

## **Brown Algae is a Natural Source Rich in Nutrients and Bioactive Compounds: Application in Balady Bread**

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**Abstract** The present study aims to determine the chemical composition, nutritional evaluation and bioactive compounds content of brown algae (*Sargassum subrepandum*) powder. Also, application in the manufacture of Balady bread by partially replacing wheat flour will be in the scope of this investigation. The contents of moisture, total protein, crude fat, crude fiber, ash and carbohydrates content of brown algae powder were 9.03, 5.14, 0.93, 8.01, 18.71 and 58.18%, respectively. Also, the total energy (Kcal/100g), the daily requirement of adult man for protein (GDR/protein) and energy (GDR/energy), percent satisfaction of the daily requirements of adult man in protein (P.S./protein) and energy (P.S./energy) which recorded 262, 1226, 1108, 6.53 and 7.22, respectively. Furthermore, bioactive compounds content of oat flour indicated that dietary fiber (g.100g<sup>-1</sup>), phenolics (mg gallic acid equivalent.100g<sup>-1</sup>), flavonoids (mg catechine equivalent.100g<sup>-1</sup>), carotenoids (mg.100g<sup>-1</sup>), polysaccharides (mg starch.100 g<sup>-1</sup>) and Tannins (mg catechine.100 g<sup>-1</sup>) were 45.21, 144.56, 31.65, 41.12, 161.17 and 30.19, respectively. The brown algae powder samples also recorded several very high biological activities which include antioxidant and radicals scavenging activities. Such important nutrients and bioactive compounds content as well as biological effects of brown algae powder played important roles in strategies to contribute a major role to bridging the nutritional gap, which is the bread industry as a partial substitute for flour. Therefore, the present study recommended like of that brown algae to be included in food processing and therapeutic applications.

**Keywords:** minerals, vitamins, antioxidant activity, radicals scavenging activity, rheological parameters, organoleptic evaluation

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## **1. Introduction**

The current and expected future climatic changes afflicting the world portend a shrinking of the available areas and a decrease in the productivity of crops, and thus a decrease in the total Egyptian production, in conjunction with the steady population increase over time. Also, the increase in the total needs of foodstuffs required by these numbers of the population, and then the decrease in the average per capita share of foodstuffs, and thus the Egyptian food security. Therefore, the attention of researchers turned to searching for new and innovative food sources to compensate for this food shortage, or what is known as the food gap. Marine algae had the largest part of this thinking because of its many advantages, represented in: the wide spread on the various Egyptian sea coasts, it multiplies naturally quickly (sexuallyasexually) without the need for special care [1] and thus the large quantitative yield, the high nutritional value as a result of its containment of macro and micro nutrients, bioactive ingredients etc., [2,3].

Algae belong to the group Heterokontophyta, a large group of eukaryotic organisms distinguished most prominently by having chloroplasts surrounded by four membranes, suggesting an origin from a symbiotic relationship between a basal eukaryote and another eukaryotic organism [4]. They are a group of living organisms that are able to capture the energy of light through the process of photosynthesis, converting inorganic substances (mostly water + carbon dioxide) into organic substances (sugars) in which they store energy [5]. Algae are also a name that denotes a group of diverse plants belonging to more than 20,000 thousand species, and these algae are found in different forms in terms of shape, size, and way of living [6]. The scholars are unanimously agreed that the word algae may denote plant groups that share a number of characteristics, the most important of which is that they do not have roots, stems, flowers, or real leaves, but rather they are a group of cells standing next to the other, living mostly in salt water (seas and oceans) and fresh (rivers), and also contain chlorophyll, and thus the ability to carry out the process of photosynthesis [7]. Some types of algae are also familiar to most people, for example seaweed, pond scum, or algae

blooms in lakes. Despite this, there is a vast and diverse world of algae that not only help us with life, but are essential to our existence in it. In general, Marine algae are classified as brown, red, or green algae [8].

Brown algae belong to Family, Phaeophyceae are a large group of mostly marine multicellular algae, including many seaweeds located in different countries around the world including Egypt. Worldwide, over 1500-2000 species of brown algae are known. Some species are important in commercial use because they have become subjects of extensive research in their own right. They have environmental importance too through Carbon fixation [9]. Most brown algae contain the pigment fucoxanthin, which gives them their name and gives them their unique greenish-brown hue [9]. In the littoral zone of the Egyptian coast, brown algae is currently the most dominant group. Members of Sargassum genus represent valuable sources of a several compounds including proteins, lipids, minerals, essential fatty and amino acids, and bioactive compounds [10,11,12]. Also, brown algae consist mainly of water (90 %) in the native state. Polysaccharides are major components and comprise alginates, cellulose, and sulfated polysaccharides such as fucoidans and laminarins. Other compounds/bioactive components include proteins, free mannitol, minerals, vitamins. polyphenols, alkaloids, peptides. fatty compounds, and various pigments [4,11,13]. Alginates, probably the most widely used of the algal extracts, are composed of block copolymers of mannuronic and guluronic acid sugars and have been adopted by the food industry as thickening agents and by the pharmaceutical industry as binders, gelling agents, and wound absorbents [13]. Such studies with the others indicated that brown algae represent valuable sources of a several nutrients and bioactive compounds. From a nutritional and therapeutic point of view, brown algae such as Sargassum genus is used dried in condiment and soup bases or eaten fresh in salads, rolls, or stews, or with rice. It is thought that the overall content of certain traditional Asian diets contributes to the low incidence of cancer, particularly breast cancer [14,15]. Also, it is apparent that the unique levels of seaweed including brown algae intake contribute to the variance in the levels of breast cancer [16]. There is a nine fold lower incidence of breast cancer in the Japanese population and an even lower incidence in the Korean population compared to the incidence in the West [17]. The relative longevity and health of Okinawan Japanese populations has been attributed in part to dietary algae in studies [18]. In Brazil, a dietary intervention for 10 weeks study, 3g of decosahexaenoic acid, 5g of seaweed (wakame) powder, and 50 mg of isoflavonoids from soybean (Glycine soja) were given daily to immigrants, at high risk for developing diseases,. This combination reduced blood pressure and cholesterol levels, suppressed the urinary markers of bone resorption, and attenuated a tendency toward diabetes. Recently, several studies indicated that consuming of brown algae (Sargassum subrepandum L.) powder of the diet was effective in protecting of some obese complications including oxidative stress, immunodeficiency, bone disorders, liver dysfunctions and hyperglycemia [11,19,12,20,21,22]. Although all of these and other previous studies have addressed the many nutritional and

medical benefits of brown algae, more and more research is still needed to explore what other roles this important nutritional and pharmaceutical sources can play. Therefore, the current study aims to estimate the nutrients, bioactive compounds and biological activities of brown algae genus *Sargassum* found on the Egyptian shores. Also, the use of brown algae powder in one of the important food industry applications that contributes a major role to bridging the nutritional gap, which is the bread industry as a partial substitute for flour, will be in the scope of this investigation.

## 2. Materials and Methods

## 2.1. Materials

#### 2.1.1. Wheat Flour

Variety Giza 155 wheat (*Triticum vulgare*) was obtained from Benha markets, Al Qalyubia Governorate, Egypt during the 2022 harvesting period. The collected samples was transported to the laboratory and stored immediately on the refrigerator at  $0^{\circ}$ C until using in preparation of flour. The grains samples were verified by the stuff in Faculty of Agriculture, Minoufiya University, Shebin El-Kom, Egypt.

## 2.1.2. Brown Algae

Brown algae (*Sargassum subrepandum*) samples were collected from the coasts of Mediterranean Sea, Alexandria, Alexandria Governorate, Egypt. The samples were drained from water and verified by the stuff in Faculty of Agriculture, Alexandria University, Alexandria, Egypt.

