{3.36 \times (mg_{sample}) \times (\%\text{dry wt})} \quad (1)$$

Where, A is the absorbance, na is the number of dilutions, 3.36 is the C-phycocyanin extinction coefficient, mg is the sample weight, % dry wt is dry matter percentage of sample (Boussiba and Richmond, 1979).

Total carotenoid content

About 15 g of the sample and 3 g of celite were mixed with 25 ml acetone to prepared paste. It was vacuum filtered and the process was continued to bleaching the paste. This extract was poured into a separatory funnel containing 40 ml petroleum ether. The acetone was removed by slow addition of deionised water and the aqueous phase was discarded. The extract was transferred to a volumetric flask containing 15 g of anhydrous sodium sulphate and the absorbance of it was read at 450 nm. The total carotenoid content was calculated as follows

$$\text{Total carotenoid } \left(\mu g/g\right) = \frac{A \times V_{(ml)} \times na \times 10^4}{A_{1cm}^{1\%} \times P_{(g)}} \quad (2)$$

Where, A is the absorbance, V is total extract volume (ml), na is the number of dilutions, P is the sample weight (g), is at 2,592 (2,592 is the β-carotene extinction coefficient in petroleum ether) (Carvalho *et al.*, 2012).

Determination of chlorophylls content

Sample was mixed with 80% acetone and the cell debris was removed by centrifugation at 4,000 rpm for 10 min. The absorbance of supernatant was measured at 646 and 663 nm against a solvent blank using a UV spectrophotometer (Unico Inc., San Diego, CA, USA). The chlorophylls content was estimated through Equations 3 and 4:

$$\text{Chlorophyll } a \text{ (mg/l)} = 127(A_{663}) - 2.69(A_{646}) \quad (3)$$

$$\text{Chlorophyll } b \text{ (mg/l)} = 22.9(A_{646}) - 4.64(A_{663}) \quad (4)$$

The content of chlorophylls *a* and *b* in mg/l in applied solutions was determined by the above equations where dilution factor in mg/g dry weight was concerned (Kumar *et al.*, 2014).

Colour measurement

The colour of the fresh and processed *Spirulina* microalgae was measured through the colourimeter (FMS Jansen GmbH & Co. KG, Murnau, Germany), in the reflectance mode at CIE L*, a*, b* colour scale (Ciurzynska *et al.*, 2014). The total colour difference was calculated through Equation 5:

$$\Delta E = \sqrt{(L_\circ - L)^2 + (a_\circ - a)^2 + (b_\circ - b)^2} \quad (5)$$

Correlation coefficients of colour indexes and pigments content of samples were also determined.

Extraction and determination of water-soluble vitamins

2.0 g of sample was poured in a 250 ml conical flask, to which 50 ml of 0.1 M hydrochloric acid was added. The suspension was placed in a water bath at 100 °C for 30 min. After cooling, the pH was adjusted to 4.5 with 2.5 M sodium acetate buffer to which a mixture of 40 mg papain and 100 mg alpha-amylase per 1 g of sample was added. After enzyme incubation at 37 °C for 18 h, samples were heated up to 100 °C for 5 min in order to inactivate the enzyme, and followed by dilution of the mixture to 100 ml with distilled water. Extraction of water-soluble vitamins was done under dim light. Before HPLC analysis, extracted samples were filtered through a Whatman 1 Chr filter paper (Lebiedzinska *et al.*, 2007).

For extraction of vitamin B12, 2 g of samples were poured in a 250 ml conical flask and 60 ml of sodium acetate buffer (pH=4) with 1 ml of a sodium cyanide 1% was added and heated at 100 °C for 30 min. After cooling, 250 mg of alpha-amylase and 100 mg of papain per gram of sample were added to the mixture. The mixture was incubated at 37 °C for 3 h. After enzyme incubation, samples were heated up to 100 °C for 5 min to inactivate the enzyme and were diluted to 100 ml with distilled water. The extracts were separated on the normal-phase C18 column 5 µm (25 cm×4.6 mm). The column was thermostated at 30 °C. The mobile phase was a series of gradient steps consisting of 20 mmol/l phosphate buffer pH=2.5 (solvent A) and 20 mmol/l phosphate-methanol buffer (60:40) (solvent B). At the beginning of experiment, up to the first 2 mins the mobile phase was solvent A. Then, a mixture of solvents A and B (20:80) was introduced to the system during the experiment (20 min). The volume of injection was 20 µl and the HPLC was operated in constant flow mode at 1 ml/min, except for vitamin B12 which was kept at 0.7 ml/min. The UV-Vis detection was set at 220 nm. Quantification was carried out by external standards for all water-soluble vitamins. Calculation of concentrations was based on linear calibration graphs (Heudi *et al.*, 2006; Jin *et al.*, 2012; Lebiedzinska *et al.*, 2007).

Extraction and determination of fat-soluble vitamins

For saponification, 1 g of sample, 4 ml ethanol 99.5%, a spatle tip of BHT and ascorbic acid (antioxidant) were poured into a 10 ml tube and it was capped, vortexed and stand for 10 min. Nitrogen was used to remove oxygen, then 0.5 ml of saturated EDTA and 0.5 ml of KOH (50%) were added to the tube. The tube was capped, shaken, and placed in a boiling water bath (20 min) and was shaken once after 10 min. Then, the tube was cooled in an ice-water bath for 10 min. After saponification and cooling of the sample, 2 ml of water and n-hexane-ethyl acetate (8+2, v/v) were added for extraction of the fat-soluble vitamins. The tube was shaken 10 min prior to separating the layers. The

extraction was repeated with another 2 ml of n-hexane-ethyl acetate (8+2). The solvent of the combined organic layer was evaporated with nitrogen at 30 °C, the residue was dissolved in 1 ml of n-hexane and filtered (0.45 µm) prior to HPLC analysis. 20 µl samples were injected on the normal-phase C18 column 5 µm (25 cm×4.6 mm). The column was thermostated at 25 °C. The mobile phase consists of methanol-water (95:5) and the HPLC was operated in a constant flow mode (1 ml/min). The wavelengths in UV-vis detection were set at 280 nm for retinol and alpha-tocopherol and 250 nm for phylloquinone and each run was performed in 20 min. Quantification was carried out by external standards and the calculation of concentrations was based on linear calibration graphs (Gimeno *et al.*, 2000; Klejdus *et al.*, 2004; Salo-Vaananen *et al.*, 2000).

