Study on the physical and antioxidant properties of gluten-free bread with brown algae

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ABSTRACT
The aim of the work was to determine the influence of brown algae on the physical, antioxidant, and sensorial properties when added directly into the gluten-free bread (GFB) recipe. Algae powder was added in the amounts of 2%, 4%, 6%, 8%, and 10% of the total flour content. Compared to the control bread, a larger volume was obtained using 4% of algae. With an increased content of brown algae, significant changes were noted in the color of the breadcrumb. Favorable changes in the texture were also observed. Brown algae addition significantly increased the antioxidant activity of GFB. Most importantly, antiradical compounds from the functional products were highly bioaccessible in vitro. The results confirm the possibility of the use of brown algae powder in the production of GFB. Because of the unpleasant taste on over-addition, the acceptable GFB can be obtained by adding 2% or 4% of the algae.

Introduction
Algae are the primary source of different nutrients. The high protein content in various species of algae is one of the main reasons to consider them as an unconventional source of proteins and oils. Because algae also represent an important source of vitamins, minerals, antioxidants, and natural colorants, the incorporation of the whole biomass in food and feed could be used to provide the color, increment the nutritional value, and improve the texture or resistance to oxidation. The incorporation of algae into the traditional food is a way to design healthy new products (Kovač, Simeunović, Babić, Mišan, & Milovanović, 2013), as plant materials are a rich source of many compounds used in human medicine (Oniszczuk & Olech, 2016; Oniszczuk & Podgórska, 2015).

Algae have been used in wheat or rye bread composition only in several studies. The possibility of using algal products rich in minerals and amino acids for increasing the nutritive value of bread was first examined by Medvedeva, Kalyuzhnaya, Panchenko, Krasil’nikova, and Petrenko (1969). The goal of other studies was to assess the protein quality of baladi bread prepared from formulas containing added fish flour and green algae (Scenedesmus obliquus) (Arafah, Absay, Morcos, & Hussein, 1980). Algae Dunaliella (Finney, Pomeranz, & Bruinsma, 1984) was used as a protein supplement in white wheat pan bread. Applications of dried blue-green algal powders in bread-making were studied using spp. Oscillatoria amphibia and Spirulina platensis. Powders were decolorized with ethanol and added to wheat flour (87.5% extraction) at 6.9 and 6.6%, respectively, to add 5 g algal protein to 100 g wheat flour (Saleh, El-Fouly, Shaheen, El-Malky, & Khorshid, 1987). Studies with spirulina added in different concentrations (2%, 2.5%, and 3% related to flour) were also made by other authors (Dinu, Vlasceanu, Dune, & Rotaru, 2012). Increase in the proteins and minerals content was observed in the final bread product compared to the control bread, without spirulina. In recent studies, other authors (Achour, Toumandji, Sadi, & Saadi, 2014) prepared...
enriched breads with 1% and 3% of dry biomass of a local microalgae spirulina. The enrichment with spirulina has improved the nutritional quality (protein, ash) of bread. For the rest of the nutrients (fat, crude fiber, and carbohydrates), the values almost remain unchanged. All enriched breads presented a good global acceptability.

The above-mentioned studies relate primarily to the wheat breads, while gluten-free bread (GFB) with algae was studied in a small range (Figueira, Crizel, Silva, & Sallas-Mellado, 2011). Recent scientific studies have increasingly focused on GFB (Dłuzewska, Marcinia-Lukasiak, & Kurek, 2015; Różyło, Rudy, Krzykowski, & Dziki, 2015; Różyło, Rudy, Krzykowski, Dziki, Gawlik-Dziki et al., 2015; Tsatsaragkou et al., 2014). GFB is unacceptable completely by consumers (Mariotti, Pagani, & Lucisano, 2013), because many studies have addressed the impact of various additives on the quality of GFB. To increase the protein content of GFB, dried *Spirulina platensis*, a microalga, was added to the products in the range of 2–5% (flour basis) (Figueira et al., 2011). The addition of *Spirulina* to GFB resulted in products with improved nutritional quality, with a significant increase in the protein content as well as in some essential amino acids (threonine, methionine, isoleucine, and leucine), when compared to bread without the addition of the algae. Authors have also observed significant changes in the volume, texture, and crumb color. No antioxidant activity was determined for GFB and also for other breads supplemented with algae.