#### 2.2.3. Chemicals

Phenolic standards ( $\alpha$ -tocopherol, BHA, BHT) and  $\beta$ carotene were purchased from Sigmae-Aldrich Chemical Co agent, Egypt; linoleic acid was from J.T. Baker Chemical Co., Phillipsburg, NJ, and Tween 20 and 2,2diphenyl-1-picrylhydrazyl (DPPH) were from BDH Chemical Co., Toronto, On. All other chemicals, solvents and buffers were of analytical Grade and purchased from AlGhomhoria Co for Trading Drugs, Chemicals and Medical Instruments, Cairo, Egypt

#### **2.3. Methods**

#### 2.3.1. Preparation of Wheat Flour

Wheat and oat grains were cleaning and sorting manually and then dried in a hot air oven (Horizontal Forced Air Drier, Proctor and Schwartz Inc., Philadelphia, PA) at 55 <sup>0</sup>C until arriving by the moisture in the final product to about 10%. The dried grains were milled into flour in a miller (Moulinex Egypt, ElAraby Co., Benha, Egypt). The flour samples were sieved in standard sieves to prepare flour sample with 85% extraction rate.

#### 2.3.2. Preparation of Brown Algae Powder

Brown algae powder and extracts were prepared according to the protocol mentioned by Abd Elalal et al., [23]. In brief, brown algae samples were manually cleaning, sorting and dried arriving by the moisture in the final product to about 8%. The dried samples were ground into a fine powder and the material that passed through an 80 mesh sieve was retained used in analysis.

#### 2.3.3. Preparation of Control and Composite Wheat Flour Extracts

Control and composite wheat flour samples were used for their aqueous extracts as follow: A 20 g from flour sample plus 180 ml water were homogenized and transferred to a beaker and stirred at 200 rpm in an orbital shaker (Unimax 1010, Heidolph Instruments GmbH & Co. KG, Germany) for 1 h at room temperature. The extract was then separated from the residue by filtration through Whatman No. 1 filter paper. The remaining residue was re-extracted twice, and then the two extracts were combined. The residual solvent of was removed under reduced pressure at 55°C using a rotary evaporator (Laborata 4000; Heidolph Instruments GmbH & Co. KG, Germany). All aqueous extracts could be ready for chemical studies.

#### 2.3.4. Chemical Analysis of Control and Composite Wheat Flour Samples

Control and composite wheat flour samples were analyzed for proximate chemical composition including moisture, protein (T.N.  $\times$  6.25, micro - kjeldahl method using semiautomatic apparatus, Velp company, Italy), fat (soxhelt miautomatic apparatus Velp company, Italy, petroleum ether solvent), ash, fiber nd dietary fiber contents were determined using the methods described in the AOAC, [24]. Carbohydrates calculated by differences: Carbohydrates (%) = 100 - (% moisture + % protein + % fat + % Ash + % fiber).

#### 2.3.5. Determination of Nutritional Value of Control and Composite Wheat Flour Samples

#### 2.3.5.1. Total Energy Value

Total energy (Kcal/100 g) of control and composite wheat flour samples was calculated according to Insel *et al*, [25] using the following equation: Total energy value (Kcal/100 g) = 4 (Protein % + carbohydrates %) + 9 (Fat %).

#### 2.3.5.2. Satisfaction of the Daily Needs of Adult Man (25-50 Year Old) in Protein

Grams consumed (G.D.R. g) of food ( wet weight basis ) to cover the daily requirements of adult man (63 g) in protein was calculated using the RDA [26] values. Percent satisfaction of the daily requirement of adult man in protein (P.S./80 g, %) when consuming the possibly commonly used portions in Egypt i.e. one loaf (80 g weight), was also calculated.

#### 2.3.5.3. Satisfaction of the Daily Requirements of Adult Man (25-50 Year Old) in Energy

Grams consumed of food (wet weight basis) to cover the daily requirements of man in energy (G.D.R., g) were calculated using the RDA (Recommended dietary allowances) which are 2900 Kcal /day for man as given by RDA [26]. The percent of satisfaction (P.S., %) of the daily needs of adult man (25 -50 year old, 79 Kg weight and 176 cm height ) in energy upon consumption the commonly used portion at homes in Egypt, i.e. i.e. one loaf(80 g weight), was also calculated.

#### 2.3.6. Minerals Determination in Control and Composite Wheat Flour Samples

Minerals content of DRSP samples were prepared and determined according to the method mentioned by Singh *et al.*, [27]. In brief, 0.5 g of defatted sample i.e. left behind for lipid estimation were transferred into a digested glass tube and 6 ml of tri-acids mixture (containing nitric acid: perchloric acid: sulfuric acid in the ratio of 20 : 4 : 1 v/v respectively) were added to each tube. The tubes content were digested gradually as follow, 30 min at  $70^{\circ}$ C; 30 min at  $180^{\circ}$ C and 30 min at  $220^{\circ}$ C. After digestion i.e. until the mixture becomes colorless, the mixture was cooled, dissolved in distilled water, and the volume was increased to 50 ml in volumetric beaker. The mixture samples were filtration in ashless filter paper and aliquots were analyzed for minerals (Na, Zn, Fe, Mn, Cu, K, Mg, P, Ca, Se) using of atomic absorption spectrophotometer.

#### 2.3.7. Vitamins Determination in Control and Composite Wheat Flour Samples

SP Thermo Separation Products Liquid Chromatography (San Jose, CA, USA) was used for vitamins determination such as mentioned in our previous study [28]. Fat soluble vitamins (A and E) were extracted from the control and composite wheat flour samples according to the methods described by Epler et al. [29] and Hung et al. [30] while water soluble vitamins (B and C) according to Moeslinger et al. [31], and analyzed by HPLC techniques. Under the chromatographic conditions used in those methods, mean values ±SD of vitamins A, C, B1, B2, B3, B6, B9, B12 and E recoveries were 93.15±1.56, 89.94 ±1.32, 88.12± 2.21, 87.36 ±1.17, 85.97±1.78%, 85.32± 3.02, 90.76 ±2.11, 91.03 ±3.04 and 89.08±1.55%, respectively.

### 2.3.8. Biological Activities

#### 2.3.8.1. Antioxidant Activity

Antioxidant activity (AA) of plant extracts and standards ( $\alpha$ -tocopherol and BHT) was determined according to the  $\beta$ -carotene bleaching (BCB) assay following a modification of the procedure described by Marco, [32].

#### 2.3.8.2. DPPH Radical Scavenging Assay

Free radical scavenging ability of control and composite wheat flour extracts was tested by DPPH radical scavenging assay as described by Desmarchelier et al. [33]. A solution was prepared, and 2.4 mL of 2,2-diphenyl-1-picrylhydrazyl (DPPH) (0.1 mM in methanol) was mixed with 1.6 mL of brown algae extract at different concentrations (12.5-150 µg/mL). The reaction mixture was vortexed thoroughly and left in the dark at room temperature for 30 min. The absorbance of the mixture was measured spectrophotometrically at 517 nm (UV-160A; Shimadzu Corporation, Kyoto, Japan). BHT was used as reference. Percentage DPPH radical scavenging activity was calculated by the following equation: DPPH radical

scavenging activity (%) =  $[(A0-A1)/A0] \times 100$ , where A<sub>0</sub>, absorbance of the control, and A<sub>1</sub>, absorbance of the BA / BHT. Then inhibition (%) was plotted against concentration, and IC<sub>50</sub> was calculated from the graph.