Quantification of all water- and fat-soluble vitamins were carried out by application of external standards of them and the calculation of concentrations were based on linear calibration graphs.

Statistical analysis

Statistical analysis of the data was performed through (ANOVA) according to completely randomised design followed by LSD's test to compare the means of the dependent variables at significant probability level through the statistical software version 9 (SAS Institute, Cary, NC, USA). All measurements were carried in triplicate, and results are presented as mean ± standard deviation. Diagrams were constructed using the Microsoft Excel 2010 software (Microsoft Corporation, Redmond, WA, USA).

4. Results and discussion

The compositions of the *Sp* are shown in Table 1.

C-phycocyanin content in fresh and processed *Spirulina*

Phycocyanin is a water soluble bluish pigment with 28,000-30,000 Da molecular weight in *Sp*. The non-protein fraction of phycocyanin is made of bilin, a type of open-chain tetrapyrrole, which is attached to the apoprotein by one or two thioether bonds in a covalent manner. Separation of chromophore from protein in different processes changes the phycocyanin structure. Phycocyanin features such as absorbent spectrum and fluorescence, optical rotatory dispersion and circular dichroism spectra and light isomer form (left or right turns) are the outcome of reactions between chromophore of a monomer with other monomers depending on the structural properties, particle size and concentration of proteins (Glazer, 1976).

According to the results, the highest level of phycocyanin was observed in the fresh sample. Among the processed ones, non-blanched frozen sample had the highest level of phycocyanin (Table 2). Little effect of this process on phycocyanin content was due to the imposed physical damages of ice crystal on cellular material and high difference between water and ice density. Concentration of intercellular material induced denaturation of protein of pigment because of changing pH. Blanching process reduced phycocyanin content of *Sp* due to the denaturation of the protein and deformation of phycocyanin at 95 °C.

Table 2. Effects of different processing methods on C-phycocyanin, total carotenoid, chlorophyll content in *Spirulina* microalgae.[1]

Sample[2]	Phycocyanin (g/100 g)	Total carotenoid (mg/g)	Chlorophyll (a) (mg/g)	Chlorophyll (b) (mg/g)
SHD	8.42±0.031^{d}	1.13±0.01^{j}	13.14±0.029^{f}	4.34±0.012^{f}
SD	5.49±0.020^{f}	0.65±0.01^{k}	3.51±0.000^{k}	1.17±0.000^{k}
OD	4.64±0.025^{g}	4.39±0.04^{i}	6.75±0.035^{j}	2.27±0.030^{j}
MD (720W)	4.54±0.010^{g}	5.66±0.03^{f}	12.27±0.029^{g}	4.07±0.012^{g}
MD (360W)	4.60±0.015^{g}	5.41±0.01^{g}	10.74±0.040^{h}	3.56±0.026^{h}
VOD	1.90±0.015^{h}	4.49±0.00^{h}	7.88±0.023^{i}	2.62±0.015^{i}
FD	8.93±0.026^{c}	5.81±0.04^{e}	18.49±0.070^{e}	6.09±0.040^{e}
SPD	8.21±0.031^{e}	16.44±0.04^{d}	22.90±0.177^{c}	7.61±0.139^{c}
NBF	9.21±0.095^{b}	23.89±0.09^{b}	24.00±0.362^{b}	7.74±0.064^{b}
BF	1.53±0.020^{i}	22.83±0.11^{c}	19.70±0.527^{d}	6.40±0.061^{d}
Fresh *spirulina*	10.22±0.161^{a}	25.47±0.06^{a}	26.57±0.161^{a}	8.69±0.115^{a}

[1] Values are presented as mean ± st. dev. (n=3); values followed by the different letter, within the same column, were significantly different (P<0.05), according to LSD's Test.

[2] 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.

In drying methods, FD was the best treatment in protecting *Sp* phycocyanin. However, an extended time of drying process, and increasing volume of ice crystals resulted physical damage of cell-wall and changed phycocyanin structure. Due to the absence of destructive factors for denaturation of proteins such as heat or microwave radiation, the loss of phycocyanin in shade-dried sample was lower in comparison with oven-, microwave- and spray-dried samples. In another study, the phycocyanin content of dried *Sp* in shade at 25 °C under air circulation was also reported higher than sun-dried (35 °C for 1 h) and water bath-dried (50 °C for 1 h) samples (Doke, 2005). It is obvious that the phycocyanin content of oven- and microwave-dried samples was reduced significantly ($P<0.05$), which attributed to the denaturation of protein fraction and bonds breakage of pigment. Phycocyanin can resist heating temperature until 60 °C. Rapid denaturation takes place at 65 °C and deterioration and reduction of pigment concentration accelerates above 65 °C (Antelo *et al.*, 2008). Denaturation of phycocyanin structure and reduction of its content was intensified by increasing process time. Therefore, despite the lower temperature condition in VOD, due to the high sensitivity of pigment to treatment condition, long processing period increased deterioration of this compound than OD. Short drying period in SPD decreased the deterioration and denaturation of protein structure than OD, MD and SD methods (Table 2).

Consequently, phycocyanin had a relatively high resistance to ultraviolet radiation, while, heating and electromagnetic waves had more adverse effects on it. Dehydration process with significant effects on denaturation of protein fraction, bonds breakage and separation of chromophore from protein changes phycocyanin structure and its concentration.

Total carotenoid concentration in fresh and processed *Spirulina*

Alteration or loss of carotenoids during processing and storage of foods occurs through physical removal, geometric isomerisation, and enzymatic or non-enzymatic oxidation. Carotenoids have a series of conjugated double bonds making them susceptible to *cis/trans* isomerisation and oxidative changes. Carotenoids are sensitive to light (solar radiation and UV), oxygen, heat, electromagnetic radiation, catalysts (metals), enzymes (especially lipoxygenase, catalase, peroxidase) and water activity of food (Simpson, 1985).

