

## CHEMICAL AND NUTRITIONAL EVALUATION OF FORTIFIED BISCUITS WITH DRIED *Spirulina* ALGAE

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### ABSTRACT

The cyanobacterium *Spirulina*, a multicellular and filamentous blue green microalgae, is used in many countries of world including Egypt as human safe food. This research aims to study the nutritional values of dried *Spirulina* algae and prepare healthy and high nutritive values biscuits blends fortified with *Spirulina* algae. This study revealed that *Spirulina* algae contained high level of protein  $67.0 \pm 5.2\%$  as dry weight and interesting amounts of bioactive compounds with antioxidative activity such as phycocyanin ( $1254 \pm 23\text{mg /100g}$ ) and  $\beta$ -carotene ( $85 \pm 5\text{ mg/100g}$ ). *Spirulina* algae was rich in minerals such as Na ( $859\text{ mg/100g}$ ), K ( $1399\text{ mg/100g}$ ), Ca ( $689\text{ mg/100g}$ ), P ( $960\text{ mg/100g}$ ), Mg ( $400\text{ mg/100g}$ ) and Fe ( $122\text{ mg/100g}$ ) as average. Results indicated that biscuit blends fortified with dried *Spirulina* algae as 0.5, 1, 2 and 3, % lead to improve the organoleptic, chemical and nutritional properties for it. Biscuit blends were organoleptically accepted for panel test as soon as demonstrated high nutritional values, bioactive, protein and mineral content when compared with unfortified biscuit samples (control).

### INTRODUCTION

The cyanobacterium *Spirulina*, a multicellular and filamentous blue green microalgae, is used in many countries of world as human safe food collected from water, at the same time in many regions of Asia it is used as human health function food (Habib *et al.*, 2008). The food potential of these algae, as source of relative complete proteins (60–70% w/w), unsaturated fatty acids and various minerals, has been reported (Belay, 2002). Apart from chlorophyll a, *Spirulina* contains Phycocyanin which is a blue pigment known to have an important antioxidative power (Chen and Wong, 2008). Many clinical studies suggest several therapeutic effects ranging from reduction of cholesterol, cardio-vascular diseases and cancers to enhancement of the immune system, an increase in intestinal lactobacilli, nephrotoxicity (Mohan *et al.*, 2006). Hernandez and Olgún (2002) reported that two different species of *Spirulina* contained a high percentage of protein ( $68.95 \pm 0.30$  and  $63.73 \pm 0.25\%$ ) as a result of being cultivated in Zarrouk medium. Results obtained by Aly and Gad (2010) revealed the *Spirulina* isolated from Egypt high crude protein content which amounted 64.00% (w/w) on Zarrouk medium.

As a result, there are many applications of *Spirulina* in human healthy food: instantaneous noodles for children (Xu, 1993), beverages (Zeng and Liang 1995) tablets (Yamaguchi, 1997), development of *Spirulina* based biscuits (Gouveia *et al.*, 2007, 2008a; Sharma and Dunkwal 2012), *Spirulina* enriched pasta (Fradique *et al.*, 2010; Zouari *et al.*, 2011), puddings/gelled desserts and mayonnaises/salad dressings (Batista *et al.*, 2008; Gouveia *et al.*, 2008, b) and low fat and high protein frozen yoghurt enriched with papaya fruit and *Spirulina* (Dubey and Kumari 2011). Biscuits are popular and convenient food products, appreciated for their accepted taste, versatility, conservation, convenience, texture and appearance. The use of natural ingredients, exhibiting functional properties and providing specific health benefits beyond traditional

nutrients, is a very attractive way to design new food products, with an important market.

The present research was aimed to study the following point:

- Determination of proximate chemical composition, minerals content and bioactive compounds of dried *Spirulina* algae.
- Prepare biscuits blends fortified with different levels of dried *Spirulina* algae as a natural source of protein, some minerals and bioactive compounds. The effect of this incorporation on the organoleptic properties and nutritional value of the biscuit blends was also evaluated.

### MATERIALS AND METHODS

#### MATERIALS:

Egyptian *Spirulina platensis* algae strain was kindly obtained from Dr. Diaa Gab Allah, National Research Center, Egypt. Wheat flour (*Triticum vulgare*), sugar, vanilla, baking powder, corn oil and salt were obtained from local market in Kafr El-Sheikh City, Egypt. Chemicals used in this study were purchased from El-Gomhoria Company for Chemicals and Drugs, Tanta, Egypt.

#### METHODS:

##### Growing *Spirulina* algae in lab conditions.

*Spirulina* algae isolate was maintained in Zarrouk's modified medium synthetic medium. *Spirulina* was carried out in continues Illumination at  $30 \pm 4^\circ\text{C}$  temperature, each further shaking of culture were carried out manually thrice a day (Mühling, 2000). To avoid bacterial contamination of axenic cultures, all media and materials used for handling cultures were autoclaved at  $15\text{ lb in}^{-2}$  for 20 minutes.

##### Preparation of *Spirulina* algae powder:

*Spirulina* algae was filtered under vacuum using filter paper (Whatman No.1) and washed several time with sterilized and distilled water. Then, the algae cells biomass were dried in thin layers 2 mm under warm air at  $40^\circ\text{C}$  and the air velocity was fixed at 1 m/s for 12 hrs. Dried *Spirulina* algae grounded in electric mill and

passed through 120 mesh sieve screen to produce a fine algae powder and kept in polyethylene bags at low temperature (5 °C) until used. All samples were analyzed in triplicate (Yamsaengsung and Bualuang, 2010).

#### **Preparation of biscuit blends fortified with dried *Spirulina* algae:**

Biscuit blends were prepared by adding powder sugar (253 g), egg (150 ml) and vanilla (0.7 g) and mechanically beaten for 5 min until they creamed. Then unsalted butter (506 g) and dough were added thoroughly and mixed with wheat flour (800 g) for 2 min. Then, 15 g baking powder was added and mixed. *Spirulina* algae powder was substituted with wheat flour at ratios 0.5, 1, 2, 3, 4, and 5% (w/w) and the blended biscuits take ovoid shape. Then, backed in an oven at 160 °C for 15 min. After cooling for one hour, biscuit blends packed in polyethylene bags. The samples were used to evaluate organoleptic properties, chemical composition and nutritive values.

