# Enrich nutritional value for baldy bread fortified with *Spirulina* (*Arthrospira platensis*) microalgae

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**Abstract:** Bread is one of the most popular bakery products, generally made from wheat flour 72% extraction which is limited in vitamins, minerals, antioxidants, and dietary fiber. Therefore, in the present study, wheat flour was fortified with 20% hull-less barley which contained *β*-glucan as a natural antioxidant and dietary fiber and also, it was supplemented with *Spirulina platensis* microalgae powder at 2.5, 5.0, 7.5 and 10.0% levels to improve its nutritional quality. The results showed that the *Spirulina platensis* is rich in proteins, antioxidants, DPPH Radical Scavenging, and dietary fiber and also hull-less barley had contained rich amounts from dietary fiber and β- glucan. The results indicated also that the water absorption, dough development time and stability time increased gradually by increasing the level of *Spirulina platensis* microalgae powder and 20% hull-less barley. However data showed that the wheat flour ws being replaced with *Spirulina platensis* microalgae powder at different levels and 20% hull-less barley minimized extensibility and dough energy.
The results indicated that 7.5 % the addition *of Spirulina platensis* have not any negative effects on the shelf life of the loaves of bread and it improved the nutritional value, protein digestibility, and biological evaluation and blood compositions in rats.
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**Key words:** *Spirulina platensis***,** DPPH Radical Scavenging, Rheological properties

## 1. Introduction

Bread is a staple food that is increasingly consumed in developing countries and is characterized by its low protein content. The two elements, increase consumption and low protein content, make bread an ideal transport for protein immunization which is one of the protein energy strategies that combat malnutrition, as well as fortify with other nutrients such as minerals and vitamins (Meite *et al*., 2008).

Microalgae are having the power to increase the nutritional content of traditional food and for this reason, to positively influence humans may be caused to their original chemical constituents (Gouveia *et al.*, 2008).

Seaweeds appear favorable known and it is the purpose of functional foods may be caused by being naturally fortified in key nutrients and in various health-promoting compounds. The main compounds of macroalgae's are natural dietary fiber, a natural antioxidant, lipids, peptides, and minerals content. It could be that can hold possibility in high nutrition value products obtained from macroalgae, thus, directly to support the cardiovascular-health activity (Cardoso *et al.*, 2015).

Algae are an essential source of various nutrients. Great protein content in different species of algae is one of the major causes of an untraditional source of proteins and oils. For this reason, the algae also appear a significant source of vitamins, minerals, natural antioxidants, and natural colorants. Therefore, it could be utilized to supply the color, increase the nutritional value, and become better the resistance of oxidation. Thus, the integration of algae into conventional food is a way to give healthy new products (Oniszczuk and Podgórski, 2015 and Oniszczuk and Olech, 2016).

The great variation of compounds that are synthesized from the various metabolic pathways of freshwater and marine algae provide favorable sources of total lipids, vitamins, total carbohydrates, total protein content, and carrageenan. Furthermore, microalgae utilized to produce biological materials which economic prominence in nutrition science, and general health (Sathasivam *et al.*, 2018). Algae are considered a solution to the lack of proper nutrition and hunger. Therefore, persons in the Gulf region have face troubles to malnutrition, thus, the entry of novel nutrition into their food, like algae, would be useful (Al-Thawadi, 2018).

At recent time, eaten seaweed products have begun to be communal in the nutrition fortification for many countries may be due to has contained high nutritional characteristics (Kılınc *et al*., 2013). n the Middle East, there has been a direction to perform nutrition utilizing algae for children at public schools that are the nutrition of algae like *Spirulina*. It had contained rich amounts of nutrient compounds like protein, mineral salts; enzymes; natural antioxidants; vitamins, and rare fatty acids (Matondo *et al.*, 2016).

Bread, which is a high carbohydrate food, is habitually traditionally consumed with almost all foods in our country. The aim of this study is to add

*Spirulina* (*Arthrospira platensis*) to bread, with the purpose of increasing its protein and enriching it in terms of calcium, iron, and magnesium.