The highest total carotenoid concentration was observed in fresh sample. The best protection of carotenoids was observed in frozen samples, and their slight loss was attributed to their enzymatic and non-enzymatic oxidation in processing (Simpson, 1985). Blanching prevents the more loss of carotenoids during preservation with inactivating the destructive enzymes like catalase, peroxidase and lipoxygenase. Although the presence of oxygen and high temperature during blanching accelerated oxidation and reduced carotenoids content and also other pigments such as *a*, *b* and *β*-carotens of blanched frozen sample (Mazzeo *et al.*, 2015; Negi and Roy, 2000). In dehydrated samples, the highest total carotenoid concentration was observed in spray-dried sample because of the low exposure time of heating an absence of oxygen. The observed decline in carotenoids content of spray-dried sample was due to isomerisation of these components during process (Grabowski *et al.*, 2008). Although, drying *Sp* in low-pressure oxygen and low-temperature conditions in FD were not appropriate for degradative reactions of carotenoids, due to the extended time of drying process, the deficiency of carotenoids in freeze-dried sample was higher than spray-dried and lower in comparison with thermal processed samples. That is demonstrated the superior of FD than hot air oven-drying in preserving carotenoids due to the high sensitivity of this pigment to oxygen and heat (Rawson *et al.*, 2011). Oxidation and isomerisation of carotenoids in high temperature and extended time of process are the most important reasons of carotenoids damage. Therefore, low-pressure oxygen and low-temperature in VOD reduced the non-enzymatic reactions (oxidation and isomerisation) intensity and total carotenoid deficiency. In this regard, it was reported boiling (20 min), frying (10 min) and drying (at 57 °C for 10 h) reduced 77-88% of *β*-carotens of sweet potato due to their isomerisation of *trans* to *cis* form (Bengtsson *et al.*, 2008). Short time of heat exposure in MD reduced the destructive effect of the electromagnetic waves on carotenoids than that of the heat exposure in oven. Low-power in comparison with high-power increased deterioration of carotenoids since the sample was exposed to electromagnetic waves for longer time. Due to the long time of drying process through the traditional drying methods (SD and SHD) and the existence of induced oxidation and isomerisation factors like light, oxygen, solar rays and UV, the least level of total carotenoid was observed in processed samples with these methods. In this regard, more reduction in carotenoids content of sun-dried (sunlight for 2-3 consecutive days) than oven-dried (50 °C for 12 h) and freeze-dried (-35 °C for 24 h) *Moringa oleifera* leaves was reported (Saini *et al.*, 2014). Drying mango through convectional heating led to isomerisation of *β*-carotens from *trans* to 13-*cis* and sun wave converted it to 9-*cis* form (Yahia and Ornelas-Paz, 2010). Therefore, differences in drying conditions like heating methods, temperature, processing time, oxygen pressure and light in addition to high water activity, presence of Fe and Cu and unsaturated fatty acids in *Sp* influence carotenoids content of it due to their enzymatic and non-enzymatic oxidation and isomerisation (Table 2).

Evaluation of chlorophylls content in fresh and processed *Spirulina*

Fresh sample contained the highest level of chlorophylls *a* and *b*. NBF treatment had the highest protection level of chlorophylls *a* and *b*. A slight loss of *a* and *b* chlorophylls in this sample was due to the presence of active degrading enzymes like chlorophyllase, peroxidase, pheophytinase and their enzymatic oxidation during storage (Kaewsuksaeng *et al.*, 2015). There was reported heating lemon in water at 50 °C reduced the chlorophyllase activity and decomposition of chlorophylls (Kaewsuksaeng *et al.*, 2015). Despite the effect of blanching on inactivation of enzymes, with respect to the blanching condition for chlorophylls oxidation in present study and formation of pheophytin, a reduction in chlorophylls content of *a* and *b* in pre-treated frozen sample was observed. There are similar report on reducing chlorophylls content of *a*, *b* and also *β*-carotens in asparagus, green beans and zucchini during blanching (Mazzeo *et al.*, 2015). The chlorophyll *a* is more sensible to treatment than *b* and an increase in temperature and time of blanching due to formation of brownish pigments like pheophytin, intensifies pigments deterioration (Mazzeo *et al.*, 2015). In dehydrated samples, the spray-dried sample had the highest level of *a* and *b* chlorophylls. Decomposition and oxidation of chlorophylls in this sample were lower than other drying methods due to short exposure of sample to heat and low-pressure of oxygen. The alkaline environment, high water activity, presence of Fe and Cu and existence of unsaturated fatty acids in *Sp* were the most important reasons of slight reduction of chlorophylls content in spray-dried sample. According to the same reasons discussed the effects of FD on reducing the total concentration of carotenoids in microalgae, reduction of *a* and *b* chlorophylls content in freeze-dried sample was more than that of the fresh and the spray-dried samples. A reduction in microwave power from 720 W to 360 W increased the loss of chlorophylls. In this regard, the lower stability of chlorophylls was reported in processed kiwifruit puree in microwave oven (340 s on 1000 W) than conventional thermal treatment (97 °C-30 s) (Benlloch-Tinoco *et al.*, 2015). Effect of thermal treatment (oven) on *a* and *b* chlorophylls was similar to its influence on carotenoids. Therefore, according to the results of present and other studies, temperature has a significant effect on reducing the pigment component. Boiling green, yellow and red bell pepper at 94 °C and frying at 210 °C reduced pigment content of them due to decomposition of chlorophylls and formation of pheophytin and also conversion of carotenoids to *cis*-isomers (Cervantes-Paz *et al.*, 2014). The least *a* and *b* chlorophylls content was observed in sun-dried sample. The stability of *a* and *b* chlorophylls in shade-dried sample was lower in comparison with microwave- and oven-dried samples. The existence of simultaneous destructive agents such as oxygen, light, high heating temperature and long processing time had more effects on damaging chlorophylls (Table 2).