#### **Organoleptic properties of biscuit blends:**

A trained twenty-member panel consisting of students and staff members (both male and female) of the Home Economics Department, Faculty of Specific Education, Kafrelsheikh University, Egypt was selected based on their experience and familiarity with blends of biscuit for the organoleptic properties evaluation. The tests were performed under fluorescent lighting in organoleptic properties laboratory. Tap water was

$$\text{AAS} = \frac{\text{Mg of indispensable amino acids in } 1\text{gm of tested protein}}{\text{Mg of amino acids in } 1\text{gm reference protein}} \times 100$$

#### **Bioactive compounds:**

##### **Total phenolic contents:**

Total phenolic compounds in *Spirulina* extracts were spectrophotometrically determined using Folin-Ciocalteu reagent following the method of Lim *et al.*, (2002).

##### **Determination of chlorophyll a:**

Four grams of algae samples were homogenized in acetone (80 ml, 80%) and allowed to stand overnight in dark at 4°C for complete extraction followed by centrifugation at 10,000 xg for 5 min. Chlorophyll\_a in the supernatant was spectrophotometrically determined according to Lichtenthaler (1987) method.

##### **Extraction and determination of phycocyanin:**

Phycocyanin was extracted by repeated freezing, sonication and thawing of samples in 50 mM phosphate buffer pH 6.8, followed by centrifugation at 4000 rpm for 20 min. The concentration of total blue pigment phycocyanin was spectrophotometrically determined in the supernatant at 615 and 652 nm; respectively, as reported by Silverira *et al.* (2007). Phycocyanin concentration = OD<sub>615</sub> - 0.474 (OD<sub>652</sub>) / 5.34 mg ml<sup>-1</sup>.

##### **Determination of total carotenoids:**

The total carotenoids in samples were spectrophotometrically determined at 450 nm according to standard methods A.O.A.C. (1995). β-carotene, as a standard compound was used for preparation of the calibration curve.

##### **Determination of β-carotene:**

About 5 mg of algae sample with glass beads were placed alternately in an ice bath and in a vortex with portions of 5 ml acetone. The extracts were

provided to rinse the mouth between evaluations. The judges evaluated the samples for taste, colour, odour, texture and overall acceptability. Each organoleptic properties attribute was rated on a 10- point hedonic scale (Abo, *et. al.*, 2014).

#### **Chemical analysis:**

##### **Gross chemical composition:**

Moisture, ash, crude protein, crude fibers and ether extract were determined according to the method of AOAC, (2000). Carbohydrates were calculated by subtracting the total ether extract, ash, crude fibers and protein contents from 100%.

##### **Energy or caloric values**

Total energy contents were determined by multiplying the values obtained for crude protein, total carbohydrates, and total lipids by factors as 4, 4, and 9kcal/g, respectively, and summing the results (Fuentes *et. al.*, 2000).

##### **Minerals:**

The minerals content of samples were determined using the method of Santoso *et. al.*, (2006).

##### **Amino acids analysis**

Amino acids content of biscuit blends were determined according to the methods of AOAC, (2000).

##### **Amino Acid Scores (A.A.S.):**

Amino acid scores (AAS) were calculated for indispensable amino acids according to the FAO/WHO /UNU (1985) procedure using the following equation:

$$\text{AAS} = \frac{\text{Mg of indispensable amino acids in } 1\text{gm of tested protein}}{\text{Mg of amino acids in } 1\text{gm reference protein}} \times 100$$

##### **centrifuged at 3500 rpm for 5 min and collected. The procedure was repeated three times, until both precipitate residue and supernatant became colorless.**

The extracts were also analyzed by reversed-phase high performance liquid chromatography (HPLC), with a C18 column (250/4.6 mm) and a UV/VIS detector, with methanol : acetonitrile (75:25 v/v) as eluent. β-carotene was eluted over 30 min with a flow rate of 1 ml/min. Peak was compared with retention time of standard β-carotene solution in acetone (AOAC, 2000).

##### **Statistical analysis:**

All the obtained data were statistically analyzed by SPSS computer software. The calculated occurred by analysis of variance ANOVA and follow up Duncan's multiple range tests by SPSS ver.11 according to Abo-Allam (2003).

## **RESULTS AND DISCUSSION**

### **Evaluation of nutritional value of *Spirulina* algae.**

#### **Gross chemical composition of dried *Spirulina*:**

Data in Table (1) showed the chemical composition and caloric values of dried *Spirulina* algae. Results indicated that protein content of *Spirulina* is especially high, 67.0%, and comprises almost all amino acids, including the essential ones. However, it should be noted that a reasonable daily consumption of *Spirulina* cannot provide more than about 15 grams of proteins. Depending to many literatures *Spirulina* has the highest crude protein concentration (60-70% of its dry weight) in the range of our results (Hernandez and Olguín, 2002; Aly and Gad, 2010)

**Table (1): Gross chemical composition (g/100g) and caloric values (k.cal/100g) of dried *Spirulina* algae (on dry weight basis).**

Components	Moisture	Crude Protein	Ether extract	Ash	Crude fiber	Carbohydrates	Total caloric values(k.cal)
Dried <i>Spirulina</i>	5.5 ±0.5	67.0±5.2	7.2±0.52	6.8±0.53	3.8±0.31	15.2±0.68	393.6

Each value is the mean of three determination ±SD.

Nevertheless, in the case of children suffering malnutrition, it would be realistic to incorporate up to 10 g of *Spirulina* in the daily ration. Depending on the weight of the child, this can amount to more than 50% of the recommended protein intake. Data in Table (1) confirmed that ether extract of *Spirulina* represented 7.2% of the dry matter. Literature reports fat contents normally lower than 8% for *Spirulina* algae (Ogbonda *et. al.*, (2007); Tokusoglu and Ünal 2003; Gouveia and Oliveira, 2009) while Batista *et. al.*, 2013 found that *Spirulina* presented relative low fat value (4%). The wide variations reported in the literature indicated that there may have been striking differences in medium of cultivation or in the procedures for the extraction or the estimation of the lipid content (Ciferri, 1983). Carbohydrates in Table (1), accounted for slightly lower value (15.2%) of the dry weight of *Spirulina* algae. These results considered acceptable as others reviewers reported that *Spirulina* carbohydrates were accounted for 15 to 20% of the dry weight (Batista *et. al.*, 2013).