## 2. Materials and Methods

**Algal species collections**

The algal species used in this study; namely, *Spirulina* (*Arthrospira platensis*) (Blue-green algae) were collected from Red Seashore at Jeddah City, Kingdom of Saudi Arabia. Algae *Spirulina platensis* was cleaned of epiphytes and necrotic parts were removed. Then the algal species were rinsed with sterile water to remove any associated debris. Half of these cleaned fresh materials were air-dried as described by González del Val *et al.* (2001), and algae *Spirulina platensis* were identified according to Coppejans *et al.* (2009). *Spirulina platensis* was dried at 60°C then fine-milled with a laboratory blender, passed through a 0.6 mm diameter hole-sieve, and kept in Stoppard bottles at room temperature until use in the diet. Wheat flour 72% extraction (*Triticum astivum L.*) and hull-less barley whole meal (*Hordeum vulgare L.*) were purchased from the local market in Jeddah City. The flour was stored in plastic airtight containers at refrigerated temperatures until used.

**Preparation of algae extracts**

Twenty grams of *Spirulina platensis* were extracted with 400 ml Methanol- $H_2O$ (70:30) at room temperature then evaporated under vacuum. The residues were extracted in 100 ml chloroform and then the solvent will be evaporated under vacuum. The residue were be dissolved in 100 ml of distilled water and stored at 20°C till use (Lima-Filho *et al*., 2002).

The total phenolic and the total flavonoids content were determined according to Qawasmeh et al. (2012) and Eghdami and Sadeghi (2010), respectively. The antioxidant activity of *Spirulina platensis* wa determined using DPPH free radical scavenging assay as described by Aksoy *et al.* (2013). Proximate analysis were determined in the Wheat flour 72% extraction, hull-less barley flour and *Spirulina platensis* algae powder according to the methods of AOAC (2005). Insoluble and soluble and total dietary fiber was determined of the raw materials according to the methods described by Prosky *et al.* (1988).

Protein digestibility in vitro was extracted in different blends according to Świeca *et al.* (2013). The digestibility of protein in the supernatant was estimated using the Kjeldahl method according to AOAC (2005).

The enzymatic degradation of the in vitro digestion to determine the availability of iron and zinc was determined as described by kiers et al. (2000). Iron and zinc contents were measured using Perkin Elmer (Model 3300, USA) Atomic Absorption Spectrophotometer.

Farinograph and Extensograph characteristics were determined according to the AACC method (AACC, 2002). The different flours and *Spirulina platensis* algae were prepared as mentioned in Table (1) according to Nazir and Nayik (2016).

Table (1): The ingredients of balady bread

| Blends | *Spirulina platensis* algae | Wheat flour 72% extraction | Hull-less barley |
|---|---|---|---|
| Blend 1 as control | ---- | 100 | ------- |
| Blend 2 | 2.5 | 77.5 | 20 |
| Blend 3 | 5.0 | 75.0 | 20 |
| Blend 4 | 7.5 | 72.5 | 20 |
| Blend 5 | 10.0 | 70.0 | 20 |

Sensory evaluation was conducted by twenty experienced panelists using modified sensory evaluation form which had been described by Özer (1998).

After baking the balady bread and cooling at room temperature, bread freshness was determined at zero time, 24, 48 and 72 hours of storage by Alkaline water retention capacity (AWRC) according to the method of Kitterman and Rubentholar (1971).

**Biological experimental**

Male Wister albino weaning rats (36 rats) with weight ranging from 50-60g were purchased from Pharmacy Collage at King Saud University and delivered to the King Fahd Medical Research Center in Jeddah. Rats were housed in individual cages with screen bottoms and fed ad *libitum* on a basal diet for one-week for acclimatization, which containing casein (20 %), corn oil (8%), corn starch (31%), sucrose (32%), cellulose (4%), salt mixture (4%) and vitamin mixture (1%) according to the method Pell *et al*. (1992).

The rats were divided into six main groups with 6 rats on each group, the first group fed on basal diet and described as control negative. The second group fed on basal diet substituted with 10% balady bread made from wheat flour. Third, fourth, fifth and sixth groups fed on basal diet substituted with 10% from different balady bread blend as shown in Table (1) for four weeks (experimental period).

Food consumed was calculates per rat daily as the difference between food allowance and food remnants. Moreover, each rat was weighted every two days and feed efficiency ratio (FER weight gained/g intake) was calculated. At the end of experimental period the protein efficiency ratio (PER) was assayed. Furthermore, the biological value was calculated according to the equation of Mitchell and Block (1946). Biological value (BV) = 49.9 + (10.53 x PFR). Blood hemoglobin (Hb) and Hematocrite (Hct) were determined according to Dacie and Lewis (1984). Red blood cells (RBCs) and white blood cells (WBCs) were measured as recommended by Riley (1960). Platelet values were determined according to the method of Monreal *et al.* (1993).