According to the results of present section, chlorophylls are sensitive pigment to light, oxygen, heat, electromagnetic radiation, catalysts (metals), acids, enzymes activity and processing condition. The difference in drying methods in their heating pattern, temperature, processing time and the presence or absence of light and oxygen are effective parameters in chlorophylls content of samples. Four different chemical reactions contribute to change in chlorophylls structure. The first reaction is pheophytinisation, where two atoms of hydrogen replace the central atom of magnesium in tetrapyrrole. The second reaction is de-esterification of the phytol chain under enzymatic or alkaline conditions. The third reaction occurs at very high temperature, which induces the loss of the carboxymethoxy group at C132, yielding pyroderivatives and the fourth is due to enzymatic reactions facilitating chlorophylls oxidisation in plant tissues, including peroxidase, oxidase, and lipoxygenase. Therefore, the production of oxidised chlorophyll derivatives can be induced in enzymatic or chemical reactions. Mild heat processing, like steaming, induces the formation of C132 epimers and pheophytins. Further thermal treatment increases pheophytinisation level and formation of pyroderivatives. In fact, difference in the intensity of chlorophylls degradation and formation of pheophytin and pyropheophytin is a function of both heat exposure and time of process (Roca *et al.*, 2016).

Colour properties of fresh and processed *Spirulina platensis*

Colour is one of the most important qualitative characteristics of dried products that change in drying process or long time of shelf life due to the chemical and biochemical reactions such as degradation, oxidation and isomerisation of pigments. Production of various coloured compounds including melanins from enzymatic reactions, melanoidins from non-enzymatic reactions, pheophytin and pheoforbid from decomposed chlorophylls are affected processed food colour (Simpson, 1985).

The appearances of fresh and processed *Sp* are presented in Figure 1. The appearance of fresh *Sp* was dark green. The L^* value of fresh sample was lower than the dried samples. Due to the more stability of pigments to cold treatment than heating, the non-blanched frozen sample had a close resemblance with its fresh sample. A slight change in L^* value was observed because of pigment oxidation and degradation during freezing process. Although blanching is known as an appropriate process for preventing vegetable and algae colour changes in preservation period, the lowest L^* was observed in blanched frozen sample. The blanching conditions, high water activity and alkalinity of sample, and also the presence of Fe and Cu in *Sp* led to formation of brownish colour due to conversion of chlorophylls to pheophytin and pheoforbid. Therefore, significant ($P<0.05$) changes in vegetables colour indexes during

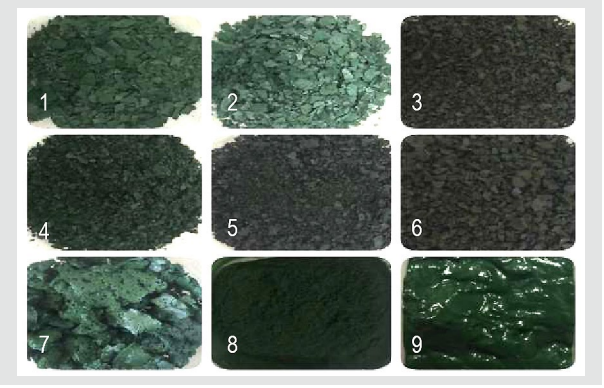

Figure 1. Appearance of fresh and processed *Spirulina* (1 = shade-dried, 2 = sun-dried, 3 = oven-dried, 4 = microwave-dried at 720 W, 5 = microwave-dried at 360 W, 6 = vacuum-dried, 7 = lyophilised-dried, 8 = spray-dried and 9 = fresh samples).

blanching and freezing processes are due to a reduction in chlorophyll *a* and formation of pheophytin and also changes in light reflection due to cellular conversions and chlorophyll derivations formation (Mazzeo *et al.*, 2015). The L^* in microwave-dried sample was lower than others and was more similar to its fresh *Sp*, since short time of drying process and low effect of electromagnetic wave on pigments variation were the main reasons of this observation. Although a reduction of power from 720 W to 360 W reduced the L^* of dehydrated *Sp*; increasing time of microwave exposure led to an increase in pigment degradation and, intensity of browning reactions (Table 3).

According to the results of carotenoids and chlorophylls content of dried microalgae through different methods, pigments damage of spray-dried sample was lower than other dried samples. On the other hand, an increase in L^* of spray-dried *Sp* compared to microwave- and vacuum oven-dried or fresh samples was due to physical and superficial properties of dried powder such as particle size distributions, porosity, adhesiveness and density. These properties of products are affected certainly with drying conditions. There was reported an increase inlet temperatures up to 160 °C in spray dryer would bright the colour of guava powder (Shishir *et al.*, 2014). With respect to pigment decomposition intensity in FD compared to SPD, the higher (L^*) value was observed in freeze-dried sample. Furthermore, the physical and superficial features of the dried samples influenced the L^* and their light dispersion. Rapid oxidation and pigment decomposition in OD increase L^* of oven-dried sample than spray-, microwave- and vacuum-dried samples. The L^* value of sun- and shade-dried samples increased significantly (P<0.05). An increase in *cis* isomer bonds in carotenoids structure and a decrease in double bonds due to oxidation were the main factors in discoloration of carotenoid compounds and intensification of L^* in dried samples subject to traditional methods.

Among the processed samples, the lyophilised sample had the highest and oven- and microwave-dried samples had the lowest resemblance to fresh sample in a^*. The sun-, shade- and spray-dried samples had a middle green index in comparison with two previous mentioned groups. The a^* value in frozen samples was significantly (P<0.05) lower than its control sample and blanching significantly (P<0.05) reduced this parameter.

In the processed samples, only the spray-dried one had no significant (P>0.05) difference with its fresh sample in b^*. A reduction in microwave power revealed a significant

Table 3. Effects of different processing methods on colour indexes of *Spirulina* microalgae.[1]

Sample[2]	L^*	a^*	b^*	ΔE
SHD	21.65±0.01^{b}	-4.37±0.03^{h}	4.05±0.03^{f}	8.60±0.01^{b}
SD	25.39±0.00^{a}	-5.51±0.02^{i}	3.89±0.02^{g}	12.17±0.02^{a}
OD	19.92±0.01^{d}	-1.37±0.03^{d}	5.78±0.03^{b}	8.26±0.02^{c}
MD (720W)	17.37±0.01^{g}	-1.31±0.04^{c}	3.76±0.04^{h}	6.47±0.05^{f}
MD (360 W)	16.86±0.01^{h}	-1.11±0.04^{b}	2.76±0.02^{j}	6.58±0.03^{e}
VOD	18.94±0.00^{f}	-0.60±0.04^{a}	4.66±0.02^{d}	7.96±0.04^{d}
FD	21.01±0.03^{c}	-6.36±0.02^{k}	6.74±0.03^{a}	7.95±0.05^{d}
SPD	19.38±0.02^{e}	-4.24±0.05^{g}	4.85±0.07^{c}	6.41±0.03^{g}
NBF	13.38±0.05^{i}	-3.25±0.04^{f}	3.03±0.02^{i}	3.49±0.03^{i}
BF	9.04±0.03^{k}	-2.46±0.02^{e}	4.31±0.05^{e}	5.68±0.02^{h}
Fresh *spirulina*	13.28±0.02^{j}	-6.20±0.01^{j}	4.90±0.02^{c}	0.00±0.00^{j}

[1] Values are presented as mean ± st. dev. (n=3); values followed by the different letter, within the same column, were significantly different (P<0.05), according to LSD's Test..