That carbohydrates represented in *Spirulina* essentially by a branched polysaccharides, composed of only glucose and structurally similar to glycogen. *Spirulina* uses glycogen as storage product and has thin and fragile peptidoglycan cell walls (Becker, 2004). Small percentage of crude fibers and ash were recorded as 3.8 and 6.8%, respectively, of studied *Spirulina* algae, while total caloric values were 393.6 k.cal./100g on dry weight basis. Tokusoglu and Ünal, (2003) reported similar results in *Spirulina* ash as 7.43-10.38%.

#### Minerals content:

Minerals content of dried *Spirulina* algae (mg/100g) is given in Table (2). *Spirulina* sample was rich in Na (859 mg), K (1399 mg), Ca (689 mg), P (960 mg), Mg (400 mg) and Fe (122 mg) as average. These data are in harmony with others obtained by Tokusoglu and Ünal, (2003) who found that minerals content in *Spirulina* were as follow; Na (929.4 mg), K (1412.9 mg), Ca (826.3 mg), P (750.7 mg), Mg (388.9 mg) and Fe (95.37 mg) as average.

**Table (2): Minerals content of *Spirulina* algae (mg/100g on dry weight basis) and required adult daily dose (RAD) as mg/ day\***

Minerals (mg/100g **)	Sodium (Na)	Potassium (K)	Calcium (Ca)	Phosphorous (P)	Magnesium (Mg)	Iron (Fe)
<i>Spirulina</i> algae	859±25	1399±19	689±10	960±16	400±28	122±8
RAD	500	3500	1200	1000	250-350	18

\* As USA National Research council (1980)

\*\*Each value is the mean of three determination ±SD.

#### Bioactive compounds:

Data in Table (3) showed some bioactive compounds of *Spirulina* algae. The most important compound in *Spirulina* algae was Phycocyanin for its health benefits and as antioxidant. Despite its low content in lipophilic pigments *Spirulina* is very rich in blue phycobiliprotein aqueous pigments, with 1254 mg/100g of Phycocyanin, which is in agreement with results of other authors (Batista *et. al.*, 2013; Ciferri, 1983). Saranraj and Sivasakthi (2014), reported that *Spirulina* and other blue-green algae contain c-phycocyanin, which acts as an accessory pigment when

light energy is captured and transferred to chlorophyll a. This is a spectrophotometry method adapted to extract and quantify a relatively pure c-phycocyanin fraction from *Spirulina*. Among the pigments, the most abundant was chlorophyll, accounts in other studies for 0.8 to 1.5% of the dry weight in *Spirulina* (Batista *et. al.*, 2013; Gouveia *et. al.* 2008c) comparing with these results which presented percentage close to 1010 mg/100g *Spirulina* algae. The conventional sources of chlorophyll are spinach and alfalfa, with approximately 0.5% (w/v) chlorophyll (Lemes, 2012). However, the *Spirulina* biomass could be an alternative source.

**Table (3): Some bioactive compounds of *Spirulina* algae (mg/100g on dry weight basis)\***

Bioactive compounds	(mg/100g)
Phycocyanin	1254±23
Chlorophyll-a	1010±19
Carotenoids	310±9
β-Carotene	85±5
Phenolic compounds (as gallic acid)	800±9.20

\*Each value is the mean of three determination ±SD.

Carotenoids and Beta-carotene represented 310 and 85 mg/100g; respectively. Beta-carotene is the major carotenoids, their content represented in many literatures approximately 0.2 to 0.4% of the dry weight. *Spirulina* is claimed to be the richest whole-food source of  $\beta$ -carotene (Christaki *et al.*, 2012; Sotiroudis and Sotiroudis, 2013). Our finding represented that the amount of Beta-carotene in *Spirulina* algae was  $85 \pm 4$  mg/100g. Habib *et. al.*, (2008) reported that, an intake of 6.0 mg  $\beta$ -carotene daily may be effective in minimizing the risk of cancer. So, anybody takes around 7.0 g of studied *Spirulina* daily that is sufficient to get 6 mg  $\beta$ -carotene. *Spirulina* also contains phenolic compounds which can act as natural antioxidant. Total phenolic compounds presented in Table (3) were 800 mg of gallic acid/100 g dry weight of *Spirulina*. Those results were in harmony with the results reported by Agustini *et. al.*, (2015).

**Evaluation of nutritional value for biscuit blends:  
Organoleptic properties of biscuit blends fortified  
with different levels of dried *Spirulina* algae:**

As the organoleptic properties of foods are of decisive importance in their acceptance, their

modification resulted through adding or replacing components should be carefully studied to access the consumer's reaction to the alterations in taste, texture and colour of the products. Organoleptic properties of the prepared biscuit blends fortified with different levels of dried *Spirulina* algae were considered the important tests affecting on a large extent, their acceptable qualities of the prepared diets. This study was undertaken to determine the effects of adding dried *Spirulina* as a function ingredient in traditional biscuits. Fortified biscuits were prepared using dried *Spirulina* algae with percentage 0.5, 1.0, 2.0, 3.0, 4.0, and 5.0 %. It was noticed that biscuit scored more points than the control of each of them in most of organoleptic characteristics that may be due to crisp texture which happened of difference levels of dried *Spirulina* used. Ismail (2007) observed that the fresh baked biscuits characterized by firm and crisp texture and received high score values. This crisp texture of biscuit was related to the used substitute materials and the moisture content of baked products. These observations are in harmony with those of Joanna *et. al.*, (1990).