**Statistical analysis**

The obtained data were exposed to the analysis of variance. Duncan's multiple range tests at ($P \leq 0.05$) level was used to compare between means. The analysis was carried out using the ANOVA procedure of Statistical Analysis System (SAS, 2004).

## 3. Results and Discussion

**Phenolics and flavonoids compounds from *Spirulina platensis***

The results in Table (2) showed that the methanol extract was the highest in phenolics and flavonoids compounds 75.3±1.5 mg GAE/g dry weight and 84.23±5.25 mg QE /g dry weigh from *Spirulina platensis*. Followed by, the chloroform extract was found 55.4±3.7 mg GAE/g and 47.32±3.5 mg QE /g, respectively; the water extract from *Spirulina platensis* algae was the lowest.

Algae had contained the polyphenols, vitamins, and flavonoids compounds indicated that to as natural antioxidants which preserve the body's tissues from oxidative stress and connected diseases like cancer and inflammation (Saeidnia *et al.*, 2009).

Table (2): Phenolics (mg GAE /g dry weight) and flavonoids compounds (mg QE /g dry weight) from *Spirulina platensis* extracts

| Extracts | Phenolics | Flavonoids |
|---|---|---|
| Methanol | 75.3±1.5$^{a}$ | 84.23±5.35$^{a}$ |
| Chloroform | 55.4±3.7$^{b}$ | 47.32±3.5$^{b}$ |
| Water | 20.9±0.94$^{c}$ | 11.62±0.82$^{c}$ |

Each value represents the mean ±SD. Mean followed by different superscript letters in each column are significantly different (p<0.05

**Antioxidant activity**

The results in Table (3) observe that the DPPH radical scavenging activity in different extracts from *Spirulina platensis* powder was dependent on the concentration. The maximum scavenging activity was observed in methanol (86.36%) followed by chloroform and water extracts (80.96 and 66.87 % respectively). Moreover, the IC50 in the different extracts were 11.2, 27.8 and 57.2 mg/ml, respectively. The free radicals are included in many pathogens comprehensive cancer, AIDS and neurodegenerative diseases. Natural antioxidants are very beneficial for the scavenging activity of control of those pathogens (Suresh *et al.*, 2009). Interestingly, the antioxidant activity of methanol extract was increased than other extracts; this could be reasons for the appearances of flavonoids and phenolics compounds in *Spirulina platensis* algae (Farasat *et al.*, 2014).

Table (3). The scavenging activity of DPPH radicals of *Spirulina platensis* extracts

| Concentration µg/ml | DPPH scavenging (%) *Spirulina platensis* extracts | | |
|---|---|---|---|
| | Methanol | Chloroform | Water |
| 000 | 000 | 000 | 000 |
| 001 | 12.98 ± 1.41 | 10.87 ± 1.50 | 4.96 ± 1.32 |
| 002 | 16.38 ± 1.44 | 12.35 ± 1.11 | 9.83 ± 1.21 |
| 004 | 62.98 ± 1.62 | 21.39 ± 1.71 | 16.61 ± 1.54 |
| 008 | 76.81 ± 1.57 | 28.09 ± 1.32 | 22.35 ± 1.33 |
| 016 | 78.72 ± 1.75 | 34.35 ± 1.91 | 27.91 ± 1.38 |
| 032 | 78.94 ± 1.51 | 55.48 ± 1.22 | 35.65 ± 1.30 |
| 064 | 80.21 ± 1.14 | 66.00 ± 1.58 | 53.83 ± 1.27 |
| 128 | 86.36 ± 1.09 | 80.96 ± 1.30 | 66.87 ± 1.12 |
| IC50 | 11.2 ± 1.55 | 27.8 ± 1.22 | 57.2 ± 1.35 |

Values are expressed as mean ± SE of 3 replicates

**Chemical composition of raw materials**

The results in Table (4) illustrated that the total protein, lipids, ash, and crude fiber were the highest in *Spirulina platensis* algae powder and their percent are 62.84, 6.93, 7.47 and 8.12%, respectively. *s a microscopic blue-green alga aspect which is present in alkaline waters*, contains 60% to 70% protein, is rich in vitamin $B_{12}$ and gamma linoleic acid and a source of calcium and iron, contains vitamin E and C, is rich in chlorophyll and phycocyanin pigments, and is used as an essential nutrition support (Morsy *et al.*, 2014).