[2] 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.

(P<0.05) difference between the microwave-dried and fresh samples in terms of b^*. The freeze- and microwave-dried samples had the most and the least quantity of yellowish, respectively. The b^* value in frozen samples was significantly (P<0.05) lower than the control and blanching was effective in protection of b^*. Changes in a^* and b^* values of processed *Sp*, was due to the effect of different processes in pigments' destruction especially chlorophylls, formation of various coloured compounds due to enzymatic and non-enzymatic reactions and physical-superficial properties of the samples. The most colour difference between fresh and processed samples was observed in sun- and shade-dried samples, respectively. The colour difference in oven-dried sample with fresh *Sp* is due to high-temperature and long time of process. A reduction in microwave power from 720 W to 360 W and an increase in process time led to an increase in colour differences between microwave-dried samples and fresh sample. Since the drying time was short in SPD and the negative impacts of process on pigments was low, the colour difference between spray-dried and fresh samples was lower than other dried samples. The least colour difference was observed in frozen samples, since pigment degradation and enzymatic and non-enzymatic browning reactions are lower than the thermal processes.

Correlation coefficients of pigments and colour properties in *Sp* are shown in Table 4. Significant (P<0.05) negative correlation coefficients were existed among L^* with chlorophylls and carotenoids, a^* value and pigments content of samples especially phycocyanin and chlorophylls and also ΔE with phycocyanin, carotenoid, chl.*a* and chl.*b* pigments content of *Sp*. Therefore, an increasing phycocyanin and chlorophylls content of samples intensified green colour and darkness of them. It can be concluded the colour indexes are suitable criteria of pigments content in processed *Sp*. Furthermore, there exist positive significant (P<0.05) correlation coefficients among phycocyanin and carotenoids with chl.*a* and chl.*b* that indicates the relatively similar effect of treatment conditions on pigments.

Water-soluble vitamins

Vitamins are a group of essential organic nutrients in metabolism. Vitamins B complex (B1, B2, B3, B5, B6, B9 and B12) and C are water-soluble vitamins. Different microalgae are valuable resources of water-soluble vitamins. *Isoehrysis galbana, Tetraselmis suecica*, *Dunaliella tertiolecta* and *Chlorella stigmatophora* have relatively high amounts of C, B1, B3 and B5 vitamins (Fabregas and Herrero, 1990).

The water-soluble vitamins' content of the fresh and processed *Sp* microalgae are shown in Table 5. Niacin, cyanocobalamin, thiamine and pyridoxine had the highest content in *Sp* in comparison with other water-soluble vitamins, respectively. Riboflavin was more sensitive to processing conditions and it destroyed in processed *Sp* with different methods. In the frozen samples, thiamine, niacin, pyridoxine and cyanocobalamin content of NBF sample were higher compared to the BF sample, while no significant difference (P>0.05) was observed between NBF and BF samples in pantothenic acid. Due to destruction of processed microalgae cell-wall, release of intracellular components and more exposure of them to oxidising agents, the oxidation intensity of vitamins increased and vitamins content of frozen *Sp* decreased significantly (P<0.05) compared to fresh sample. Increasing volume of intracellular fluids during ice crystals formation is the main reason of physical damage of *Sp* cell-wall in frozen sample. As well as, the nature of packaging and its permeable characteristics against oxidising agents (light and oxygen) affected the intensity of auto-oxidation, destruction and loss of water-soluble vitamins in freezing period. A more reduction in the BF sample was due to the effect of heating at 95 °C, in the presence of oxygen in blanching process on degradation and oxidation of vitamins' content in *Sp*. Slupski (2012) had reported the quantities of thiamine and riboflavin decreased in different varieties of frozen beans. A decline in niacin content occurs through leaching during cooking and blanching of food. These processes also reduced 10-30% of pantothenic acid content of vegetables (Deman,

Table 4. Correlation coefficients of pigments and colour properties in *Spirulina* microalgae.[1]

	L^*	a^*	b^*	ΔE	Phycocyanin	Chlorophyll (*a*)	Chlorophyll (*b*)	Carotenoid
L^*	1							
a^*	-0.23	1						
b^*	0.24	-0.37*	1					
ΔE	0.78**	0.10	0.09	1				
Phycocyanin	0.14	-0.75**	0.16	-0.39*	1			
Chlorophyll (*a*)	-0.64**	-0.43*	0.08	-0.83**	0.59**	1		
Chlorophyll (*b*)	-0.63**	-0.43*	0.08	-0.82**	0.59**	1.00**	1	
Carotenoid	-0.83**	-0.20	-0.10	-0.85**	0.28	0.87**	0.86**	1

[1] *P<0.05; **P<0.01

1999). Temperature and time of processes are effective factors in destruction of vitamins. Loss of water-soluble vitamins was reduced in quick drying at higher temperature compared to slow drying at lower temperature. Long drying process increased thermal degradation of vitamins. Therefore, among the different dehydrated samples, due to short exposure time to heat and low pressure of oxygen, reduction of thiamine, pantothenic acid, pyridoxine and cyanocobalamin in spray-dried sample was lower than that of the freeze-, microwave-, oven-, shade- and sun-dried samples, while loss of niacin in SPD was more than MD (720 W). The levels of thiamine, niacin, pantothenic acid, pyridoxine and cyanocobalamin in lyophilised sample was reduced significantly (P<0.05) because of freezing process effect on destruction of microalgae cell-wall. However, loss intensity in FD was lower than SHD, SD, OD and MD due to low temperature and low pressure of oxygen in vacuum chamber of freeze dryer. Therefore, directly exposure of samples to oxygen during processing is one of the main reasons of more water-soluble vitamins losses (Deman, 1999).