#### 2.3.9. Determination of Bioactive Compounds

Throughout this study absorbance for different assays were measured using UV-160A; Shimadzu Corporation, Kyoto, Japan. Total phenolics in brown algae powder were determined using Folin-Ciocalteu reagent according to Singleton and Rossi, [34] and Wolfe *et al.*,[35] and were expressed as gallic acid equivalents (GAE). Total flavonoids contents were estimated using colorimetric assay described by Zhisen *et al.*, [36] and expressed as catechin equivalent, CAE. Total polysaccharides were measured according to the method of Vazirian *et al.*, [37] and were expressed as mg of starch equivalents. The total carotenoids were determined by using the method reported by Litchenthaler, [38]. Tannins were determined by the method of Van-Burden and Robinson [39] and expressed as catechine equivalents.

#### 2.3.10. Preparation of Wheat Flour and Balady Bread

The wheat kernels samples were go out the refrigerator and sieved to get rid of any foreign matters or dust. Kernels were then dried to below 10 % moisture content and were then adjusted to the moisture content required which was 14%. The quantity of water added to the quantity of grains to reach the level 14% moisture content was according to the formula mentioned by Ahmed *et al.*, [40] as follow: X = 100 x: B - A/100 - B Where:\_X, the quantity of water to be added to 100 grams of wheat; A, the initial moisture content and B, the moisture content required. The kernels was milled by using of laboratory mill and sieved through 60 and 50 meshes screen to obtain wheat flour extraction rate 72.

The Balady bread samples were prepared according to the common method. Formulation of the bread is applied as follow: wheat flour, 1000 g; salt, 20g; and dries yeast, 2 g; and water 500 g. Yeast was mixed with water  $(25^{\circ}C)$  to form a suspension, to which the other ingredients were then added and kneaded to form smooth dough. Substitution of wheat flour with brown algae powder were conducted based on 10 and 20% of the weight of the wheat flour. The dough was later proofed for 2 hours in a proofer (Bakbar E81, New Zealand), then cut into loafs 80 g prior to baking at 170 ° C for 10 min.

## 2.3.11. Water (WHC) and oil (OHC) Holding Capacity

Water (WHC) and oil (OHC) holding capacity were determined according to the method of Larrauri *et al.*, [41]. Twenty-five milliliters of distilled water or commercial corn oil were added to 0.5 g of MPP or MKP, shacked vigorously for 1 min and then centrifuged for 15 min at 10,000g. The residue was weighed and the WHC and OHC were calculated as g water or oil per g of dry sample, respectively.

#### 2.3.12. Determination of Physical Properties of Dough (Rheology)

Both wheat flour control sample and samples with

additions of selected food by-products were determined by using of farinograph, and extensograph tests according to the methods of **A.A.C.C.** [42] as the following: the individual measurements were performed in brown algae powder in 5, 10 and 15% additions. The amounts of the 5, 10 and 15% of brown algae powder were selected according to their chemical structure and their effect on the rheological properties of dough.

#### 2.3.12.1. Farinograph Measurement

Before the actual farinograph measurement, determination of moisture is necessary. The measurement was performed by means of ISO norm 5530-1 (1997). The moisture of flour was determined according to ISO norm 712 (2009). The measurement of water absorption, dough development time, dough stability and farinograph quality number was carried out on a Brabender R Farinograph (BrabenderR GmbH & Co, Duisburg, Germany). Visual comparison of the curves was performed in Farinograph Data Correlation program (BrabenderR GmbH & Co, Duisburg, Germany).

#### 2.3.12.2. Extensograph Test

Extensograph test was carried out on a Brabender R Extensograph (BrabenderR GmbH & Co, Duisburg, Germany) to determine the maximum resistance to extension extensibility and strength of the dough (energy) of wheat flour control sample and samples with additions of brown algae.

#### 2.3.13. Sensory Evaluation

Sensory evaluation was carried out with 15 panelists comprising of postgraduate students from Minoufiya University, Shebin El-Kom, Egypt. Each panelist was served with 5 randomly arranged bread samples on a rectangular plastic tray. The loaves' were individually sealed in a pouch and coded with a three-digit number prior to testing. The 5 samples consisted of 4 types of composite flour loafs and a control (100% wheat flour). Water was provided for rinsing between the samples. Panelists were required to evaluate the colour, taste and overall acceptance of the bread using the 9-point hedonic scale with 1=dislike extremely, 2=dislike very much, 3=dislike moderately, 4=dislike slightly, 5=neither like nor dislike, 6=like slightly, 7=like moderately, 8=like very much, and 9=like extremely.

#### **2.4. Statistical Analysis**

All tests/measurements were done in triplicates and presented as mean $\pm$  standard deviations (SD). Statistical analysis was performed using Student *t*-test and MINITAB 12 computer program (Minitab Inc., State College, PA).

## 3. Results and Discussion

## 3.1. Bioactive Compounds and Dietary Fiber Content in Wheat Flour and Brown Algae

| Component   | Wheat flour (WF, 85%)        | Brown algae (BA)           |
|---|------------------------------|----------------------------|
| Dietary fiber (g.100g <sup>-1</sup> )                       | $7.87 \pm 0.67$ <sup>b</sup> | $45.21 \pm 0.1.02^{a}$     |
| Phenolics (mg gallic acid equivalent. 100 g <sup>-1</sup> ) | $3.69 \pm 0.37$ <sup>b</sup> | $144.56 \pm 19.43$ a       |
| Flavonoids (mg catechin equivalent. 100 g <sup>-1</sup> )   | $2.06 \pm 0.42$ <sup>b</sup> | $31.65 \pm 5.56^{a}$       |
| Carotenoids (mg.100g <sup>-1</sup> )                        | $3.86 \pm 0.27 \ ^{\rm b}$   | 41.12± 5.21 <sup>a</sup>   |
| Polysaccharides (mg starch. 100 g <sup>-1</sup> )           | 9.34± 1.67 <sup>b</sup>      | 161.17± 10.51 <sup>a</sup> |
| Tannins (mg catechine. 100 g <sup>-1</sup> )                | $6.25 \pm 0.98$ <sup>b</sup> | $30.19 \pm 5.09^{a}$       |

Table 1.Total content of bioactive constituents and dietary fiber (Mean  $\pm$ SD) in wheat and brown algae

\*Each value represents the mean of three replicates  $\pm$ SD. Means with the superscript letters in the same raw were significant different at  $p \leq 0.05$ .

Bioactive constituents and dietary fiber content in wheat flour and brown algae were shown in Table 1. Such data indicated that dietary fiber was the most largest compound is dietary fiber (7.87 and 45.21 g. 100  $g^{-1}$ 1) followed by polysaccharides (9.34 and 161.17 mg starch.100g<sup>-1</sup>), phenolics (3.69 and 144.56 mg gallic acid equivalent. 100 g<sup>-1</sup>), carotenoids (3.86 and 41.12 mg.100g<sup>-1</sup>) ), flavonoids (2.06 and 31.65 mg catechin equivalent. 100  $g^{-1}$ ) and Tannins (6.65 and 30.19 mg catechine.  $g^{-1}$ ) in wheat flour and brown algae powder, respectively. It is also clear from the results that brown algae are much richer in the percentages of estimated active constituents when compared to wheat flour. Such data are in a accordance with that reported in several studies have been analyzed brown algae samples in Egypt [11,20,21] [22,23,43,44]. All of these bioactive constituents play important roles in applications of food processing and human nutrition. Dietary fibers are good for human health as they make an excellent intestinal environment by favoring the growth of intestinal microflora, including probiotic species so they can be considered as prebiotic [45]. Also, Elbasouny et al., [46] reported that fibers are primarily insoluble, and can bind bile acids. It is believed that binding of bile acids is one of the mechanisms whereby certain sources of dietary fibers lower plasma cholesterol. Furthermore, high intake of dietary fibers has a positive influence on blood glucose profile and it is related health complications, through altering the gastric emptying time and affect the absorption of other simple sugars [47,48]. In general, several studies reported that brown algae carbohydrates possessing a fiber level greater than those recorded for many common vegetables or fruits [49,50]. Other bioactive constituents such polysaccharides are using as thickening and gelling agents, and emulsion stabilizers as well as exhibited several biological activities including anticoagulant, antithrombotic, antiinflammatory, anti-obesity, antiviral, anti-osteoporosis, antioxidant and antimicrobial activities [11,12,13] [20,21,22,23] [43,51,52]. Also, polysaccharides play an important role in hypocholesterolemic and hypolipidemic phenomena through absorbing the substances like cholesterol, which are then eliminated from the digestive system [53,54]. Other brown algae active compounds such phenolics, flavonoids and carotenoids play important biological roles in preventing and/or treating many diseases such as diabetes, atherosclerosis, cancer, obesity, bone, anemia and aging. Such previous effects of these compounds are due mainly to their magical biological/antioxidant activities [12] [22,23,28] [55,56,57]. Finally, tannins exhibited antimicrobial, antiinflammatory,

antidiabetic, cardioprotective, antitumor, antioxidant effects [58]. It is utilized as a food preservative and packaging material in the food industry [59,60].