**Table: (4). Organoleptic properties of biscuit blends fortified with different levels of dried *Spirulina* algae.**

<b>Biscuit blends</b>	<b>Taste</b>	<b>Colour</b>	<b>Organoleptic properties</b>		
			<b>Odour</b>	<b>Texture</b>	<b>Overall Acceptability</b>
Control	9.20 <sup>a</sup>	10.00 <sup>a</sup>	9.70 <sup>a</sup>	9.48 <sup>a</sup>	9.50 <sup>a</sup>
Biscuit 1	9.30 <sup>a</sup>	8.00 <sup>b</sup>	9.50 <sup>a</sup>	9.49 <sup>a</sup>	9.40 <sup>ab</sup>
Biscuit 2	8.50 <sup>ab</sup>	7.40 <sup>bc</sup>	9.10 <sup>a</sup>	9.49 <sup>a</sup>	8.50 <sup>b</sup>
Biscuit 3	8.50 <sup>ab</sup>	6.10 <sup>c</sup>	6.50 <sup>b</sup>	9.50 <sup>a</sup>	8.50 <sup>b</sup>
Biscuit 4	7.10 <sup>b</sup>	5.60 <sup>c</sup>	5.20 <sup>c</sup>	9.50 <sup>a</sup>	7.40 <sup>b</sup>
Biscuit 5	3.30 <sup>cd</sup>	3.50 <sup>d</sup>	4.30 <sup>d</sup>	9.54 <sup>ab</sup>	4.40 <sup>c</sup>
Biscuit 6	3.20 <sup>cd</sup>	1.10 <sup>e</sup>	3.10 <sup>e</sup>	9.55 <sup>b</sup>	3.50 <sup>c</sup>

Where: Control: Biscuit without dried *Spirulina* algae.

- Biscuit 1: Fortified with 0.5% dried *Spirulina* algae.
- Biscuit 2: Fortified with 1.0% dried *Spirulina* algae.
- Biscuit 3: Fortified with 2.0% dried *Spirulina* algae.
- Biscuit 4: Fortified with 3.0% dried *Spirulina* algae.
- Biscuit 5: Fortified with 4.0% dried *Spirulina* algae.
- Biscuit 6: Fortified with 5.0% dried *Spirulina* algae.

Mean values in each column designated by the same letter are not significantly different at 5.0% level using Duncan's multiple range tests.

Data in Table (4) and Fig (1) revealed that biscuit blends fortified with high levels of dried *Spirulina* algae had not accepted especially in taste, colour, odour, texture and overall acceptability. The organoleptic quality start dramatically decreased in biscuit 5 and biscuit 6 with lowest values (less than 5) which we eliminated in further studies. For a product to be considered approved in relation to its organoleptic properties, it is necessary that the acceptability index be at least 70%, adequate for consumption, with a good marketability (Santos *et. al.*, 2011) so we decided the above decisions. In otherwise, those results not agreed

with Sharma and Dunkwal (2012) who prepared biscuits with 10 % dried *Spirulina* algae in India. They found that mean score for overall acceptability of value added biscuit was 7.5 against the control sample, 7.9 on nine point hedonic ranking scale. Accepted high percentage (10% dried *Spirulina* algae) in the Indian study and unaccepted even half of it (4% dried *Spirulina* algae) in the Egyptian study could be attributed for panel members food culture and habit. The eating habits of an individual are acquired depending on one's environment or family experiences.



**Fig (1): Prepared biscuit blends fortified with different percentage of *Spirulina* algae.**

Where Control: Biscuit without dried *Spirulina* algae.

Biscuit 1: Fortified with 0.5% dried *Spirulina* algae.

Biscuit 2: Fortified with 1.0% dried *Spirulina* algae.

Biscuit 3: Fortified with 2.0% dried *Spirulina* algae.

Biscuit 4: Fortified with 3.0% dried *Spirulina* algae.

Biscuit 5: Fortified with 4.0% dried *Spirulina* algae.

Biscuit 6: Fortified with 5.0% dried *Spirulina* algae.

Traditionally, biscuits exhibit light yellow colorations, derived from its tradition ingredients. So, Egyptian people not accepted the green colour especially with high tone in the high concentration of *Spirulina* algae. Colour factor was the crucial variable in the organoleptic properties evaluation. In other hand, the texture of the biscuit blends in Table (4), was also evaluated, and a significant increase of their firmness was evidenced with an increase of added dried *Spirulina* algae. These results evidence the positive effect of the algae in the biscuit structure, reinforcing the short dough system. Biscuits are considered solid emulsions of sucrose, lipids and non-gelatinized starch, being this morphology is responsible for the biscuits structure and

texture. The main factor affecting these properties is the moisture content and water mobility, which are highly affected by the interaction with hydroxyl groups present in the matrix. The replacement of a small amount of flour by dried *Spirulina* algae resulted in the inclusion of a complex biomaterial, rich in different proteins and polysaccharides. These molecules have an important role on the water absorption process, which promote the increase of biscuits firmness, resulting in more compact structures (Gouveia *et. al.*, 2008c).

#### **Chemical composition of biscuit blends:**

Biscuits are the most popular bakery products consumed nearly by all sections of the society in Egypt. Some of the reasons for such wide popularity are low cost as

comparing with other processed foods affordable cost, good nutritional quality and availability in different forms, varied taste and longer shelf life. Bakery products are sometimes used as a vehicle for incorporation of different nutritionally rich ingredients (Sudha *et. al.*, 2007 and Zedan, 2012). Data given in Table (5) showed the centesimal chemical composition of biscuits fortified with different levels of dried *Spirulina* algae, it could be noticed that the moisture content for biscuits fortified with dried *Spirulina* algae was less than biscuits without dried *Spirulina* (control). Moisture content is measured for a number of reasons including legal and label requirements, economic importance, food quality better processing operations and storage stability considerations. The moisture content in biscuits was ranged between (3.90 to 4.90%), the highest value was in control biscuit and the lowest

in biscuit 4 fortified with 3.0% dried *Spirulina* algae. Moisture content showed slight and gradually decreased in all biscuit blends as comparing with control samples, but it was not significant increase P< 0.05. For protein content, the results cleared that the percentage of crude protein was increased in a noticed degree by adding dried *Spirulina* algae. Crude protein content in biscuits was ranged from (6.8 to 7.8 %). The highest crude protein content was found in Biscuit 4 followed by Biscuit 3, Biscuit 2 and Biscuit 1 as (7.8, 7.4, 7.2, and 6.9 %), respectively. Crude protein showed significant increase (P< 0.05) in all biscuit blends except with Biscuit 1 as comparing with control biscuits. Fat, ash, crude fibers and carbohydrates in both control biscuits and biscuits blended with dried *Spirulina* algae were similar and there were no significance differences between them.