The second nutrition value was Hull-less barley and their chemical analysis were 18.90, 2.54, 3.86 and 7.28%, respectively for total protein, lipids, ash content, and crude fiber, meanwhile, wheat flour was the lowest in the chemical constituencies. Whole barley grain has contained about from 10% to 17% protein content, β-glucan was found from 4% to 9 %, free lipids indicated from 2% to 3% and finally, minerals content was 1.5% to 2.5%, respectively (Quinde *et al.*, 2004). Concerning, soluble, insoluble and total dietary fiber the hull-less barley were the highest and found to be 4.82, 9.79 and 14.61%, these results confirmed to Březinová Belcredi *et al.* (2009) considered that the barley (*Hordeum vulgare* L.), had an excellent source of the nutritional value as soluble, insoluble and total fibers, vitamin B complex, minerals content, and natural antioxidant as phenolic and flavonoids compounds. The nutritional value has been connected with β-glucans, the main fiber ingredients in barley.

Moreover, the *Spirulina platensis* powder algae were rich in soluble, insoluble and total dietary fiber and the results found were 3.73, 6.82 and 10.55%, respectively, these results confirm that the *Spirulina platensis* is digestible because 86% of its cell wall is composed of digestible polysaccharide (Li and Qi, 1997). The general purpose of *Spirulina* production is to provide protein resource for people and also to benefit from the richness of its total dietary fiber. *Spirulina* has a special value among the other algae types. *Spirulina* contains a high amount of iron, which makes it important in anemia disease (Takeuchi, 1978).

Wheat flour 72% extraction was the lowest in total fiber fractions; meanwhile, it was the highest in total carbohydrates was 85.58% followed by hull-less barley 72.42% and *Spirulina platensis* was the lowest (14.64%).

Table (4): Chemical composition of raw materials based on a dry weight

| Chemical composition | *Spirulina platensis* | Wheat flour 72% extraction | Hull-less barley |
|---|---|---|---|
| Total solids | 95.36±5.27 | ---- | ------ |
| Protein | 62.84±3.18 | 11.95±2.57 | 13.9±3.41 |
| Lipids | 6.93±2.62 | 1.24±0.91 | 2.54±0.93 |
| Ash | 7.47±1.54 | 0.51±0.07 | 3.86±1.14 |
| Crude fiber | 8.12±2.76 | 0.72±0.06 | 7.28±2.15 |
| Total carbohydrates | 14.64±3.29 | 85.58±7.63 | 72.42±5.48 |
| Total dietary fiber | 10.55±3.69 | 3.17±1.28 | 14.61±3.17 |
| Soluble dietary fiber | 3.73±1.18 | 1.03±0.73 | 4.82±1.46 |
| Insoluble dietary fiber | 6.82±2.63 | 2.14±0.82 | 9.79±2.38 |

Values are expressed as mean ± SE of 3 replicates

**Protein digestibility in vitro and bioavailability of iron and zinc for different blends:**

The results present in Table (5) observed that the determination of protein digestibility in vitro and bioavailability for iron and zinc in blendes made with substitution wheat flour with hull-less barley and *Spirulina platensis* powder algae at different levels. From the obtained results it could be noticed that the protein digestibility of blend (5) was low (65.19 %) and Urooj (2011) confirmed our results. Moreover, control as a blends (1) and blends (2, 3 and 4) were significantly increased the protein digestibility. These increases can be referred to greater soluble proteins may be caused to partial hydrolysis of strong proteins by endogenous proteases enzyme (Bhise *et al.*, 1988).

The same table it was shown the bioavailability iron (Fe) and zinc (Zn) of different blends, and the results were parallel and confirmed to the protein digestibility for different blends. Iron is not suitably absorbed in some nutritional complements. Meanwhile, iron in algae *Spirulina* is 60% best absorbed compared with ferrous sulfate and other supplements. Therefore, algae *Spirulina* could appear enough source of iron in anemic for pregnant women (Carolin Joe Rosario and Mary Josephine, 2015).