## **3.2. Biological Activities of Control and** Composite Wheat Flour Extracts

#### 3.2.1. Antioxidant Activities of Aqueous Control and Composite Wheat Flour Extracts

Data in Table 2 are shown the antioxidant activity of aqueous control and composite wheat flour extract. Such data indicated that brown algae samples showed high antioxidant activity (AA,  $78.22 \pm 3.15\%$ ) and wheat flour showed low one (AA,  $28.56 \pm 2.12\%$ ). With the increasing of brown algae powder level addition to the wheat flour, the value of antioxidant activity in the composite samples were significant ( $p \le 0.05$ ) increased. The increasing of antioxidant activity in composite flour is correlated with its reasonable content of different bioactive compounds such as polysaccharides, phenolics, flavonoids, carotenoids and tannins. The variation in the antioxidant activity values in composite flours may be possible due to the presence of different quantities of such specific bioactive constituents [61,62].Such data are in accordance with that observed by several authors who reported that brown algae are rich in and bioactive compounds (polysaccharides, polyphenols, triterpenoids, tannins, flavonoids, carotenoids  $(19.11 \pm 3.27 \text{ mg}.100\text{g}^{-1})$ , kaempherol and anthocyanin's subsequently antioxidant activities [21,22,43,44]. Also, Abd Elalal et al., [23] reported that brown algae contain a large amount of unique vitamins and minerals which exhibited different biological roles including the antioxidant activity.

Table 2. Antioxidant activity  $(\mathbf{A}\mathbf{A})$  of control, composite wheat flour and standards

| Parameter                                 | Value (Mean ±SD)              |
|---|-------------------------------|
| Antioxidant activity (Wheat flour, WF)    | $28.56\pm2.12^{\text{g}}$     |
| Antioxidant activity (Brown algae, BA)    | $78.22 \pm 3.15^{\circ}$      |
| Antioxidant activity BA+ 5% BA)           | $33.16 \pm 1.93^{\rm \ f}$    |
| Antioxidant activity (BA+ 10% BA)         | $39.25 \pm 2.63^{e}$          |
| Antioxidant activity (BA+ 15% BA)         | $43.93 \pm 4.33$ <sup>d</sup> |
| α-tocopherol (Standard, 50 mg/L)          | $98.79 \pm 0.72^{a}$          |
| Butalyted hydroxyl toluene (BHT,Standard, | $88.91 \pm 0.54$ <sup>b</sup> |
| 50 mg/L)                                  |                               |
| Butalyted hydroxyl toluene (BHT,Standard, | $97.68 \pm 0.32^{a}$          |
| 200mg/L)                                  |                               |

<sup>\*</sup> Each value represents the mean of ten replicates  $\pm$ SD. Mean values with the different superscript letters in the same column mean significantly different at level p $\leq$ 0.05.

#### 3.2.2. DPPH Radical Scavenging Activity of Aqueous Control and Composite Wheat Flour Extracts

Data in Figure 1 and Table 3 were showed the free radical scavenging activity (RSA, %) of aqueous control and composite wheat flour extract and standard (BHT). From such data it could be noticed that brown algae powder possessed the highest scavenging activity while wheat flour samples exhibit the lowest one. With the increasing addition of brown algae up to 15%, the value of RSA in the samples were significant ( $p \le 0.05$ ) increasing in comparison with the control sample (wheat flour). At a concentration of 100 µg/mL, the RSA of wheat flour and brown algae powder were 26.83 and 80.41%, respectively, whereas at the same concentration, the standard BHT was 91.55%. For the IC50, the brown algae was recorded 20.21µg/mL while the control and composite wheat flour samples not detected. The IC50 of BHT (standard) was 9.73 µg/mL. The free radical scavenging activity of different tested samples and standard was in the following order: standard (BHT) > brown algae > composite flour > wheat flour. Several studies indicated that DPPH methodology has been used successfully to evaluate the antioxidant activity oxidative stability of different plant parts including algae [20,21,22,23,43]. Several authors reported that the free radical scavenging activity are very important to prevent the adverse role of free radicals in diseases including obesity, different cancer. cardiovascular, diabetes, neurological, pulmonary diseases [2] [20,21,22] [43,44,46] [63,64]. The results of this study suggest that brown algae showed free radical scavenging activity which due to their high content of different categories of bioactive constituents/antioxidants including polysaccharides, phenolics, flavonoids, carotenoids, tannins etc.



Figure 1. DPPH radical scavenging activity (%) of control, composite wheat flour and standard (BHT) Each value represents the mean value of three replicates

# **3.3.** Chemical Composition of Control and Composite Wheat Flour

Data in Table 4 showed the approximate chemical

composition of control and composite wheat flour samples. Such data indicated that wheat flour samples recorded high values for total protein, crude fat and carbohydrates while brown algae powder samples recorded high values for the rest of the components (crude fiber and ash).With the increasing of brown algae powder level addition to the wheat flour, the value of crude fiber and ash in the composite samples were significant ( $p \le 0.05$ ) increased. While, total protein and carbohydrates contents were recorded the opposite direction. On the other side, nutritional properties of control and composite wheat flour samples may be altered as the result of such chemical composition alterations (Table 6). These properties include the total energy (Kcal/100g), the daily requirement of adult man from energy (GDR/energy) and from protein (GDR/protein), and percent satisfaction of the daily requirements of adult man in energy (P.S./energy) and protein (P.S./protein). With the increasing of brown algae powder level addition up to 15%, to the wheat flour, the value of total energy, GDR/energy and GDR/protein in the composite samples were significant ( $p \le 0.05$ ) altered. Data of this study are in accordance with that obtained by several authors who found that brown algae contain high levels of diverse essential nutrients [23,43]. Subsequently, the addition of brown algae to wheat flour samples leads to highly significant increasing in their crude fiber and mineral content. This property could be played many nutritional and therapeutic benefits. Soluble fiber, which dissolves in water, can help lower blood glucose and cholesterol levels [47,48]. Insoluble fiber, which does not dissolve in water, can help food move through your digestive system, promoting regularity and helping prevent constipation [65]. Thus, fiber contributes to reduce the risk of developing various diseases including cardiovascular disease, diabetes, and constipation. Also, several studies suggest that higher intake of fiber may offer protective benefits through manipulate several parameters include high blood pressure, high insulin levels, excess weight (especially around the abdomen), high levels of triglycerides, and low levels of HDL (good) cholesterol. [2] [20,21] [43,44] [63,66]. Furthermore, in Type 2 diabetes, diets low in fiber and high in foods that cause sudden increases in serum glucose may increase the risk of developing type 2 Diabetes [67,68,69]. Additionally, Farvid ey al., [70] reported that higher fiber intake reduces breast cancer risk, suggesting that fiber intake during adolescence and early adulthood may be particularly important. Finally, Constipation is the most common gastrointestinal complaint in worldwide and consumption of fiber seems to relieve and prevent constipation. In general, the fiber in brown algae is considered more effective than fiber from fruits and vegetables [2,3] [22,44]. In this way, nutrition experts recommend increasing fiber intake gradually rather than suddenly, and because fiber absorbs water, beverage intake should be increased as fiber intake increases [65].