In our study we proposed, for the first time, the addition of brown alga to GFB; besides the qualitative assessment of bread, we also have evaluated the antioxidant activity in the light of potential bioaccessibility of active compounds.

**Material and methods**

**Materials**

The raw materials for making GFB included white rice flour, corn flour, millet flour, and brown algae. The white rice and corn commercial flours were sourced from Melvit (Warsaw, Poland) and the millet whole grain flour was sourced from Bio Raj (Pokrzydowo, Poland). The brown algae (*Ascothylum nodosum*) powder was sourced from Nat Vita, Radgeb (Wrocław, Poland).

The protein, the ash, and the fat contents were evaluated according to ISO standards (ISO 20483:2006; ISO 2171:2007; ISO 11085:2015). Total carbohydrates were calculated as the difference between the protein, fat, ash, and moisture contents in flour.

The dried instant yeasts (Instaferm) were obtained from Lallemand Iberia, SA. Salt was purchased from a local market.

**Baking methods**

GFB was baked in a laboratory oven equipped with a fermentation chamber (Sadkiewicz Instruments, Bydgoszcz, Poland). The bread formulations were based on white rice flour, corn flour, and millet flour (45:45:10) (Control probe C). Loaves of bread were prepared with different additions of brown algae in an amount of 2%, 4%, 6%, 8%, and 10% per the amount of the flour mass used in the bread recipe (Table 1). In addition, the flour in the formulation salt (2%), yeast (1%), and water were used (according to baking practice – the amount of flour is considered as 100% and the ratio of the other components is converted to the weight of flour). The optimal water usage level was determined using empirical trial-and-error testing (Hager et al., 2012). The gluten-free dough was prepared after the mixing (five-speed mixer, Kitchen Aid, St. Joseph, MI, USA) of all the ingredients for 5 min. After mixing, the dough was immediately transferred into a pan (loaves of equal mass 300 g), and then subjected to proofing (30 °C and 75–88% RH) for 40 min.

The loaves were baked at 230 °C for 45–50 min in a laboratory oven (live steam was injected immediately after the loaves were placed in the oven) (Sadkiewicz Instruments, Bydgoszcz, Poland). After baking and cooling the GFB, loaves were wrapped in polyethylene bags and stored in a glass cabinet at room conditions (20 °C and 50% RH).

Baking tests were performed three times in three repetitions obtaining nine loaves of bread.

**Evaluation of the physical and sensory properties of bread**

GFB loaf volume was measured (one day after baking) using the millet seed displacement method. The pH of the dough and the breadcrumb was measured using a pH meter (TESTO 206-ph2, Pruszków, Poland) with a penetration probe for semi-solid substances.

The color of the breadcrumb (central part) samples was measured using a Chromometer HP 2132 (Anticorr, Gdarisk, Poland). The analysis was based on the *L*°*a*°*b*° system for breads defined by the CIE (International Commission on Illumination), where *L*° is the luminance or lightness component (from 0 to 100), and parameters *a*° (from green to red) and *b*° (from blue to yellow) are color-opponent dimensions. According to the manufacturer’s declaration, the calibration was performed using 100 color patterns, which a colorimeter has in its memory, enabling the execution of 20 000 measurements.

The samples (30 × 30 × 20 mm) (1 and 3 days after baking) for textural measurements were compressed (ZWICK Z020/TN2S strength tester) using a capital equipped with a 30 mm plug until a 50% depth at a crosshead speed of 1 mm s⁻¹ was achieved (Różyło, 2014; Różyło, Gawlik-Dziki et al., 2014). The samples were compressed twice (curves 1 and 2) to give a two-bite TPA (texture profile analysis) (Gámbaro, Gimenez, Ares, & Gilardi, 2006), from which textural parameters were obtained: firmness (peak force 1), elasticity (length of the base of area 2), and cohesiveness (area 2/area 1). The texture tests were performed in nine replicates.