Microwave heating is the conversion of electromagnetic energy into thermal energy through direct interaction of the radiation with material molecules. The differences in the source and transformation of energy are the main reasons in superiority of microwave heating compared to other heating methods (Deman, 1999). With respect to rapid nature of drying process through microwave, the intensity of destruction and loss of water-soluble vitamins in this method was lower than heating with conventional oven. In this regard, in different methods of rice cooking, microwave heating had the least impact on destruction of thiamine due to the short cooking time (8 min) (Silveira *et al.*, 2016). A reduction in microwave power from 720 W to 360 W increased the oxidation and degradation of thiamine, niacin, pantothenic acid, pyridoxine and cyanocobalamin vitamins in microwave-dried sample. Therefore, destructive effect of electromagnetic waves at low power increased, due to high exposure time to microwave. Evaluation of vitamins content of other processed food also demonstrated the significant influence of them to processing methods. Niacin content of baked in an oven (180 °C, 30 min), boiled (5 min), fried (150 °C, 15 min) and microwaved (2,450 MHz, 13 min) fish fillets decreased significantly compared to the raw sample. The least level of niacin was observed in boiled and microwaved samples (Hosseini *et al.*, 2014).

Heat processing in oven-dried sample decreased thiamine, niacin, pantothenic acid, pyridoxine and cyanocobalamin content of *Sp* significantly (P<0.05). Free form of pantothenic acid in hygroscopic oil appearance is very unstable form of vitamin. It is relatively stable to air exposure at pH=4-7, and susceptible to dry heat processing (Deman, 1999). The content of these vitamins in vacuum oven-dried sample was preserved better than oven-dried sample due to the simultaneous application of vacuum and low temperature. Increasing temperatures and time of drying accelerated the oxidation and degradation of water-soluble vitamins. Increasing oven temperature to 70 °C in hot air drying of tarhana reduced the content of thiamine, riboflavin, niacin, pantothenic acid, pyridoxine and folic acid significantly (Ekinci, 2005). Kadakal *et al.* (2007) also reported the high drying temperature reduces

Table 5. Effects of different processing methods on water-soluble vitamins of *Spirulina* microalgae.[1]

Samples[2]	Amounts of water-soluble vitamins (mg/100 g)						
	B1	B2	B3	B5	B6	B9	B12
SHD	0.03^{d}±0.00	0.00^{b}±0.00	1.28^{h}±0.00	0.01^{f}±0.00	0.00^{i}±0.00	0.00^{c}±0.00	2.34^{f}±0.01
SD	0.00^{f}±0.00	0.00^{b}±0.00	0.03^{k}±0.00	0.00^{g}±0.00	0.00^{i}±0.00	0.00^{c}±0.00	1.64^{j}±0.01
OD	0.00^{f}±0.00	0.00^{b}±0.00	0.82^{j}±0.00	0.00^{g}±0.00	0.00^{i}±0.00	0.00^{c}±0.00	1.96^{i}±0.00
MD (720W)	0.03^{d}±0.00	0.00^{b}±0.00	4.34^{d}±0.00	0.11^{e}±0.00	0.52^{f}±0.00	0.00^{c}±0.00	2.35^{f}±0.01
MD (360W)	0.00^{f}±0.00	0.00^{b}±0.00	1.30^{g}±0.00	0.01^{f}±0.00	0.31^{g}±0.00	0.00^{c}±0.00	2.29^{g}±0.00
VOD	0.00^{f}±0.00	0.00^{b}±0.00	0.88^{i}±0.00	0.00^{g}±0.00	0.26^{h}±0.00	0.00^{c}±0.00	2.22^{h}±0.00
FD	0.06^{c}±0.00	0.00^{b}±0.00	3.60^{f}±0.00	0.12^{d}±0.00	0.81^{e}±0.00	0.00^{c}±0.00	3.07^{d}±0.00
SPD	0.84^{b}±0.00	0.00^{b}±0.00	3.91^{e}±0.00	0.20^{b}±0.00	1.04^{d}±0.00	0.01^{b}±0.00	3.67^{b}±0.01
NBF	0.03^{d}±0.00	0.00^{b}±0.00	6.92^{b}±0.00	0.16^{c}±0.00	1.53^{b}±0.00	0.00^{c}±0.00	3.21^{c}±0.01
BF	0.01^{e}±0.00	0.00^{b}±0.00	6.59^{c}±0.00	0.16^{c}±0.00	1.50^{c}±0.00	0.00^{c}±0.00	2.94^{e}±0.01
Fresh *spirulina*	3.40^{a}±0.00	0.03^{a}±0.00	7.34^{a}±0.00	0.33^{a}±0.00	2.13^{a}±0.00	0.02^{a}±0.00	3.88^{a}±0.01

[1] Values are presented as mean ± st. dev. (n=3); values followed by the different letter, within the same column, were significantly different (P<0.05), according to LSD's Test.

[2] 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.

the water-soluble vitamins and the maximum reduction of water-soluble vitamins was occurred in oven- and sun-dried samples. They introduced hot air drying at 60 °C as the best way in protection of water-soluble vitamins compared to drying with sun and hot air at 70-80 °C and also cooking at 90, 100 and 121 °C. Proteins of food such as egg albumin and casein protect vitamins in processing. Heat processing in the presence of glucose induces a browning analogous to a maillard reaction that is effective in the loss of thiamin during heat processing (Ottaway, 1993). Thiamine with a molecular weight of about 300.8 Da contains pyrimidine and thiazol moieties that link to the base structure with a methylene group. Thermal processing degrades thiamine and creates various derivatives such as thiazole and pyrimidine (Deman, 1999). Dehydration and canning meat decreased significantly the water-soluble vitamins, especially riboflavin and pantothenic acid (Rice and Robinson, 1944). Loss of pyridoxine during heat treatment was attributed to conversion of pyridoxal to pyridoxamine and formation of bis-4-pyridoxal disulphide (Deman, 1999). The major reason of water-soluble vitamins reduction in foods during cooking is solubility and releasing them to cooking water that increase their thermal degradation (Silveira *et al.*, 2016). Thiamin is very sensitive to alkali and the thiazol moiety ruptures easily at room temperature and high pH (more than 7). The destruction of thiamin in heat processing occurs rapidly in food with high water activity. B12 is relatively stable at pH=4.5, but it destroys in alkaline condition and in the presence of reducing agents such as ascorbic acid and sulphur dioxide. Therefore, oven heating accelerated oxidation and degradation of water-soluble vitamins (B1, B3, B5, B6 and B12) due to sensitivity of them to alkaline condition in the presence of Fe and Cu (catalysts) and high water activity.