Table (5): Protein digestibility in vitro and bioavailability iron and zinc of different blends (g/100g).

| Blends | Protein digestibility | Bioavailability | |
|---|---|---|---|
| | | Iron | Zinc |
| Control as Blend (1) | 78.91± 2.51$^{a}$ | 21.73 ± 2.94$^{a}$ | 18.31± 0.33$^{a}$ |
| Blend (2) | 75.23 ± 1.91$^{b}$ | 19.38± 0.37$^{a}$ | 18.10 ±1.07$^{a}$ |
| Blend (3) | 72.51 ± 2.39$^{b}$ | 17.38± 2.37$^{b}$ | 17.16 ±0.81$^{a}$ |
| Blend (4) | 70.67± 2.23$^{b}$ | 16.17± 0.73$^{b}$ | 15.72± 2.19$^{b}$ |
| Blend (5) | 65.19 ± 3.16$^{c}$ | 13.82 ± 1.22$^{c}$ | 11.74 ± 2.87$^{c}$ |

Each value represents the mean ±SD. Mean followed by different superscript letters in each column are significantly different ($p<0.05$)

**Rheological properties of dough**

From Table (6), it could be found that the water absorption, dough development time and stability time were increased gradually by increasing of the substitution ratios from *Spirulina platensis* powder algae than that found in the control sample. The increasing water absorption might be due to the addition of hull-less barley and *Spirulina platensis* powder algae which containing more fiber content, which retained more water.

These results were confirmed by those obtained by Hemery *et al*. (2011) who pointed out that the increase in water absorption probably due to their higher fiber content. Meanwhile, the substitution of wheat flour with hull-less barley and *Spirulina platensis* powder algae caused a gradual reduction in the degree of weakening. This clear decrease might due to dilution of gluten protein from wheat flour with the increase fiber content from hull-less barley and *Spirulina platensis* powder algae at different levels.

Table (6): Farinograph parameters of wheat flour and hull-less barley fortified with different levels of *Spirulina platensis*

| Blends | Water absorption % | Dough development (min) | Dough stability (min) | Degree of weakening (B.U.) |
|---|---|---|---|---|
| Control | 58.9 | 1.5 | 8.0 | 70.00 |
| Blend 2 | 59.5 | 1.5 | 8.5 | 67.25 |
| Blend 3 | 61.5 | 2.00 | 9.0 | 64.00 |
| Blend 4 | 61.8 | 1.5 | 9.0 | 60.00 |
| Blend 5 | 66.1 | 2.50 | 9.5 | 50.00 |

Rosell *et al.* (2001) found that the differences in basic water absorption could be the reason for the largest number of hydroxyls present in the fiber formulation and allow the additional effect of water during hydrogen bonding.

The extensograph reading in the Table (7) observed that the value of resistance to extension and proportional number illustrate ascending order with enhancing of the value of hull-less barley and *Spirulina platensis* powder algae at different levels. Meanwhile, the value of extensibility and energy was gradually reduction by increasing the substitution ratios of *Spirulina platensis* powder algae at different levels than that found in the control sample may be caused by the extensograph energy of wheat flour dough as control was considerably stronger, therefore, the deformation of wheat dough was needed more energy. The foundation on the outcomes of extensographic measurements, in general, it can be known the alterations of dough uniformity, the effect of promoting products, a total volume of products, etc., confirmed on this to choose the additives and other quality-improving products for the final wanted characteristics of bakery products (Bojňanská *et al*., 2013).

Table (7): Extensograph parameters of wheat flour and hull-less barley fortified with different levels of *Spirulina platensis*

| Blends | Resistance to extension (B.U.) | Extensibility (min.) | Proportional number (R/E) | Energy ($cm^2$) |
|---|---|---|---|---|
| Control | 150 | 130 | 1.15 | 45 |
| Blend 2 | 160 | 125 | 1.28 | 39 |
| Blend $_3$ | 180 | 110 | 1.64 | 35 |
| Blend 4 | 180 | 115 | 1.57 | 35 |
| Blend 5 | 200 | 105 | 1.90 | 32 |