The percentage changes in hardness caused by storage were calculated (BS₄₀, degree of staling bread) as described
Estimation of antioxidant activity of bread

Extraction procedures

Samples of bread (1 g dry mass) were homogenized for 1 min with a laboratory blender, extracted in 20 mL PBS buffer (phosphate buffered saline: pH 7.4) for 30 min at room temperature (RT), centrifuged (15 min, 3000 x g, RT), and followed with supernatant recovery. This procedure was repeated to obtain 40 mL of the combined buffer extract. The extract was stored in darkness at -20 °C.

Digestion in vitro

In vitro digestion was carried out according to the method described by Gawlik-Dziki et al. (2013) with slight modification. To obtain the digestion extracts, the samples were homogenized in the presence of 15 mL of simulated salivary fluid (prepared by the following procedure: 1) dissolving 2.38 g Na2HPO4, 0.19 g KH2PO4, 8 g NaCl, and 100 mg of mucin in 1 L of distilled water (2) adjusted to pH = 6.75, and (3) addition of α-amylase (E.C. 3.2.1.1) to obtain the final units of activity of 200 U/mL with a stomacher laboratory blender for 1 min to simulate mastication. The extract of 15 mL of extract was adjusted to pH 2 with 5.0 M HCl. After pH adjustment, 0.75 mL of pepsin solution (1 g pepsin dissolved in 50 mL of 0.1 M HCl) was added to each tube. The extracts were adjusted to pH 6 with 0.1 M NaHCO3, then 3.75 mL of bile extract (0.05 g pancreatic and 0.3 g of bile extract in 35 mL of 0.1 M NaHCO3) was added to each extract; the extracts were again adjusted to pH 7 with 1 M NaOH, and finally 5 mL of 120 mM NaCl and 5 mL of 5 mM KCl were added to each extract. The final extract concentration was 20 mg/mL.

Analytical procedures

Total phenolics content (TPC) was estimated according to Singleton and Rossi (1965) and calculated as gallic acid equivalent (GAE) in mg/g dm.

Determination of antioxidant activity

For antiradical activity (AA) analyses, the improved 2,2'-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS) decolorization assay was performed according to Re et al. (1999). Chelating (CHEL) (Guo, Lee, Chiang, Lin, & Chang, 2001) and ferric reducing antioxidant power (FRAP) (Oyaizu, 1986) were also determined. The FRAP was calculated as the EC50 value (mg/mL; the effective concentration at which the absorbance was 0.5 for reducing power and was obtained by interpolation from linear regression analysis). Hydroxyl radical (OH) scavenging assay (Su, Wang, & Liu, 2009) was also performed. Antioxidant activities were determined as EC50 – extract concentration that provided 50% of activity based on a dose-dependent mode of action.

Theoretical approach

The active compounds bioaccessibility (ACB) index and the antioxidant bioaccessibility (BAC) index were determined to better understand the relationships between active compounds in the light of their potential bioaccessibility (Gawlik-Dziki et al., 2013).

The ACB index can be described as follows:

\[
ACB = \frac{C_{BE}}{C_{DE}}
\]

where:

- \(C_{BE}\) is the concentration of active compounds in the extract after digestion in vitro,
- \(C_{DE}\) is the concentration of active compounds in the buffer extract,

so that ACB \(\geq 1\) indicates high bioaccessibility, ACB value \(< 1\) indicates low bioaccessibility.

Statistical analysis

Statistical analysis was performed at a significance level α = 0.05 using Statistica by Statsoft (analysis of variance (ANOVA) and Tukey’s test).

Results and discussion

Chemical composition of raw materials

White rice flour was characterized by protein content of 7.1 ± 0.34%, carbohydrate content of 79.5 ± 3.58%, ash content of 0.28 ± 0.02%, and fat content of 0.7 ± 0.07%. Corn flour was characterized by protein content 5.8 ± 0.22%, carbohydrate content 78.2 ± 3.20%, ash content 0.44 ± 0.04%, and fat content of 2.9 ± 0.18%. The millet whole grain flour was characterized by protein content 9.4 ± 0.41%, carbohydrate content 72.6 ± 3.67%, ash content 2.0 ± 0.05%, and fat content 3.5 ± 0.09%. The brown algae powder was characterized by protein content 6.6 ± 0.31%, carbohydrate content 56.7 ± 0.31%, ash content 22.01 ± 0.12%, and fat content 3.1 ± 0.04%.