In SHD, loss of thiamine, niacin, pantothenic acid and cyanocobalamin was lower than that of the OD and SD methods, while there were no significant differences (P>0.05) among shade-, sun- and oven-dried samples in terms of pyridoxine. However, a further decline was recorded in SHD in comparison with SPD, FD and MD methods due to the long duration of drying process in the presence of oxygen. Pyridoxine is recognised as a relatively stable vitamin against heat and strong acidic, while sensitive to light, especially in alkaline condition. Cyanide group in the structure of cyanocobalamin is easily broken in light and removed from molecule (Deman, 1999). The least content of thiamine, niacin, pantothenic acid, pyridoxine and cyanocobalamin vitamins was observed in traditional method like SD due to the long time of drying process and presence of oxidising agents such as solar rays especially UV and oxygen.

Different processing methods had significant (P<0.05) impacts on reducing riboflavin and folic acid in microalgae, so that these vitamins destroyed completely in various processed samples and folic acid was observed only in spray-dried sample. The riboflavin molecule consists of a D-ribitol unit in connection to an isoalloxazine ring. Various processes with breakage of riboflavin molecule, separating ribitol of its structure, accelerate oxidation, thermal degradation and reduction of riboflavin content in processed samples. According to scientific literature review, freezing of vegetables like spinach and broccoli reduced their folic acid content. Furthermore, blanching before freezing, depending on the temperature and time of process was effective on reduction of folic acid (Czarnowska and Gujska, 2012). A significant (P<0.05) reduction was observed in riboflavin content of dried microalgae with oven heating. Heat treatment under acidic and neutral conditions is not destructive on riboflavin, while heating in alkaline conditions destroyed relatively high amount of it (Deman, 1999). Due to the sensitivity of riboflavin and folic acid to heat in alkaline medium, and alkalinity of *Sp*, the rate of these vitamins degradation at high processing temperature increased significantly. In this situation, riboflavin was converted to an inactive derivative named lumiflavin (Deman, 1999).

Fat-soluble vitamins

Sp is a good source of fat-soluble vitamins (A, E, D, K) (Fahmy Shabana *et al.*, 2017). *Isoehrysis galbana, Tetraselmis suecica*, *Dunaliell tertiolecta* and *Chlorella stigmatophora* are other microalgae that are recognised as excellent sources of fat-soluble vitamins, especially A and E (Fabregas and Herrero, 1990).

The quantities of fat-soluble vitamins in processed and fresh microalgae are shown in Figure 2 (A, B and C). In quantitative terms, retinol and phylloquinone were in maximum and minimum values in *Sp*, respectively. The fat-soluble vitamins' levels in processed microalgae were decreased significantly (P<0.05) compared to the fresh sample.

In freezing, loss intensity of retinol and α-tocopherol vitamins in NBF sample was lower than that of the BF sample. However, no significant reduction was observed in phylloquinone content when NBF and BF samples were compared. With respect to unsaturated structure of fat-soluble vitamins, a further decline in BF sample was observed due to the oxidation of them in heating process at 95 °C in the presence of oxygen. Destructive effects of heating process on retinol were more than freezing. Therefore, the loss of this vitamin in frozen samples was lower compared to that of the dehydrated samples. Destruction of cell-wall structure with formation of ice crystals led to more susceptibility of released sensitive components such as vitamins to oxidation and degradation. The presence of Fe and Cu (pro-oxidants), high water activity in the *Sp* and the permeability characteristics of