**Sensory characteristics of balady bread**

Data in Table (8) showed that no significant differences were observed for balady bread fortified with 2.5, 5.0 and 7.5% levels in their crumb texture, crust color, taste, diameter, separation, crust texture, crumb color, flavor and overall scores compared with control bread. On the other hand sensory properties of balady bread were decreased with increasing the levels of *Spirulina platensis* powder algae at 10% level and significant quality reduction. These results confirmed with Gómez *et al.*, (2003) found there was no connection between of the sensory score and the added of fiber whilst depended on the type of fiber experiments. Little additions of dietary fiber to wheat flour to produce very similar white wheat bread and acceptability. Meanwhile, the addition of wheat fiber until 10%, with the inherent promoted positive nutritional influence, the results reported that the highest acceptability for sensory evaluation compared with control. Noort *et al*. (2010) reported that most consumers prefer the product of fine wheat flour more than whole-grain products for the reason that they become aware of the textural characteristics of the whole grain products to be lowered attractive.

Under nutrition shape a common health problem, especially in improving countries. The using of algae, especially *Spirulina*, as a functional food was reported decades ago may be caused the fact that it is not only a protein-dense food source whilst for the reason that its amino acid profile is believed as a great biologic-value of protein content. *Spirulina* had contained necessary fat acids like gamma-linolenic oleic acids, associated with low content nucleic acids. Also, it had contained vitamin B12, is a good source of beta-carotene and minerals. Furthermore, *Spirulina* has also confirmed to have a good agreement as of its organoleptic characteristics and it has exhibited neither heavy nor chronic toxicities, therefore it safe for human consumption (Gutiérrez-Salmeán *et al.*, 2015).

Table (8): Sensory evaluation of balady bread

| Items | Control | Blend 2 | Blend 3 | Blend 4 | Blend 5 |
|---|---|---|---|---|---|
| Crumb texture (5) | 4.9 ±0.11[a] | 4.8±0.07[a] | 4.7±0.08[a] | 4.3±0.09[b] | 4.0±0.08[b] |
| Crust color (5) | 4.9±0.07[a] | 4.9±0.05[a] | 4.8 ±0.05[a] | 4.3±0.03[b] | 3.5.±0.10[b] |
| Taste (5) | 4.9 ±0.07[a] | 4.9 ±0.05[ab] | 4.7±0.13[a] | 4.1±0.10[b] | 3.7±0.20[b] |
| Diameter (5) | 4.9±0.05[a] | 4.7±0.10[a] | 4.6±0.90[ab] | 4.5±0.10[bc] | 4.0±0.15[c] |
| Separation (5) | 4.9±0.05[a] | 4.8 ±0.10[a] | 4.7±0.00[ab] | 4.6±0.30[ab] | 3.7±0.05[c] |
| Crust textures (5) | 4.9±0.00[a] | 4.8±0.20[a] | 4.7±0.30[a] | 4.2±0.20[b] | 4.0±0.00[b] |
| Crumb color (5) | 4.9±0.10[a] | 4.8±0.20[a] | 4.7±0.20[a] | 4.3±0.30[b] | 4.0±0.10[b] |
| Flavor (5) | 4.9±0.08[a] | 4.8±0.07[a] | 4.7±0.08[a] | 4.4±0.10[b] | 4.2±0.10[c] |
| Overall Score (40) | 39.2±0.30[a] | 38.50±0.34[a] | 37. 6±0.74[a] | 34.70±0.26[b] | 31.1±0.14[c] |

Each value represents the mean ±SD. Mean followed by different superscript letters in each column are significantly different ($p<0.05$)

**The freshness of produced balady bread**

From results (Table 9), it could be noticed that the substitution wheat flour with hull-less barley and *Spirulina platensis* powder algae at different levels in the various balady bread formulations contributed to the increase in alkaline water retention capacity (AWRC) compared with the control, due to hull-less barley and *Spirulina platensis* powder algae which had to contain more fiber content. The freshness of all balady bread blends was lowering at various durations in addition to bread control than zero time periods. Also, the rate of decrease of all blends in balady bread was decreased during storage at 24, 48 and 72h of storage at room temperature. The crumb firmness development through storage duration of 3 days observed the sufficiently great influence of fiber addition on bread lifetime. This influence is connected to the fiber's known ability to bind to water which maintains water loss through storage and the potential interaction among the fiber and starch that might delay the regression of starch (Gómez *et al.*, 2003).