Physical and sensory properties of GFB with brown algae

The pH of dough and bread changed significantly as a result of algae addition (Figure 1a); this parameter increased with increasing in the content (from 4% to 10%) of brown algae. No significant differences were found in the pH of bread with 0% and 2% share of algae in bread formulation. In another study, the pH value of GFB with algae was not determined. It is important to determine the pH of the dough and bread. The pH of the dough affects the pH of the bread and this parameter determines the growth of...
yeast and other microorganisms. It also determines the taste of the bread as well as the suitability for long storage when the demand is lower. The results of the volume of loaves of GFB produced with different additions of brown algae in the range of 0–10% are shown in Figure 1(b). In comparison with the control bread (0% algae), a larger volume was obtained using 4% brown algae. No significant differences were observed in the volume of GFB with 4%, 6%, 8%, and 10% share of algae in bread formulation. The formation of gluten-free dough structure consists of many changes, such as hydration and swelling of various components of flour, fermentation and retention of CO₂ in a network formed during protein denaturation, and starch gelatinization (Diowksz, Sucharzewska, & Ambroziak, 2009). Some authors (Renzetti, Dal Bello, & Arendt, 2008) suggest that both the deamidation and cross-linking reaction might be involved in the improvement of the water-holding capacity of different gluten-free doughs.

In our studies, the addition of algae powder for 4% has significantly affected the reduction in bread volume, but that could have been caused by many other factors. Algae components, compared to the flour base, could undergo a slower rate and lesser degree of hydration, swelling, gelatination, and denaturation. A larger share (6–10%) of algae powder could cause interactions between algae and other recipe components, which may not allow for further reduction in the volume of bread. In some of the available studies, regarding GFB it was shown that the volume was not affected by the addition of up to 4% of algae (dried Spirulina platensis), but with the addition of 5% of algae a decrease of 22% in the volume of bread was noted down (Figueira et al., 2011). Saleh et al. (1987) observed favorable changes in the volume of wheat bread after adding 5 g algal protein to 100 g wheat flour. Enrichment with algal protein improved the rheological properties of dough, increased the gas retention capacity, and thereby led to increased loaf volume. In other studies, Scenedesmus acutus, a dried green alga added at levels of 5%, 10%, or 15%, reduced the volume of white wheat bread loaf slightly, but the same levels of Spirulina platensis reduced the loaf volume markedly. Loaf volume and porosity of wholemeal breads were not affected by algal additions (Nigam et al., 1985). In other studies, it was noticed that the volume of enriched breads was slightly influenced (reduced) by the 3% addition of spirulina. The decrease in the volume of bread was explained in this study by the action of the enzyme transglutaminase, which catalyzes the formation of the covalent bonds in proteins (Achour et al., 2014). Finney et al. (1984) observed that 10% replacement of wheat flour by algae Dunaliella significantly reduced the bread loaf volume.

With an increased content of brown algae, significant changes were noted down in the color of breadcrumb (Figure 2(a–c)). Lightness component L* decreased with the addition of brown algae in the range of 0–10% (Figure 2(a)). Parameter a* increased with the addition of brown algae in the range of 0–6% (Figure 2(b)). The highest redness (a*) of crumb color was achieved with 4% or 6% addition of brown algae (Figure 2(c)). A greater, 8–10%, brown algae addition influenced the parameter a* unfavorably, suggesting the exposure to the green color. Color b* value of the crumb (yellowness) decreased with increase in the content (from 0% to 10%) of brown algae.

Algae contain pigments that may affect crumb color changes. In our study the L*, and b* color parameters changed with increased addition of algae. In contrast, a unique relationship was obtained for parameter a*. Those changes...
Figure 3. Texture properties of gluten-free breadcrumb baked with different amounts of brown algae addition (a) firmness of breadcrumb, (b) elasticity of breadcrumb, (c) chewiness of breadcrumb.