Table 3. IC  $_{50}$  (DPPH) of control, composite wheat flour and standard (BHT)  $^{*}$ 

| Tested Materials  | Butylated hydroxytoluene | Wheat flour | Brown algae        | WF+ 5% | WF+ 10% | WF+15% BA  |
|-------------------|--------------------------|-------------|--------------------|--------|---------|------------|
|                   | (BHT)                    | (WF)        | (BA)               | BA     | BA      | W1+1570 DA |
| $IC_{50}$ (µg/mL) | 9.73 <sup>b</sup>        | ND          | 20.21 <sup>a</sup> | ND     | ND      | ND         |

\* Each value represents the mean value of three replicates  $\pm$ SD. Values with different superscript letters in the same raw are significantly did different at  $p \le 0.05$ .

| Component     | XX                 | Brown              | Composite wheat flour |             |                     |             |                    |             |  |
|---------------|--------------------|--------------------|-----------------------|-------------|---------------------|-------------|--------------------|-------------|--|
|               | (WF 85%)           | algae              | BA                    | BA (5%)     |                     | BA (10%)    |                    | BA (15%)    |  |
|               | (11,0570)          | (BA)               | g/100g                | % of change | g/100g              | % of change | g/100g             | % of change |  |
| Water         | 10.98 <sup>a</sup> | 9.03 <sup>a</sup>  | 10.90 <sup>a</sup>    | -0.73       | 10.70 <sup>a</sup>  | -2.55       | 10.67 <sup>a</sup> | -2.82       |  |
| Total Protein | 11.99 <sup>a</sup> | 5.14 <sup>b</sup>  | 11.71 <sup>a</sup>    | -2.36       | 11.35 <sup>a</sup>  | -5.34       | 10.98 <sup>a</sup> | -8.42       |  |
| Fat           | 1.32 <sup>a</sup>  | 0.93 <sup>b</sup>  | 1.31 <sup>a</sup>     | -0.72       | 1.25 <sup>a</sup>   | -5.30       | 1.24 <sup>a</sup>  | -6.06       |  |
| Fiber         | 1.17 °             | 8.01 <sup>a</sup>  | 1.53 <sup>b</sup>     | 30.77       | 1.88 <sup>ab</sup>  | 60.68       | 2.23 <sup>a</sup>  | 90.60       |  |
| Ash           | 0.96 <sup>d</sup>  | 18.71 <sup>a</sup> | 1.87 °                | 94.53       | 2.77 <sup>b</sup>   | 188.02      | 3.68 <sup>b</sup>  | 283.33      |  |
| Carbohydrates | 73.58 <sup>a</sup> | 58.18 <sup>b</sup> | 72.81 <sup>a</sup>    | -1.05       | 72.055 <sup>a</sup> | -2.07       | 71.2 <sup>a</sup>  | -3.23       |  |

Table 4. Chemical composition of control and composite wheat flour

\* Each value represents the mean value of three replicates. Values with different superscript letters in the same raw are significantly did different at  $p \le 0.05$ .

|  |                    |                   | Composite wheat flour |             |                    |             |                    |             |  |
|--|--------------------|-------------------|-----------------------|-------------|--------------------|-------------|--------------------|-------------|--|
| Component  | (WE 85%)           | Brown algae       | BA                    | (5%)        | BA (10%)           |             | BA (15%)           |             |  |
|  | (W1,0570)          | (BA)              | g/100g                | % of change | g/100g             | % of change | g/100g             | % of change |  |
| Energy (Kcal/100g)                               | 354 <sup>a</sup>   | 262 <sup>b</sup>  | 349 <sup>a</sup>      | -1.31       | 345 <sup>a</sup>   | -2.61       | 340 <sup>a</sup>   | -3.92       |  |
| G.D.R. (g) for<br>protein (63 g)                 | 525 °              | 1226 <sup>a</sup> | 561 <sup>d</sup>      | 6.66        | 596°               | 13.33       | 631 <sup>b</sup>   | 20          |  |
| G.D.R. (g) for<br>energy (2900 Kcal)             | 819 <sup>b</sup>   | 1108 <sup>a</sup> | 833 <sup>b</sup>      | 1.77        | 848 <sup>b</sup>   | 3.54        | 862 <sup>b</sup>   | 5.30        |  |
| P.S./ 80 g (One<br>loaf, %) For protein<br>(63g) | 15.23 <sup>a</sup> | 6.53 <sup>b</sup> | 14.79 <sup>ª</sup>    | -2.86       | 14.41 <sup>a</sup> | -5.34       | 13.94 <sup>a</sup> | -8.42       |  |
| P.S./80 g (One<br>loaf, %) For energy<br>(63g)   | 9.77 <sup>a</sup>  | 7.22 °            | 9.64 <sup>b</sup>     | -1.31       | 9.51 <sup>b</sup>  | -2.61       | 9.39 <sup>b</sup>  | -3.92       |  |

\* Each value represents the mean value of three replicates. Values with different superscript letters in the same raw are significantly did different at  $p \le 0.05$ . G.D.R., grams consumed of food ( wet weight basis ) to cover the daily requirements of adult man ( 63 g) in protein and energy, P.S., percent satisfaction of the daily requirement of adult man in protein and energy.

|           |                     | D                   | Composite wheat flour |         |                      |          |                     |          |  |
|-----------|---------------------|---------------------|-----------------------|---------|----------------------|----------|---------------------|----------|--|
| Mineral   | Wheat flour         | algae               | BA (S                 | 5%)     | BA (1                | BA (10%) |                     | BA (15%) |  |
| (WF, 85%) | (BA)                | mg/100g             | % of<br>change        | mg/100g | % of<br>change       | mg/100g  | % of change         |          |  |
| Ca        | 20.67 <sup>a</sup>  | 33.56 <sup>a</sup>  | 21.55 <sup>b</sup>    | 4.25    | 22.43 <sup>b</sup>   | 8.54     | 23.08 <sup>b</sup>  | 11.64    |  |
| K         | 133.67 <sup>a</sup> | 37.67 °             | 130.29 <sup>a</sup>   | -2.53   | 126.69 <sup>ab</sup> | -5.22    | 121.76 <sup>b</sup> | -8.91    |  |
| Mg        | 26.15 <sup>b</sup>  | 31.14 <sup>a</sup>  | 26.69 <sup>b</sup>    | 2.06    | 27.29 <sup>b</sup>   | 4.36     | 27.56 <sup>b</sup>  | 5.39     |  |
| Р         | 118.23 <sup>d</sup> | 209.12 <sup>a</sup> | 125.13 °              | 5.83    | 129.90 <sup>bc</sup> | 9.87     | 134.62 <sup>b</sup> | 13.86    |  |
| Fe        | 1.48 <sup>d</sup>   | 7.79 <sup>a</sup>   | 1.82 <sup>d</sup>     | 22.65   | 2.19 <sup>cd</sup>   | 48.23    | 2.48 <sup>b</sup>   | 67.38    |  |
| Zn        | 1.89 °              | 3.41 <sup>a</sup>   | 1.99 <sup>bc</sup>    | 5.17    | 2.08 <sup>b</sup>    | 10.24    | 2.18 <sup>b</sup>   | 15.46    |  |
| Na        | 4.19 <sup>d</sup>   | 23.56 <sup>a</sup>  | 5.31 °                | 26.62   | 6.28 <sup>bc</sup>   | 49.91    | 7.24 <sup>b</sup>   | 72.88    |  |
| Mn        | 1.82 °              | 2.73 <sup>a</sup>   | 1.89 °                | 3.63    | 1.97 <sup>bc</sup>   | 8.23     | 2.00 <sup>b</sup>   | 9.75     |  |
| Cu        | 0.71 <sup>b</sup>   | 0.85 <sup>a</sup>   | 0.72 <sup>b</sup>     | 2.10    | 0.74 <sup>b</sup>    | 4.04     | 0.76 <sup>b</sup>   | 6.52     |  |
|           |                     |                     |                       |         |                      |          |                     |          |  |

Table 6. Mineral composition of control and composite wheat flour

\* Each value represents the mean value of three replicates. Values with different superscript letters in the same raw are significantly did different at  $p \le 0.05$ .