Table (9) Percent of Freshness properties of balady bread stored at room temperature as determined by alkaline water retention capacity (AWRC).

| Blends | Zero time | After 24 hour | After 48 hour | After 72 hour |
|---|---|---|---|---|
| Control | 345.12 | 15.83 | 28.68 | 39.59 |
| Blend 2 | 356.82 | 13.65 | 26.20 | 37.67 |
| Blend 3 | 362.52 | 12.76 | 25.35 | 34.23 |
| Blend 4 | 370.73 | 10.21 | 24.65 | 32.23 |
| Blend 5 | 380.56 | 8.24 | 20.43 | 25.65 |

**Biological experimental**

**Effect of different diets on weight gain, feed quantity, and feeding efficiency ratio in weaning rats**

Increases in weight, feed consumption and feed efficiency rate were determined and calculated in all rats groups fed on the different diets compared with rats fed on basal diet and the results are presented in Table (10). The gain in the body of the animals showed that the growth patterns of animals fed the different diets were lower than those of animals fed on basal diet. Moreover, the decrease in feed intake and feed efficiency ratio were parallel the results from the gain in body weight. These decreases could be caused to the presence of relatively great content of the fibers in the different diets which led to full the stomach of rats and reduce the feed intake.

Table (10) body weight gain, feed intake and feed efficiency ratio in weaning rats

| Groups | Initial body weight | Final body weight | body weight Gain | Feed intake | Daily feed intake | Feed efficiency ratio |
|---|---|---|---|---|---|---|
| Control negative | 44.25±2.38[a] | 122.50±9.24[a] | 78.25±4.27[a] | 417.0±10.26[a] | 14.89±0.81[a] | 18.8±1.15[a] |
| Control wheat flour | 45.28±4.82[a] | 121.18±10.74[a] | 75.9±6.34[a] | 406.0±20.54[a] | 14.5±1.12[a] | 18.7±1.24[a] |
| Group 3 | 45.17±3.17[a] | 115.0±7.38[b] | 69.83±5.38[b] | 378.0±15.38[b] | 13.5±0.91[b] | 18.5±0.98[b] |
| Group 4 | 44.00±3.45[a] | 110.6±9.73[b] | 66.6±5.28[b] | 364.0±18.27[b] | 13.0±0.94[b] | 18.3±0.73[b] |
| Group 5 | 45.17±3.24[a] | 104.87±8.34[b] | 59.7±4.36[b] | 332.0±16.34[b] | 11.86±0.83[c] | 18.0±1.05[b] |
| Group 6 | 45.34±3.24[a] | 97.93±10.12[c] | 52.59±3.18[c] | 294.0±12.94[c] | 10.5±0.85[d] | 17.9±1.14[b] |

Each value represents the mean ±SD. Mean followed by different superscript letters in each column are significantly different ($p<0.05$)

**Effect of different dies protein effectiveness and their biological significance in weaning rats**

Protein assimilation, protein effectiveness, and their biological significance results in experimental animals fed on the different diets are reported in Table (11). As stated by their physical appearance, the rats utilized in the research illustrated perfect growth and advancement with the basal diets. Sufficiently higher ($p<0.05$) protein efficiency ratio and biological value (1.88 and 69.65%) found for the basal diet in this research would be attributed to increased efficiency of animal protein and the best distribution of absolutely necessary amino acids.

Moreover, the biological value in rats fed on different diets was reduced from 69.35 to 68.73% and also protein efficiency ratio was decreased from 1.85 to 1.79%, respectively. These decrease caused by the different diets that had contained high amounts crude fiber had low feed assimilation, perhaps caused taste, for one of the major influences of fiber-rich diets are increasing in satiety and, consequently, the lowering in appetite (Pinto *et al.*, 2015 and Esme-Fuller *et al.*, 2016)

Table (11): Protein efficiency ratio and biological value in weaning rats

| Groups | Protein intake | Protein efficiency ratio | Biological value |
|---|---|---|---|
| Control negative | 41.70±2.86$^{a}$ | 1.88±0.05$^{a}$ | 69.65±3.27$^{a}$ |
| Control wheat flour | 40.60 ±2.05$^{b}$ | 1.87±0. 01$^{a}$ | 69.59±8.57$^{a}$ |
| Group 3 | 37.80 ±1.54$^{c}$ | 1.85±0. 02$^{b}$ | 69.35±7.36$^{b}$ |
| Group 4 | 36.40 ±1.83$^{c}$ | 1.83±0. 65$^{b}$ | 69.17±5.19$^{b}$ |
| Group 5 | 32.20 ±1.63$^{d}$ | 1.80±0.37$^{b}$ | 68.85±9.15$^{b}$ |
| Group 6 | 29.40 ±1.29$^{e}$ | 1.79±0.29$^{c}$ | 68.73±6.94$^{c}$ |