**Table (5): Chemical composition of biscuit blends fortified with different levels of dried *Spirulina* algae (g/100g dry weight basis).**

Biscuit blends	Moisture	Crude Protein	Fat	Ash	Crude fibers	Carbohydrates
Control	4.90 <sup>a</sup>	6.80 <sup>c</sup>	24.94 <sup>ab</sup>	0.55 <sup>a</sup>	•.8 <sup>ab</sup>	66.87 <sup>a</sup>
Biscuit 1	4.70 <sup>a</sup>	6.90 <sup>bc</sup>	26.16 <sup>a</sup>	0.61 <sup>a</sup>	•.12 <sup>ab</sup>	65.73 <sup>a</sup>
Biscuit 2	4.10 <sup>ab</sup>	7.20 <sup>b</sup>	20.8 <sup>ab</sup>	0.62 <sup>a</sup>	•.19 <sup>ab</sup>	70.59 <sup>a</sup>
Biscuit 3	3.91 <sup>ab</sup>	7.40 <sup>ab</sup>	22.58 <sup>ab</sup>	0.59 <sup>a</sup>	•.13 <sup>a</sup>	68.09 <sup>a</sup>
Biscuit 4	3.90 <sup>ab</sup>	7.80 <sup>a</sup>	24.74 <sup>ab</sup>	0.45 <sup>a</sup>	•.11 <sup>ab</sup>	66.20 <sup>a</sup>

Where: Control: Biscuit without dried *Spirulina* algae.

Biscuit 1: Fortified with 0.5% dried *Spirulina* algae.

Biscuit 2: Fortified with 1.0% dried *Spirulina* algae.

Biscuit 3: Fortified with 2.0% dried *Spirulina* algae.

Biscuit 4: Fortified with 3.0% dried *Spirulina* algae.

Mean values in each column designated by the same letter are not significantly different at 5.0% level using Duncan's multiple range tests.

Carbohydrates mean values in studied biscuits were ranged from 66.20 to 70.59%. The highest carbohydrates content of biscuits may be due to high content of wheat flour and this agree with that reported by Arafa, (2009) and Ahmed (2012), who mentioned that wheat flour at extraction ratio 72% involves high amount of total carbohydrates. The increase in crude protein of biscuits fortified with dried *Spirulina* can be attributed to the high content of ingredient in *Spirulina* algae. These results are confirmed with the results of Hooda and Jood (2005) and Eissa *et. al.* (2007) they reported that high protein content of biscuits prepared from blends of wheat-raw and germinated leguminous flour. Our results in general were in the same line of others obtained by Sharma and Dunkwal (2012) who prepared biscuits with 10 % dried *Spirulina* algae. The developed value added biscuit contained 2.9% moisture, 19.6g protein, 26.71g fat, 2.08g crude fiber, 1.83g ash, 46.83 g carbohydrates

and 506.11 kcal energy/100g on dry weight basis. And also with results obtained by Singh *et. al.*, 2015 who found that addition of *Spirulina* powder in biscuit increased its protein as well as antioxidant potential in proportion to the level of *Spirulina* powder added.

#### Caloric values of biscuit blends:

Data in Table (6) showed the caloric values of biscuits fortified with different levels of *Spirulina* algae according to nutrient sources. It is noticed the calories become from the protein as a source increased with the increasing of the percentage of dried *Spirulina* algae in biscuit blends. It start from 27.2 in control biscuit and reached to 31.2 in biscuit 4 which contain 3% dried *Spirulina* algae. In other wise, it were clear that both fat or carbohydrates not play an important role as results not correlate with the percentage of algae

**Table (6): Caloric values of biscuit blends fortified with different levels of *Spirulina* algae (kcal. /100 g of biscuit blends)**

Biscuit blends	Sources of calories (k.cal.)			Total caloric values (k.cal.)/100 g
	Crude Protein	Fat	Carbohydrates	
Control	27.2 <sup>b</sup>	224.46 <sup>a</sup>	267.48 <sup>a</sup>	519.14a
Biscuit 1	27.6 <sup>b</sup>	235.44 <sup>a</sup>	262.92 <sup>a</sup>	525.96 <sup>ab</sup>
Biscuit 2	28.8 <sup>ab</sup>	187.2 <sup>a</sup>	282.36 <sup>a</sup>	598.36 <sup>a</sup>
Biscuit 3	29.6 <sup>ab</sup>	203.22 <sup>a</sup>	272.36 <sup>a</sup>	505.18 <sup>b</sup>
Biscuit 4	31.2 <sup>a</sup>	222.66 <sup>a</sup>	264.81 <sup>a</sup>	518.66 <sup>ab</sup>

Where: Control: Biscuit without dried *Spirulina* algae.

Biscuit 1: Fortified with 0.5% dried *Spirulina* algae.

Biscuit 2: Fortified with 1.0% dried *Spirulina* algae.

Biscuit 3: Fortified with 2.0% dried *Spirulina* algae.

Biscuit 4: Fortified with 3.0% dried *Spirulina* algae.

Mean values in each column designated by the same letter are not significantly different at 5.0% level using Duncan's multiple range tests.

Ingredients used for making biscuits are considered of low nutritive and biological values, for example soft wheat flour used is deficient in several nutrients including some vitamins, minerals and dietary fibre and contains only 7 to 10 % of protein. Besides, wheat flour lacks certain essential amino acids such as lysine, tryptophan and threonine (Erben *et. al.*,2014).

#### Amino acids composition of biscuit blends.

Amino acids composition of biscuit blends fortified with different levels of dried *Spirulina* algae are shown in Table (7). The biscuits samples contained all detected indispensable amino acids. It could be noticed that, all indispensable amino acids contents of tested biscuits were found to be higher than that of reference protein recommended by FAO/WHO/UNU (1985). It could be observed that, biscuits blends fortified with different levels of dried *Spirulina* had the highest amount of indispensable amino acids (individual and total) than control biscuit.