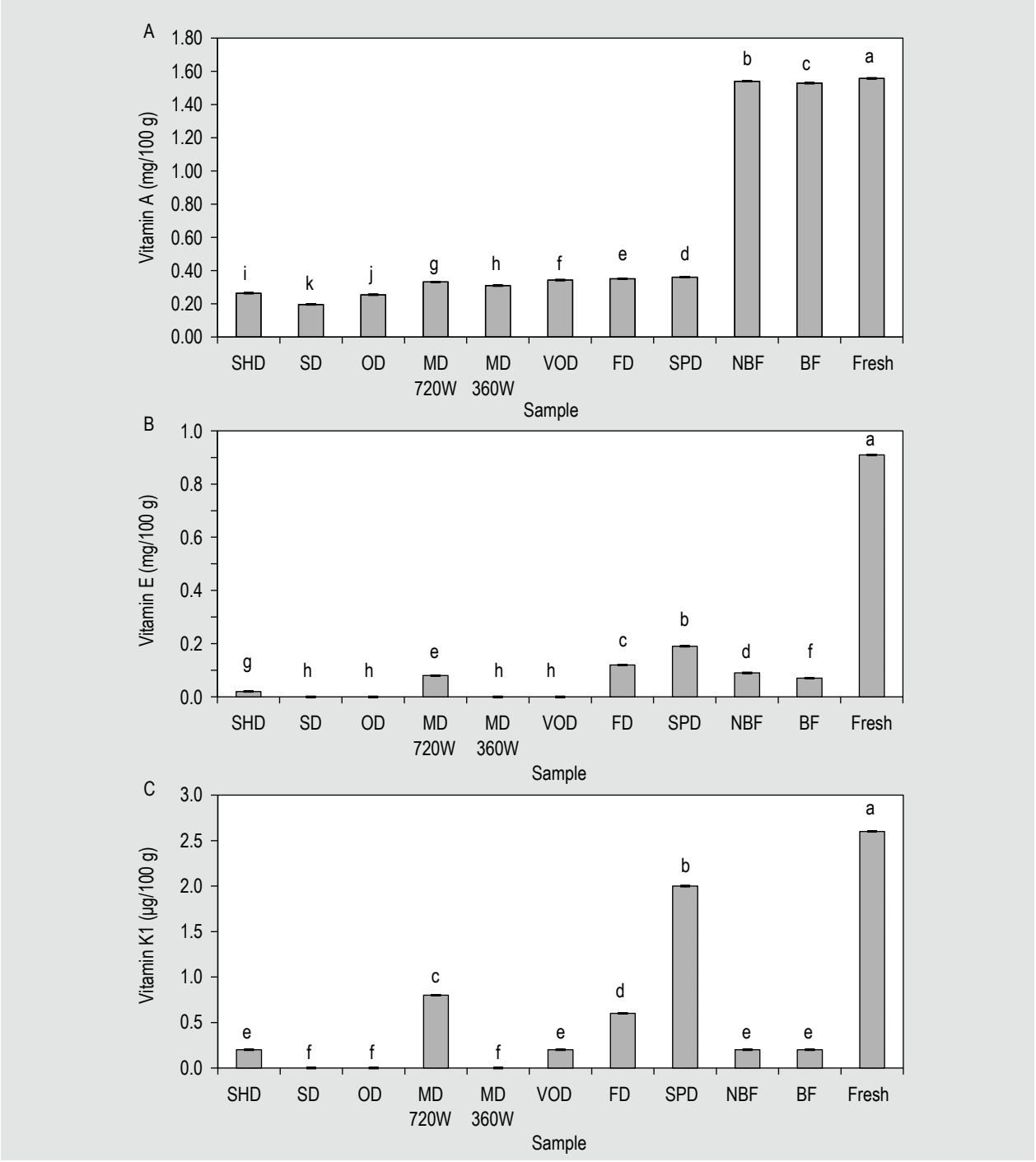

Figure 2. Effect of different processing methods on fat-soluble vitamins of *Spirulina* microalgae. 2A = vitamin A; 2B = vitamin E; 2C = vitamin K1. Values are presented as mean ± SD (n=3). Values followed by the different letter, within the same column, were significantly different (P<0.05), according to LSD's 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; control = fresh *Spirulina platensis*.

packaging affected fat-soluble vitamins reduction during freezing.

Loss of fat-soluble vitamins in SPD was lower than that of the other dehydration methods due to the short time of processing and low pressure oxygen. The content of retinol, α-tocopherol and phylloquinone in freeze-dried sample were decreased significantly (P<0.05). However, due to the low pressure oxygen and low temperature, damaged vitamins in this method was lower than in shade-, sun-,

oven- and microwave-dried samples. However, a further reduction in content of phylloquinone was observed in freeze-dried sample compared to high power microwave-dried sample. The destructive effect of electromagnetic waves on retinol, α-tocopherol and phylloquinone was lower than oven heating due to short exposure time in MD. Heating at low power microwave intensified oxidation and isomerisation reaction; so that this method destroyed α-tocopherol and phylloquinone competently. In another research, the impact of increased microwave heating time on destruction intensity of α-tocopherol was also reported (Malheiro *et al.*, 2009). The presence of double bonds in the structure of fat-soluble vitamins reduces their stability to heating processes especially in alkaline pH (Ottaway, 1993). The destructive effect of oven heating was significantly on retinol, α-tocopherol and phylloquinone content of processed *Sp* (P<0.05). While, loss of retinol and phylloquinone in vacuum oven-dried sample was lower than oven-dried sample due to low pressure oxygen and low temperature. No significant difference (P>0.05) in the content of α-tocopherol was observed when the oven- and vacuum oven-dried samples were compared, that is, this vitamin destroyed in OD and VOD methods competently. Destruction and degradation of retinol in the presence of oxygen and high temperatures at long period is a result of oxidation, isomerisation and fragmentation reactions. Oxidative degradation of retinol breakdown its structure and create volatile compounds (Deman, 1999; Ottaway, 1993). The presence of oxidising agents such as UV and oxygen in traditional drying methods (SHD and SD) reduced the content of retinol, α-tocopherol and phylloquinone in sun- and shade-dried samples significantly (P<0.05). However, reduction of these vitamins in shade-dried sample was lower than that of the sun- and oven-dried samples due to the absence of light and heat. The observed reduction in shade-dried sample was due to oxidation of vitamins in long periods of drying process in the presence of oxygen. α-tocopherol is readily oxidised when directly exposed to air and is stable to heat in the absence of air. Phylloquinone is sensitive to light and alkali and is rapidly oxidised and decomposed in these situations (Deman, 1999). The content of retinol in sun dehydrated leafy vegetables was lower compared to solar energy cabinet dryers due to possibility of more oxidation and isomeration reactions (Mulokozi and Svanberg, 2003).

4. Conclusions

Photochemical components of *Sp* are affected to treatments condition significantly. The quantity of colour compounds and water- and fat-soluble vitamins in *Sp* and their sustainability are subjected to processing conditions including: light, oxygen, time and temperature of processes, electromagnetic waves and also pH, enzymes and the water activity of sample during processing. In dehydrating processes, due to short time of heat exposure in SPD, spray-dried sample is preferred than others in term of carotenoids, chlorophylls, final colour properties and content of water- and fat-soluble vitamins. Due to the high sensitivity of protein segment in phycocyanin structure, FD can protect the highest levels of phycocyanin in *Sp*. Low-power in comparison to high-power of microwave irradiation has more detrimental effect on pigments and water- and fat-soluble vitamins. High temperature and long time of heating process in OD had significant (P<0.05) effects on destruction of *Sp* pigments. Destructive effect of VOD on vitamins was lower than OD method. According to the results, NBF is considered as the best method to protect phycocyanin, carotenoids, chlorophylls pigments and colour properties of *Sp*. It's due to the slow rate of pigments destructive processes such as degradation, oxidation, isomerisation and enzymatic and or non-enzymatic browning reactions in freezing conditions. Furthermore, NBF was determined as the best processing method to maintain the maximum content of niacin, pyridoxine and retinol in *Sp*. Blanching process increased pigments deterioration in the frozen sample significantly (P<0.05). SD, OD and MD (360 W) were the inappropriate methods in protecting vitamins of *Sp*.

In general, due to high sensitivity of pigments and vitamins to processing conditions, the content of pigments and water- and fat-soluble vitamins in processed samples were reduced compared to control. However, non-blanched frozen sample had the least difference than control in pigments and vitamins.

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