## 3.4. Mineral Composition of Control and Composite Wheat Flour

Data in Table 6 showed the mineral content of control and composite wheat flour. Such data indicated that with the except of K brown algae powder samples recorded high values for all estimated mineral including Ca, Mg, P, Fe, Zn, Na, Mn and Cu than wheat flour. With the increasing of brown algae powder level addition to the wheat flour, the value of the all estimated minerals except K in the composite samples were significant ( $p \le 0.05$ ) increased. The high significant increasing was recorded for Fe and Na which recorded 67.38 and 72.88% when compared with the control wheat flour samples. Also, increasing was noticed for some important trace metals such Zn, Mn and Cu. The present data are accordance with that reviewed by Abd Elalal et al., [23] and Gad Alla, [22]. In general, Fe has a main biological role through carry oxygen in the hemoglobin of red blood cells in the body so cells can produce energy. It improves oxygen storage through myoglobin, a protein containing iron which transports and stores oxygen within the muscles. Also, Fe is necessary for DNA synthesis and plays an important role in the human immune system [71]. For P, it is a mineral that naturally occurs in many foods and is also available as a supplement. Phosphorus is a key element of bones, teeth, and cell membranes. It helps to activate enzymes, and keeps blood pH within a normal range. It regulates the normal function of nerves and muscles, including the heart, and is also a building block of the genes, as it makes up DNA, RNA, and ATP, the body's major source of energy [72]. Magnesium is a key factor

in making several parts of the body run smoothly: the heart, bones, muscles, nerves, and others. Without enough Mg, these areas malfunction i.e. deficiency or low magnesium diet leads to health problems. Epidemiological studies show that Mg-rich diet is often higher in other nutrients, which collectively work together in disease prevention as opposed to a supplement containing a single nutrient [73]. For traces elements i.e. meaning that the body only needs small amounts, Mn has an important role in the metabolism of lipids and lipoproteins and it participates in the pathogenesis of atherosclerosis as well as numerous other cardiovascular diseases [74]. Also, Cu works together with Fe to help the body in formation the red blood cells. It also helps keep the blood vessels, nerves, immune system, and bones healthy. Cu also aids in iron absorption [75]. Furthermore, Zn is necessary for several enzymes to carry out vital chemical reactions. It is a major player in the creation of DNA, growth and multiply of cells, building proteins, healing damaged tissue, supporting a healthy immune system, and involved with the senses of taste and smell [76,77].

## 3.5. Vitamins Content of Control and Composite Wheat Flour

Data in Table 7 showed the vitamins content of control and composite wheat flour. Such data indicated that with brown algae powder samples recorded high values for all estimated vitamins including A, B complex, C and E than wheat flour. With the increasing of brown algae powder level addition to the wheat flour, the value of the all estimated vitamins in the composite samples were significant ( $p\leq0.05$ ) increased by different rates ranged 71.29 to 84301%. The present data are accordance with that reviewed by Abd Elalal et al., [23] and Gad Alla, [22] who reported that brown algae are rich in different nutrients including vitamins (A, B complex, C and E). In general, Vitamins B are a diverse group in terms of structure and function [78]. For example, Vitamin B1 (Thiamin) plays a vital role in the growth and function of various cells. Because it is involved in several basic cell functions and the breakdown of nutrients for energy, a deficiency can lead to various problems in the brain and heart that require a constant supply of energy) [79]. Riboflavin (B<sub>2</sub>) plays a vital role in maintaining the body's energy supply, convert carbohydrates into adenosine triphosphate (ATP), storing energy in muscles, growth and red blood cell production and. fight free radicals and may reduce or help prevent some of the damage they cause [80]. Vitamin B9 (folate) which provide methyl groups necessary for DNA methylation, play an important role in the pathogenesis of neurological diseases and involved in the metabolism of several amino acids, including, serine, methionine, glycine and histidine. The roles of folate and vitamin B<sub>12</sub> in the conversion of homocysteine to methionine, along with the role of vitamin B6 (Pyridoxine) in the conversion of homocysteine to cystathionine, continue to receive considerable attention because low intakes of these three vitamins (B6, B9 and B12), especially B9 (folate), are inversely associated with plasma homocysteine concentrations, and elevated plasma homocysteine concentrations (>15  $\mu$ ) are associated with several dangerous diseases including premature coronary artery disease, premature occlusive vascular disease, or peripheral vascular disease, cerebral cancer. Alzheimer's [81,82]. On the other side, Vitamin A is involved in vision, growth, and reproduction [83]. Finally, brown algae powder is rich in vitamin E content which including in the cell antioxidant defence system and is exclusively obtained from the diet. Oxidation has been linked to numerous possible conditions and diseases, including cancer, ageing, arthritis, platelet hyperaggregation and cataracts; vitamin E has been shown to be effective against these [84].

|                                       | Wheat              |                    | Composite wheat flour |             |                    |             |                    |             |  |
|---------------------------------------|--------------------|--------------------|-----------------------|-------------|--------------------|-------------|--------------------|-------------|--|
| Component                             | flour              | algae              | BA                    | (5%)        | BA (               | 10%)        | BA (15%)           |             |  |
| component                             | (WF,<br>85%)       | (BA)               | mg/100g               | % of change | mg/100g            | % of change | mg/100g            | % of change |  |
| B1 (Thiamine, mg/100g)                | 0.160 <sup>b</sup> | 0.911 <sup>a</sup> | 0.21 <sup>a</sup>     | 32.32       | 0.24 <sup>a</sup>  | 51.52       | 0.28 <sup>b</sup>  | 74.99       |  |
| B2 (Riboflavin,<br>mg/100g)           | 0.196 <sup>e</sup> | 18.0 <sup>a</sup>  | 1.11 <sup>d</sup>     | 467         | 2.14 °             | 993         | 2.95 <sup>a</sup>  | 1405        |  |
| B3 (Niacin, mg/100g)                  | 0.310 <sup>e</sup> | 21 <sup>a</sup>    | 1.38 <sup>d</sup>     | 344         | 2.46 °             | 693         | 3.68 <sup>b</sup>  | 1085        |  |
| B6 (Pyridoxine,<br>mg/100g)           | 0.088 °            | 0.415 <sup>a</sup> | 0.13 <sup>b</sup>     | 42.63       | 0.13 <sup>b</sup>  | 52.80       | 0.15 <sup>b</sup>  | 71.29       |  |
| B9 (Folate, µg/100g)                  | 0.310 <sup>d</sup> | 109 <sup>a</sup>   | 5.93 <sup>d</sup>     | 1812        | 11.60 °            | 3640        | 17.16 <sup>b</sup> | 5436        |  |
| B12 (Cyancobalamin, mg/100g))         | 0.039 °            | 0.149 <sup>a</sup> | 0.05 <sup>b</sup>     | 16.51       | 0.06 <sup>b</sup>  | 57.85       | 0.07 <sup>b</sup>  | 76.92       |  |
| C (Ascorbic acid, mg/100g)            | 0.017 <sup>d</sup> | 2.11 <sup>a</sup>  | 0.12 <sup>c</sup>     | 631         | 0.26 <sup>bc</sup> | 1449        | 0.35 <sup>b</sup>  | 1958        |  |
| A ( $\beta$ -carotene, $\mu g/100g$ ) | 0.038 <sup>e</sup> | 208 <sup>a</sup>   | 10.89 <sup>d</sup>    | 28548       | 21.58 <sup>c</sup> | 56700       | 32.07 <sup>b</sup> | 84301       |  |
| E (Tocopherols, mg /100g)             | $0.054^{d}$        | 0.650 <sup>a</sup> | 0.09 °                | 58.46       | 0.12 <sup>b</sup>  | 116.93      | 0.17 <sup>b</sup>  | 209.74      |  |

Table 7. Vitamins content of control and composite wheat flour

\* Each value represents the mean value of three replicates. Values with different superscript letters in the same raw are significantly did different at  $p \le 0.05$ .