FIGURA 3. Propiedades texturales de la molla del pan sin gluten horneado con diferentes cantidades de adición de algas pardas (a) firmeza de la molla del pan, (b) elasticidad de la molla del pan, c) masticabilidad de la molla del pan.

were related to the porosity of the crumb, with a larger amount of algae addition making the structure of bread more porous than a smaller amount of algae. In research presented by Nigam et al. (1985), use of algae (5%, 10%, and 15%) markedly colored the breadcrumb (gray with S. acutus, greenish-brown with blue-green alga S. platensis, dried green alga Scenedesmus acutus, and sun-dried S. platensis) of all test loaves. Achour et al. (2014) also noticed that the color of the fortified samples attained more green color as the fortification with spirulina was increased. Figueira et al. (2011) demonstrated a decrease in the luminosity with increase in the addition of the alga, and a displacement of the hue angle toward a green color.

With an increased content of brown algae, significant changes were observed in the textual properties of breadcrumb. The firmness (Figure 3(a)) decreased significantly with increasing algae addition (in the range of 0–10%), as compared to control bread. A lower degree of gluten-free crumb starching (change in crumb firmness) was also observed with increasing level of algae addition (Table 2). Elasticity increased with algae addition (Figure 3(b)). Compared with the control bread (0% algae), a larger elasticity was obtained while even using 2%, 4%, and 6% of brown algae. No significant differences were found in the elasticity of GFB with 2%, 4%, and 6% share of algae in bread formulation. The significantly greatest elasticity was obtained after using 8% and 10% of algae. Chewiness of crumb (Figure 3(c)) significantly decreased after adding 4% of brown algae. No significant differences were observed in the chewiness of GFB with 4%, 6%, 8%, and 10% share of algae in bread formulation. In some of the available studies, it was shown that the crumb hardness was not affected by the addition of up to 4% of algae (Figueira et al., 2011). Enrichment with algal protein after adding 5 g algal protein to 100 g wheat flour did not adversely affect the crumb texture (Saleh et al., 1987). In our research, a control GFB with no additives was characterized with high hardness. Addition of algae had a positive influence on reducing the hardness and the degree of staling of GFB, which could also be due to the presence of natural hydrocolloids in algae.

Sensory evaluation showed that control GFB was classified with the highest scores for taste (Table 2). Taste evaluation scores decreased with the addition of brown algae. An acceptable GFB can be obtained by adding 2% of the algae (no statistical difference with the control). Significantly lower evaluation results on the taste of bread was obtained by adding 4% (some consumers still accept it) of the algae, but the most unpleasant bread was obtained with greater levels of algae (6–10%). It was also demonstrated that the sensory evaluation of the crumb texture of GFB made with increasing additions of algae increased slightly but significantly. Finally, it was concluded that an acceptable GFB can be obtained by adding 2% of the algae.

In the study presented by Saleh et al. (1987), enrichment with algal protein after adding 5 g algal protein to 100 g wheat flour gave lower ratings for flavor. For the sensory evaluation, no significant difference was found between the bread with 3% and 5% of spirulina in the formulation (Figueira et al., 2011). The sensory analysis in another study (Achour et al., 2014) showed that bread with 1% of spirulina was the best and the bread with 3% was less appreciated because of the green color. Otherwise, all enriched bread presented a good global acceptability.

Table 2. Staling degree and sensory attributes of gluten-free bread made with the addition of brown algae.

| Amount of algae addition | Staling degree of bread | Taste | Flavor | Texture | Overall |
|--------------------------|-------------------------|-------|--------|---------|---------|
| 0                        | 99.1 ±4.78              | 6.2 ±0.51 | 6.5 ±0.33 | 5.1 ±0.31 | 5.5 ±0.58 |
| 2                        | 89.8 ±3.19              | 5.9 ±0.51 | 5.8 ±0.49 | 5.3 ±0.92 | 5.8 ±0.63 |
| 4                        | 75.3 ±3.76              | 4.6 ±0.50 | 4.9 ±0.65 | 6.1 ±0.55 | 4.8 ±0.39 |
| 6                        | 63.1h ±2.75             | 2.3 ±0.63 | 2.5 ±0.63 | 6.3h ±0.77 | 2.9 ±0.32 |
| 8                        | 61.9h ±1.62             | 1.4 ±0.21 | 1.9 ±0.31 | 6.5 ±0.61 | 1.6 ±0.25 |
| 10                       | 58.3 ±2.03              | 1.1 ±0.24 | 1.8 ±0.22 | 6.5 ±0.22 | 1.3 ±0.16 |

*S-Nine-point hedonic scale with 1, 5, and 9 representing extremely dislike, neither like nor dislike, and extremely like, respectively. Means with different letter in the same column are significantly different (p < 0.05).