Each value represents the mean ±SD. Mean followed by different superscript letters in each column are significantly different ($p<0.05$)

**Effect of different diets on complete blood picture in weaning rats:**

Effect of the different diets fortified with *Spirulina platensis* powder algae at 2.5, 5.0, 7.5 and 10.0% levels on the measurements of hematological (blood compositions) in the rats, the results are reported in Table (12). The results showed that the hemoglobin and red blood cells of the rats fed with the different diets were considerable higher than control rats. The hemoglobin and red blood cells of the rats were within acceptable range propose sufficient iron status. This could be connected with the iron content of *Spirulina platensis* powder algae which are a good source of nonheme iron.

The leukocyte (WBC) and the hematocrit (RBC) of the rats fed on different diets all fell within normal ranges. Normal leukocyte and hematocrit counts are 5x109 to 9 x 109/L (Skala *et al.*, 1981) and 5x10$^{12}$/L (Bender, 1965), respectively.

The complete blood picture, recognized as hemogram, it is one of the components of the routine physical examination. The complete blood picture test measures the quantity of all the cellular part of the blood: the leukocyte (WBC) and the hematocrit (RBC) and platelets (PLT). Additionally, the complete blood picture estimates some valuable facts provided on other indices concerning each kind of the blood cell. Any abnormities (increases or decreases) in blood cell counts as detected by the CBC may show an implicit medical situation that immediate and justifies further, additional specific, laboratory search to the agreement the diagnosis (George-Gay and Parker, 2003).

Table (12): Level of hemoglobin, hematocrit, red blood cells, white blood cells, and platelets levels in the rats.

| Groups | Hemoglobin (g/dl) | Hematocrit (%) | Red blood cells ($10^{12}$/L) | White blood cells ($10^{9}$/L) | Patelets ($10^{3}$/ mm$^{3}$) |
|---|---|---|---|---|---|
| Control negative | 13.6±0.48$^{ab}$ | 40.7±1.37$^{ab}$ | 7.74±0.24$^{ab}$ | 6.45±0.74$^{ab}$ | 910.2±52.35$^{ab}$ |
| Control wheat flour | 12.7 ±0.81$^{c}$ | 38.0 ±2.41$^{c}$ | 6.97±0.31$^{c}$ | 5.67 ±0.931$^{c}$ | 753.3 ±37.81$^{c}$ |
| Group 3 | 13.0 ±0.87$^{b}$ | 39.5 ±2.6$^{b}$ | 7.22±0.53$^{b}$ | 5.93 ±0.85$^{b}$ | 816.3±51$^{b}$ |
| Group 4 | 13.5 ±0.79$^{ab}$ | 40.4 ±2.4$^{ab}$ | 7.54±1.02$^{ab}$ | 6.23 ±0.47$^{ab}$ | 889.3 ±67.3$^{b}$ |
| Group 5 | 13.7±0.28$^{ab}$ | 40.9±2.1$^{ab}$ | 7.91±0.28$^{ab}$ | 6.57±0.92$^{ab}$ | 920.4±66.4$^{a}$ |
| Group 6 | 13.9±0.53$^{a}$ | 41.2±2.3$^{a}$ | 8.12±.04$^{a}$ | 6.92±10.58$^{a}$ | 970.6±71.28$^{a}$ |

Each value represents the mean ±SD. Mean followed by different superscript letters in each column are significantly different ($p<0.05$).

**Conclusions**

The bread was blended with different concentrations of *Spirulina platensis* powder algae and hull-less barley that resulted in an increase in the content of proteins, a natural antioxidant, β-glucan and dietary fibers in loaves of bread with increasing *Spirulina platensis* powder algae concentration. Rheological, sensory and freshness characteristics in bread with *Spirulina platensis* powder algae were modified with respect to the control bread. Moreover, the addition of *Spirulina platensis* powder algae rich in nutrients indicates a new alternative in the enrichment of loaves of bread and consumer acceptance. From this study, it was found the best concentrations from *Spirulina platensis* powder algae was 7.5% did not impair the technological and sensory characteristics of the loaves of bread.

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