## **3.6.** Physical Properties of Brown Algae Powder and Wheat Flour

Data in Table 8 shown the water (WHC) and oil (OHC) holding capacity of brown algae powder compared to wheat flour. Such data indicated that brown algae powder recorded higher WHC and OHC being 8.94  $\pm$  0.41 g  $H_2O.g^{-1}$  and 4.01  $\pm$  0.33 g oil.g<sup>-1</sup>, respectively than wheat flour which recorded  $6.35 \pm 0.25$  g H<sub>2</sub>O.g<sup>-1</sup> and  $2.61 \pm 0.31$ g oil.g<sup>-1</sup>, respectively. Such data are in accordance partially with several authors who reported that the plant parts have high WHC is mainly attributed to high fiber content [85,86,87]. The higher in WHC recorded in brown algae powder could be attributed to the higher fiber content which hold more water compared to wheat flour. In similar study, Ashoush and Gadallah, [88] reported that mango peel powder was higher than that of mango kernel powder being 5.08 and 2.08 g water/g, respectively indicating that the higher fiber content in mango peel powder hold more water compared to mango kernel powder. Also, this observation is agreed with that reported by Ajila et al., [89], Mashal, [85] and Aly and Sadeek, [87].

Table 8. Physical properties of brown algae powder and wheat flour

| Parameters  | Brown algae (BA)  | Wheat flour (WF, 85%)        |
|---|-------------------|------------------------------|
| Water holding<br>capacity (WHC, g<br>H <sub>2</sub> O.g <sup>-1</sup> ) | $8.94\pm0.41~^a$  | $6.35 \pm 0.25^{b}$          |
| Oil holding capacity<br>(OHC, g oil.g <sup>-1</sup> )                   | $4.01\pm0.33^{a}$ | $2.61 \pm 0.31$ <sup>b</sup> |

Each value represents the mean of three replicates  $\pm$ SD. Mean values with different superscript letters in the same row were significant different at p $\leq$ 0.05.

## 3.7. Effect of Brown Algae Powder Mixing on the Rheological Parameters of Wheat Flour Bread

#### 3.7.1. Farinograph Parameters

Data in Table 9 showed all farinograph parameters of wheat and wheat flour mixing with brown algae powder. The incorporating of brown algae powder in dough increased the water absorption, arrival time, dough development time, dough stability and farinograph quality number. The rate of increasing in all farinograph parameters levels were exhibited a dose-dependent increase with brown algae powder incorporation. Such data are in accordance partially with that obtained by Aly and Sadeek, [87] who reported that the incorporating of plant powder other than algae i.e. quinoa powder by 10 and 20% in dough increased its all farinograph parameters for cake dough. Also, Sayed-Ahmed [86] found that the incorporating of plant parts by-products i.e. 5% of potato

peel, cauliflower, onion and mango peels powder in dough increased its water absorption. This increment in dough water absorption may be due to high content of dietary fiber in brown algae powder which with significant difference with control. The increasing in dough development time and dough stability increased with 5-15% incorporation of brown algae powder may be due to its high content of dietary fibers and pectin which act as a food hydrocolloid. Dough stability in minutes is the most important index for dough strength. Incorporation of brown algae powder to flour samples showed markedly longer stability periods than the control samples (wheat flour). This affect was significantly with the addition of 5 to15% of brown algae powder to the wheat flour. This affect could be attributed to the effect of brown algae powder incorporation on the quality of protein and dietary fiber flour in particular the binding force property [85,87,90,91,92]. From the viewpoint of dough farinograph quality number (FQN), statistically significant difference was found between the control sample and the dough with incorporation of 5 to 15% of brown algae powder. It is meaning that an improvement in the quality of the dough occurred after the addition of brown algae powder, when the FON value significantly increased in comparison with the control sample.

#### 3.7.2. Extensograph Parameters

Data in Table 10 showed all extensograph parameters of wheat and wheat flour mixing with brown algae powder. The incorporating of brown algae powder in dough increased the extensibility, relative resistance to extension, proportional number and energy. The rate of increasing in all extensograph parameters levels were exhibited a doseincrease with brown dependent algae powder incorporation. Such data are in accordance partially with that reported by Aly and Sadeek, [87] who reported that the incorporating of quinoa powder in dough increased the all extensograph parameters. Also, Sayed-Ahmed, [86] reported the incorporating of plant parts by-products in dough increased such all parameters by different rates. The effect of quinoa powder on increasing the extensibility of the wheat flour may be due to the alteration of the viscosity and forced the gluten network [93]. Also, several reports suggest that different plant parts by-products such as potato and onion peels, cauliflower leaves, and prickly pear have antioxidant activity which could be easily prevented the oxidation process usually decreases dough extensibility [91,85,86]. Finally, data of the rheological studies reported that in order to improve the quality of bakery products such breads additions of the brown algae powder by rate up to 15% to the dough are recommended.

Table 9. Farinograph parameters of the control and composite wheat flour bread with brown algae

|                           |                               |                           | -                                       | -                            |                                  |
|---------------------------|-------------------------------|---------------------------|---|------------------------------|----------------------------------|
| Treatment                 | Water absorption<br>(WA, %)   | Arrival time<br>(AT, min) | Dough development<br>time<br>(DDT, min) | Dough stability<br>(DS, min) | Farinograph quality number (FQN) |
| Control wheat bread (CWB) | $41.45 \pm 1.23^{d}$          | $1.33 \pm 0.09^{c}$       | $2.52\pm0.12^{d}$                       | $3.58\pm0.35^{\ c}$          | $132\pm2.98^{\rm d}$             |
| CWB + 5% BA               | $51.65 \pm 2.71$ <sup>c</sup> | $2.01\pm0.23^{\text{ b}}$ | $2.80\pm0.31^{\text{ c}}$               | $4.81\pm0.99^{\text{ b}}$    | $143 \pm 4.01$ <sup>c</sup>      |
| CWB + 10% BA              | $59.17 \pm 1.96^{b}$          | $2.89\pm0.18^{\ b}$       | $3.81 \pm 0.41^{\ b}$                   | $6.43\pm0.47^{\:a}$          | $155\pm3.19^{b}$                 |
| CWB + 15% BA              | $66.33 \pm 3.03^{a}$          | $3.64 \pm 0.11^{a}$       | $4.66\pm0.29^{a}$                       | $7.09\pm0.69^{\:a}$          | $163\pm2.94^{\text{ a}}$         |

Each value represents the mean of three replicates  $\pm$ SD. Mean values with the different letters in the same column mean significantly different at level  $p \leq 0.05$ .