It was clear that leucine was the highest amino acids in control biscuit (6.85), followed by phenylalanine (5.75), while lysine was the lowest value

**Table (7): Amino acids composition of biscuit blends (g/100g protein).**

Amino acids	Biscuit blends	Control	Biscuit 1	Biscuit 2	Biscuit 3	Biscuit 4	FAO/WHO/UNU pattern (1985)
Indispensable (Essential) Amino Acids							
Threonine	3.09 <sup>c</sup>	3.21 <sup>ab</sup>	3.19 <sup>ab</sup>	3.46 <sup>a</sup>	3.19 <sup>ab</sup>	3.19 <sup>ab</sup>	0.90
Valine	4.20 <sup>ab</sup>	4.39 <sup>ab</sup>	5.06 <sup>a</sup>	5.03 <sup>a</sup>	4.73 <sup>a</sup>	4.73 <sup>a</sup>	1.30
Methionine + Cystine	3.31 <sup>b</sup>	3.64 <sup>a</sup>	3.85 <sup>a</sup>	3.46 <sup>ab</sup>	3.47 <sup>ab</sup>	3.47 <sup>ab</sup>	1.70
Isoleucine	3.54 <sup>ab</sup>	3.53 <sup>ab</sup>	4.07 <sup>a</sup>	3.98 <sup>a</sup>	3.67 <sup>a</sup>	3.67 <sup>a</sup>	1.30
Leucine	6.85 <sup>b</sup>	7.07 <sup>b</sup>	7.70 <sup>a</sup>	7.75 <sup>a</sup>	7.43 <sup>ab</sup>	7.43 <sup>ab</sup>	1.90
Histidine	2.43 <sup>ab</sup>	2.46 <sup>ab</sup>	2.75 <sup>a</sup>	2.62 <sup>a</sup>	2.32 <sup>ab</sup>	2.32 <sup>ab</sup>	1.60
Tyrosine	4.09 <sup>a</sup>	3.96 <sup>a</sup>	4.29 <sup>a</sup>	4.29 <sup>a</sup>	3.96 <sup>a</sup>	3.96 <sup>a</sup>	1.90
Phenylalanine	5.75 <sup>a</sup>	5.67 <sup>ab</sup>	5.94 <sup>a</sup>	5.86 <sup>a</sup>	5.31 <sup>b</sup>	5.31 <sup>b</sup>	1.90
Lysine	2.65 <sup>c</sup>	2.78 <sup>ab</sup>	2.97 <sup>a</sup>	2.93 <sup>a</sup>	2.70 <sup>ab</sup>	2.70 <sup>ab</sup>	1.60
Total indispensable A.A	35.91 <sup>b</sup>	36.72 <sup>ab</sup>	39.82 <sup>a</sup>	39.37 <sup>a</sup>	36.18 <sup>ab</sup>	36.18 <sup>ab</sup>	
Disposable Amino Acids							
Aspartic acid	5.30 <sup>ab</sup>	5.78 <sup>ab</sup>	6.27 <sup>a</sup>	6.39 <sup>a</sup>	5.98 <sup>ab</sup>	5.98 <sup>ab</sup>	
Glutamic acid	26.00 <sup>a</sup>	24.90 <sup>a</sup>	26.10 <sup>a</sup>	25.30 <sup>a</sup>	23.90 <sup>a</sup>	23.90 <sup>a</sup>	
Serine	4.97 <sup>ab</sup>	4.71 <sup>ab</sup>	4.62 <sup>ab</sup>	5.34 <sup>a</sup>	4.34 <sup>ab</sup>	4.34 <sup>ab</sup>	
Glycine	3.54 <sup>b</sup>	3.64 <sup>a</sup>	3.96 <sup>a</sup>	3.98 <sup>a</sup>	3.57 <sup>ab</sup>	3.57 <sup>ab</sup>	
Arginine	4.09 <sup>ab</sup>	4.28 <sup>ab</sup>	4.62 <sup>a</sup>	4.82 <sup>a</sup>	4.54 <sup>a</sup>	4.54 <sup>a</sup>	
Alanine	3.43 <sup>b</sup>	3.53 <sup>ab</sup>	3.96 <sup>a</sup>	4.08 <sup>a</sup>	3.76 <sup>a</sup>	3.76 <sup>a</sup>	
Proline	5.30 <sup>b</sup>	9.85 <sup>a</sup>	9.90 <sup>a</sup>	9.53 <sup>a</sup>	8.88 <sup>a</sup>	8.88 <sup>a</sup>	
Total dispensable A.A	52.59 <sup>b</sup>	56.75 <sup>ab</sup>	59.41 <sup>a</sup>	59.48 <sup>a</sup>	55.02 <sup>ab</sup>	55.02 <sup>ab</sup>	
Total A.A.	88.51 <sup>b</sup>	93.47 <sup>a</sup>	99.23 <sup>a</sup>	98.85 <sup>a</sup>	91.22 <sup>ab</sup>	91.22 <sup>ab</sup>	

Where: Control: Biscuit without dried *Spirulina* algae.

Biscuit 1: Fortified with 0.5% dried *Spirulina* algae.

Biscuit 2: Fortified with 1.0% dried *Spirulina* algae.

Biscuit 3: Fortified with 2.0% dried *Spirulina* algae.

Biscuit 4: Fortified with 3.0% dried *Spirulina* algae.

Mean values in each column designated by the same letter are not significantly different at 5.0% level using Duncan's multiple range tests.

#### Amino acids score of biscuit blends

The amino acids scores (AAS) of the indispensable amino acids in control biscuit was ranged from 1.52 to 5.18, the highest value was detected in phenylalanine + tyrosine followed by leucine (3.61) and the lowest value was presented in histidine. Biscuit blends fortified with dried *Spirulina* algae showed increase in A.A.S., it ranged between 1.45 to 5.38. The highest values were in phenylalanine +tyrosine in biscuit 2 followed by leucine

(2.65) of essential amino acids. Biscuit 2 recorded total indispensable A.A. (39.82) as the highest biscuit blends compared with the lowest one which was the biscuit control (35.91).

All biscuits blends samples contained more amounts of dispensable amino acids than indispensable amino acids.