* Escala hedónica de nueve puntos con 1, 5 y 9 representando disgustar extremadamente, ni gusta ni disgusta y gustar extremadamente. Los promedios con diferentes letras en la misma columna son significativamente distintos (p < 0.05).
**TPC and antioxidant activity of GFB with brown algae**

Many studies have demonstrated a high antioxidant potential of brown seaweeds (Auezova, Najjar, Selivanova, Moussa, & Assaf, 2013; Heo & Jeon, 2008; Senevirathne et al., 2006; Wang, Jónsdóttir, & Ólafsdóttir, 2009). The antioxidant activity is mainly due to the presence of phlorotannins, a major group of phenolic compounds in brown algae (Kumar et al., 2011). However, no works are present that focused on the antioxidant activity of bread supplemented with algae. As presented in Table 3, algae are good sources of buffer-extractable phenolics. Their level was not changed significantly after digestion, *in vitro*. On the contrary, GFB contains a low amount of hydrophilic phenolics; however, their level increased significantly after simulated digestion, which can be confirmed by the ACB value. From Table 3 it can be concluded that the addition of 2–% of algae had no significant effect on the TPC (vs. control). By the addition of 6% and above amount of algae, the enrichment in buffer-extractable phenolics was significant; however, the content of TPC was lower than expected. Contrary to the expectation, this tendency is not visible in extracts after *in vitro* digestion. This may be a result of the formation of protein-phenolic complexes. This hypothesis may be confirmed by ACB values – the highest was obtained for control bread.

Most importantly, algae were found to be a rich source of buffer-extractable and potentially bioaccessible compounds with AA (ABTS assay) compared to control bread. However, their potential bioaccessibility expressed in BAC was lower than those determined for the control bread. Algae addition significantly increased the antiradical potential of GFB. Most importantly, antiradical compounds from functional products were highly bioaccessible *in vitro*. This evidence may suggest their pro-health properties.

Both algae and control bread contained buffer-extractable compounds able to chelate metal ions. As expected, higher activity was determined for algae samples; however, active compounds derived from control bread were more bioaccessible *in vitro* than those derived from the functional supplement. Algae addition enriched GFB with chelating-active compounds, wherein the increase of activity was proportional to the amount of additive.

Interesting results were obtained for the ability of hydroxyl radical scavenging. Algae were a very rich source of...
consequences of such scavenging activity. Also in this case, simulated digestion did not cause its changes. More importantly, no effect of bread supplementation was found on the ability of OH radicals’ neutralization.

The proposed functional supplement and control bread contained buffer-extractable compounds with reducing ability (FRAP assay). However, the activity of the compounds derived from algae was significantly higher than those determined for the control sample. Algae addition significantly influenced the FRAP activity demonstrated by extracts obtained from bread. In buffer extracts, a positive correlation between FRAP activity and percentage of addition was found. Also with potentially bioaccessible compounds, a similar tendency was observed. Active compounds from the proposed functional product were highly bioaccessible in vitro.

Conclusions
The results of the study confirm the possibility of the use of brown algae in the production of GFB. In comparison with the control bread, a larger volume was obtained using 4% of brown algae. No significant differences were found in the volume of GFB with 4%, 6%, 8%, and 10% share of algae in bread formulation. With an increased content of brown algae, significant changes were observed in the color of the breadcrumb. Lightness and yellowness of breadcrumb decreased with the addition of brown algae. With an increased content of brown algae in bread recipe, the firmness decreased significantly compared to the control bread. A lower degree of crumb staling was also observed with increasing level of algae addition. Elasticity of breadcrumb increased with algae addition. Algae addition significantly increased the antiradical potential of GFB. Most importantly, antiradical compounds from functional products were highly bioaccessible in vitro. Finally, it was concluded that an acceptable GFB could be obtained by adding 2% or 4% of the algae.

Disclosure statement
No potential conflict of interest was reported by the authors.

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