| Treatment                 | Extensibility<br>(mm)        | Relative resistance to extension (BU) | Proportional number       | Energy<br>(cm <sup>2</sup> ) |  |  |  |  |
|---------------------------|------------------------------|---------------------------------------|---------------------------|------------------------------|--|--|--|--|
| Control wheat bread (CWB) | $158.55 \pm 4.98^{d}$        | $481.18 \pm 7.87^{d}$                 | $2.33\pm0.25^{\text{ d}}$ | $99\pm4.21^{\text{d}}$       |  |  |  |  |
| CWB + 5% BA               | $171.68 \pm 6.31^{\rm c}$    | $510.76 \pm 9.12^{\circ}$             | $2.86 \pm 0.44$ °         | $117\pm5.02^{c}$             |  |  |  |  |
| CWB + 10% BA              | $180.25\pm3.44^{\mathrm{b}}$ | $520.22 \pm 7.44^{b}$                 | $3.10\pm0.50^{\text{ b}}$ | $126\pm3.97^{\text{ b}}$     |  |  |  |  |
| CWB + 15% BA              | $191.11 \pm 5.21^{a}$        | $553.19 \pm 7.41{}^{\rm a}$           | $3.52 \pm 0.33^{a}$       | $133 \pm 3.01^{a}$           |  |  |  |  |

Table 10. Extensograph of the control and composite wheat flour bread with brown algae

Each value represents the mean of three replicates  $\pm$ SD. Mean values with the different letters in the same column mean significantly different at level  $p \leq 0.05$ .

| Table 11. Sensory | v evaluation o | of Balady | bread incor | porated with | brown algae |
|-------------------|----------------|-----------|-------------|--------------|-------------|
|                   | ,              |           |             |              |             |

| Treatment                     | Crust appearance        | Crust colour            | Texture                 | Taste and Flavor       | Mouth feel              | Overall acceptability   |
|-------------------------------|-------------------------|-------------------------|-------------------------|------------------------|-------------------------|-------------------------|
| Control Balady<br>bread (CBB) | 9.21±0.39 <sup>a</sup>  | 8.69±0.75 <sup>a</sup>  | 8.50±0.39 <sup>a</sup>  | 8.83±0.21 <sup>a</sup> | 9.07±0.12 <sup>a</sup>  | 9.23±0.24 <sup>a</sup>  |
| CBB + 5% BA                   | 8.81±0.25 <sup>ab</sup> | 8.48±1.15 <sup>a</sup>  | 8.02±0.33 <sup>a</sup>  | 8.78±0.14 <sup>a</sup> | 8.61±0.11 <sup>a</sup>  | 8.91±0.44 <sup>a</sup>  |
| CBB + 10% BA                  | 7.98±0.54 <sup>b</sup>  | 7.79±1.09 <sup>ab</sup> | 7.78±0.25 <sup>ab</sup> | $8.70 \pm 0.44^{a}$    | 8.50±0.44 <sup>ab</sup> | 8.44±0.39 <sup>ab</sup> |
| CBB + 15% BA                  | 7.62±0.54 <sup>b</sup>  | 7.54±1.09 <sup>b</sup>  | 6.98±0.25 <sup>b</sup>  | 8.55±0.44 <sup>a</sup> | 8.20±0.44 <sup>b</sup>  | 8.23±0.39 <sup>b</sup>  |

\* Each value represents the mean of ten replicates ±SD. Mean values with the different letters in the same column mean significantly different at p≤0.05.

## 3.8. Sensory Evaluation of Balady Bread Incorporated with Brown Algae Powder

Results of sensory evaluation of Balady bread incorporated with brown algae powder in terms of appearance, colour, texture and flavor, mouth feel and overall acceptability are presented in Table 11 and Figure 2. Colour, taste and flavor, mouth feel and overall acceptability were not significantly different between the control and brown algae powder incorporated Balady bread at the level5%. At the high level of brown algae powder incorporation i.e.10 and 20%, the sensory evaluation parameters were not significant ( $p \le 0.05$ ). The present data are in accordance with that obtained by Aly and Sadeek, [87] with the incorporation of other plant powder i.e. quinoa powder with cake. In similar study, Broyart, [94] reported that the initial acceptance of baked products is much influenced by colour, which can also be an indicator of baking completion. The desirable colour of bread is mainly due to the Millard browning during baking. However, in brown algae powder blended Balady bread, the colour could be partially contributed by the phenolics and carotenoids in algae powder which imparts a yellowish/brownish colour to the final bread. Similar data were reported by Brannan et al., [95] who observed that an increased flour and thus muffin visual lightness (with more yellowness and brownness rather than dark and vellow green) yield a higher aroma, texture and colour acceptability scores. Also, in such data there was no significant difference in texture amongst the different samples with different composite flour bread types. Furthermore, no significant difference was observed in terms of taste and flavor between the control and brown algae powder incorporated bread. This could probably be due to the nature of brown algae powder which did not impart any additional flavor to the bread. Finally, there was partially no significant difference in term of overall acceptability among the control and brown algae powder incorporated bread. This could be attributed to the close resemblance of the breads types in terms of the colour and taste/flavor of the commercial breads in the market, for example, bread with bran wheat.



**Figure 2.** Photos of Balady Bread (CBB) mixed with brown algae powder. A) Control Balady Bread (CBB), B) CBB + 5% brown algae powder, C) CBB + 10% brown algae powder, D) 15% brown algae powder

## 4. Conclusion

The chemical composition of brown algae powder showed that it is a good source of protein, ash, dietary fibers, carbohydrates and bioactive compounds such as total phenolics, alkaloids, polysaccharides etc. Incorporation of brown algae powder with Balady bread flour improved the rheological properties of the dough including farinograph and extensograph parameters subsequently their baking characteristics. Bread samples enriched with brown algae powder showed higher crude fiber, control breads. Increasing of such bioactive compounds in bread samples probably exhibited significant improving in their biological activities such antioxidant and scavenging of the free radicals activities. The brown algae powder incorporated bread up to 15% doesn't affect on their quality/sensory evaluation parameters. Thus, the use of brown algae powder in one of the important food industry applications that contributes a major role to bridging the nutritional gap, which is the bread industry as a partial substitute for flour.

## **Ethical Considerations**

The ethical issues of this study was reviewed and approved by the Scientific Research Ethics Committee (SREC, Approval #14-SREC-09-2021), Faculty of Home Economics, Menoufia University, Shebin El-Kom, Egypt.

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## **Conflict of Interests**

Authors declared no competing of interest whatsoever.

## **Authors' Contribution**

Yousif Elhassaneen participated in proposing and developing the study protocol, following up on the laboratory experimental part, retrieving conceptual information, reviewing and validating the results and statistical analyses, preparing a draft of the manuscript, conducting a critical review to intellectually organize the content and granting approval to publish the final version of the manuscript. Fatma Alsobky conducted laboratory experiments, collected, tabulated, analyzed and interpreted the results. She was also involved in retrieving conceptual information and preparing the draft of the manuscript. Ghada ELBassouny participated in proposing the study protocol, retrieving conceptual information, validating the study results, and preparing the draft manuscript. Omar Emam participated in proposing the study protocol, retrieving conceptual information, and providing contributions to the concept and design of the work.

## Abbreviations

AT: arrival time, ATP: adenosine triphosphate, BA: brown algae, BHT: butalyted hydroxyl toluene, CBB: control balady bread, CWB: control wheat bread, DDT: dough development time, DNA: deoxyribonucleic acid, DPPH: 2,2-diphenyl-1-picrylhydrazyl, DS: dough stability, FQN: farinograph quality number, G.D.R.: grams consumed of food ( wet weight basis ) to cover the daily requirements of adult man ( 63 g) in protein and energy, P.S.: percent satisfaction of the daily requirement of adult man in protein and energy, RNA: ribose nucleic acid, OHC: oil holding capacity, WA: water absorption, WF: wheat flour, WHC: water holding capacity.

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