The nutritive value of dietary protein depends on its indispensable amino acids composition .The amino acids scores can be considered as an imperfect indicator of protein quality, but it still the best one based on amino acids. The nutritive value of a protein depends primarily on its efficiency to satisfy the requirements for essential amino acids. Thus, the amino acids requirements are the logical yard -stick by which protein quality can be measured (Abdou ,2004 and Zedan 2012). This is due to the fact that protein quality as determined by biological procedures depends on the limiting indispensable amino acids .The amino acid scores were calculated to throw light on the quality of bakery products as affected by of dried *Spirulina* additives.

(4.08) in biscuit 3 and the lowest value was presented in histidine in biscuit 4 (Table 8).

The first limiting amino acids in all biscuit blends was the histidine, while lysine was the second limiting amino acids. The AAS of threonine, valine, methionine + cystine, leucine, Histidine and lysine in all biscuit blends fortified with (0.5-3.0%) dried *Spirulina*, showed some improvement.

**Table (8): Amino acids scores\* (A.A.S.) of biscuit blends fortified with different levels of dried *Spirulina* algae.**

Amino acids	Biscuit blends					FAO/WHO/UNU pattern (1985)*
	Control	Biscuit 1	Biscuit 2	Biscuit 3	Biscuit 4	
Threonine	3.44	3.57	3.54	3.84	3.54	0.90
Valine	3.23	3.38	3.89	3.87	3.64	1.30
Methionine + Cystine	1.95	2.14	2.26	2.03	2.04	1.70
Isoleucine	2.72	2.72	3.13	3.06	2.82	1.30
Leucine	3.61	3.72	4.05	4.08	3.91	1.90
Histidine	1.52	1.54	1.72	1.64	1.45	1.60
Phenylalanine + Tyrosine	5.18	5.07	5.38	5.35	4.88	1.90
Lysine	1.66	1.74	1.86	1.83	1.69	1.60
First limiting amino acid	Histidine	Histidine	Histidine	Histidine	Histidine	-
Second limiting amino acids	Lysine	Lysine	Lysine	Lysine	Lysine	-

Where: Control: Biscuit without dried *Spirulina* algae.Biscuit 1: Fortified with 0.5% dried *Spirulina* algae.Biscuit 2: Fortified with 1.0% dried *Spirulina* algae.Biscuit 3: Fortified with 2.0% dried *Spirulina* algae.Biscuit 4: Fortified with 3.0% dried *Spirulina* algae.

\*Chemical scores were calculated as amino acid ratio of food protein to reference protein FAO/WHO/UNU (1985)

**Minerals content of biscuit blends:**

Data in Table (9) illustrated minerals content of biscuit blends fortified with different levels of dried *Spirulina* algae, including sodium, potassium, calcium, phosphorus, magnesium and iron. Generally, adding dried *Spirulina* algae to biscuit blends with all added percentages (0.5 to 3.0%) increased minerals content of all samples. It was noticed that there were significant difference between all biscuits blends and the control in all studied minerals except for phosphorus. Sodium values showed significantly different among all biscuit blends. Biscuit 4 showed the highest sodium content (150 mg/100g), followed by Biscuit 3 (131.10

mg/100g), Biscuit 2 (95.9 mg/100g) and Biscuit 1 (72 mg/100m); while control biscuit recorded the lowest sodium content (47.2 mg/100g). Biscuits blends contained high levels of potassium began dramatically with Biscuit 1 (878 mg/100g) and reached to the maximum value with Biscuit 4 (1149 mg/100g) which recorded 13 fold potassium content of biscuit control. Potassium content was highly significance between all biscuit blends and biscuit control. Those results were much more than with others reported by Sharma and Dunkwal (2012) who found potassium content in fortified biscuits was 292 mg/100g.

**Table (9): Minerals contents of biscuit blends fortified with different levels of dried *Spirulina* algae (mg/100g, dry weight basis)**

Biscuit blends	Minerals					
	Sodium (Na)	Potassium (K)	Calcium (Ca)	Phosphorous (P)	Magnesium (Mn)	Iron (Fe)
Control	47.20 <sup>a</sup>	84.90 <sup>e</sup>	162.35 <sup>c</sup>	0.22 <sup>b</sup>	154.70 <sup>c</sup>	22.10 <sup>b</sup>
Biscuit 1	72.00 <sup>b</sup>	878.00 <sup>d</sup>	194.80 <sup>b</sup>	0.23 <sup>b</sup>	167.70 <sup>b</sup>	26.34 <sup>a</sup>
Biscuit 2	95.90 <sup>c</sup>	1007.00 <sup>c</sup>	182.70 <sup>b</sup>	0.12 <sup>bc</sup>	168.80 <sup>b</sup>	31.72 <sup>a</sup>
Biscuit 3	131.10 <sup>d</sup>	1046.00 <sup>b</sup>	234.80 <sup>a</sup>	0.26 <sup>a</sup>	188.10 <sup>a</sup>	26.58 <sup>a</sup>
Biscuit 4	150.00 <sup>e</sup>	1149.00 <sup>a</sup>	277.20 <sup>a</sup>	0.27 <sup>a</sup>	196.80 <sup>a</sup>	31.40 <sup>a</sup>

Where: Control: Biscuit without dried *Spirulina* algae.Biscuit 1: Fortified with 0.5% dried *Spirulina* algae.Biscuit 2: Fortified with 1.0% dried *Spirulina* algae.Biscuit 3: Fortified with 2.0% dried *Spirulina* algae.Biscuit 4: Fortified with 3.0% dried *Spirulina* algae.

Mean values in each column designated by the same letter are not significantly different at 5.0% level using Duncan's multiple range tests.

Both of Biscuit 3 and Biscuit 4 were very important sources of calcium as (234.8 and 277.2 mg/100g); respectively, with being not significant difference between all of them. The lowest calcium content was recorded in control biscuit (162.35 mg/100g). Regarding phosphorus content, there were not significant differences among control biscuit, Biscuit 1 and Biscuit 2 without trend of increase. However, phosphorus contents start to be significant more than control in Biscuit 3 and Biscuit 4 (0.26 and 0.27 mg/100g). Magnesium content increased slowly as a function of the increase of add dried algae percentage. There were significant differences between biscuit

blends (3 and 4) and biscuit blends (1, 2 and control). The highest value was detected in Biscuit 4 (196.8 mg/100g) which record to be 1.3 fold in magnesium content more than the biscuit control. Iron content differed significantly in biscuit blends fortified with dried algae comparing with biscuit control. However, there were no significant differences among those biscuit blends. But as others studied elements, Biscuit 4 recorded the maximum contents of iron (31.4 mg/100g). The obtained results recorded iron content much more than that reported by Sharma and Dunkwal (2012) who prepared biscuits with 10 % dried *Spirulina* algae and found that iron content recorded about 17.62 mg/100g.

It was cleared that biscuit blends containing dried *Spirulina* algae at different levels were a good source for sodium, potassium, calcium, phosphorus, magnesium and iron.

#### Bioactive compounds of biscuit blends:

Data in Table (10) showed total chlorophyll-a, total carotenoids, phycocyanin and total Phenolic compounds of biscuit blends fortified with different levels of dried *Spirulina* algae. Chlorophyll-a increased upon the increase of dried *Spirulina* algae levels.

**Table (10): Bioactive compounds of biscuit blends fortified with different levels of dried *Spirulina* algae.**

Biscuit blends	Bioactive compounds	Carotenoids ( $\mu\text{g g}^{-1}$ )			
		Chlorophyll-a ( $\mu\text{g g}^{-1}$ )	Total carotenoids	$\beta$ -carotene	Phycocyanin ( $\mu\text{g g}^{-1}$ )
Control		0.00 <sup>d</sup>	29.30 <sup>d</sup>	10.90 <sup>d</sup>	0.00 <sup>d</sup>
Biscuit 1		31.70 <sup>c</sup>	58.80 <sup>c</sup>	21.40 <sup>c</sup>	22.10 <sup>c</sup>
Biscuit 2		29.70 <sup>c</sup>	63.90 <sup>bc</sup>	20.10 <sup>c</sup>	23.90 <sup>c</sup>
Biscuit 3		39.40 <sup>b</sup>	69.00 <sup>b</sup>	29.10 <sup>b</sup>	30.70 <sup>b</sup>
Biscuit 4		46.10 <sup>a</sup>	72.00 <sup>a</sup>	31.10 <sup>a</sup>	35.00 <sup>a</sup>

Where: Control: Biscuit without dried *Spirulina* algae.

Biscuit 1: Fortified with 0.5% dried *Spirulina* algae.

Biscuit 2: Fortified with 1.0% dried *Spirulina* algae.

Biscuit 3: Fortified with 2.0% dried *Spirulina* algae.

Biscuit 4: Fortified with 3.0% dried *Spirulina* algae.

Mean values in each column designated by the same letter are not significantly different at 5.0% level using Duncan's multiple range tests.

In other compounds like carotenoids, results indicated that control biscuit have a little amount which considered lowest amount with significant different regarding others biscuit blends. Total carotenoids ranged between 29.3 in control biscuit and increase upon the increase of dried *Spirulina* algae level to reach the maximum with 3.0% in biscuit 4 as 72.0.  $\beta$ -carotene started also with small amount in control biscuit as 10.9 then increased to reach a 31.10 in biscuit 4. Those results were less than others obtained by Sharma and Dunkwal (2012) found that  $\beta$ -carotene in biscuits fortified with 10 % dried *Spirulina* and contents was in the range of 349.75  $\mu\text{g}/100\text{g}$ . The major differences in biochemical composition of studied biscuit blends were related to the present of phycocyanin and chlorophyll-a in *Spirulina* algae as markable compounds. Phycocyanin and chlorophyll amount in biscuit control were 0, whereas those amount increased upon the increase of dried *Spirulina* algae to reach the maximum as 80.9 and 35.00  $\mu\text{g g}^{-1}$ , respectively, in Biscuit 4. Total phenolic increased slowly with no significant deference between control biscuit (55.2) and biscuit 1 (61.20), then increased with high ratio significantly to reach the maximum in biscuit 4 (99.2). Those results were agreed with Rodriguez De Marco *et. al.*, (2014) who reported that pasta with *Spirulina* exhibited high phenolic compounds content and antioxidant activity compared to control pasta, which could be used to enhance the nutritional profile of the product.

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upon the increase of dried *Spirulina* algae levels. Results indicated that chlorophyll-a ranged between 0.0 and 46.1  $\mu\text{g g}^{-1}$ . It is noticed that the lowest results were for control biscuit and highest values were for biscuit 4. Data reveled that there were high significance between control biscuit (0 of Chlorophyll-a) and others biscuit blends.

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**التقييم الكيميائي وال الغذائي للبسكويت المدعوم بطعم الاسبيرولينا الجاف**  
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يستخدم طلب الاسبيرولينا كغذاء أمن للانسان في العديد من الدول ومن بينها مصر وهو من الطحالب الخضراء المزرعة عبيده الخلايا خطي. يهدف هذا البحث لدراسة القيمة الغذائية لطلب الاسبيرولينا واعداد خلطات البسكويت المدعوم به ذات القيمة الغذائية المرتفعة حيث أوضحت هذه النتائج احتواء طلب الاسبيرولينا على نسب مرتفعة من البروتين(٦٧%) ومن المركبات الحيوية الفعالة والتي لها قدره عالية كمضادات للأكسدة من خلال خصائصها مثل الفيوكسيانين (١٢٥٤ ملجم/١٠٠ جم) والبيتاكاروتين (٨٥ ملجم/١٠٠ جم). والطلب غني بالعناصر المعدنية مثل الصوديوم (٨٥٩ ملجم)-اليوتاسيوم (٦٨٩ ملجم)-الكالسيوم (١٣٩٩ ملجم) كما أوضحت النتائج أن تدعيم خلطات البسكويت بطلب الاسبيرولينا الجاف بنسبة ١، ٢، ٣، ٤% أدى إلى تحسين الخواص الحسية والكميائية والغذائية لها حيث كانت الخلطات مقوله حسياً لدى المحكمين ومترتفعة القيمة الغذائية من المركبات الحيوية الفعالة والبروتين والعناصر المعدنية مقارنة بعينات البسكويت الغير مدعوم بطلب الاسبيرولينا (الكترون).