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INVESTIGATION OF CHANGES IN ANTIOXIDANT ACTIVITY AND PROTEIN DIGESTIBILITY OF WHEAT BREADS INCLUDING *SPIRULINA PLATENSIS* AND PROTEIN EXTRACTS FROM *SPIRULINA PLATENSIS* DURING IN VITRO DIGESTION

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ABSTRACT
In this study, wheat breads including *Spirulina platensis* (SP) and protein extracts from *Spirulina platensis* (SPE) at levels of 0.125%, 0.25% and 0.50% were prepared and textural, volume, color and sensory properties were determined. Moreover, changes in total phenolic content (TPC), antioxidant activity (AOA) and in vitro protein digestibility (PD) of breads were investigated during in vitro digestion. The sample with the highest firmness value was control (919.4 g, $p \leq 0.05$). Breads with SP and SPE had higher volume than control ($p \leq 0.05$). The L* (52.2±1.0) and b* (18.9±0.2) values were the lowest for bread with SPE-0.25 ($p \leq 0.05$). The AOA of breads (SP-0.125, SP-0.5 and SPE-0.25) increased after in vitro gastric digestion ($p \leq 0.05$). The SPE-0.50 was the most preferable in terms of sensory properties. The SPE-0.125 had the highest TPC and breads including SPE at all levels had higher AOA than control and breads with SP after in vitro intestinal digestion ($p > 0.05$).
Keywords: *Spirulina platensis*, bread, enrichment, textural characteristics, phenolic content, sensory properties.

SPIRULINA PLATENSIS VE *SPIRULINA PLATENSIS* PROTEİN EKSTRAKTLARI İÇEREN BUĞDAY EKMEKLERİNİN IN VITRO SİNDİRİM SIRASINDA ANTİOKSİDAN AKTİVİTE VE PROTEİN SİNDİRİLEBİLİRLİĞİNDEKİ DEĞİŞİMİN ARAŞTIRILMASI

ÖZ
Bu çalışmada, %0.125, %0.25 ve %0.50 oranlarında *Spirulina platensis* (SP) ve *Spirulina platensis* (SPE)'den elde edilen protein ekstraktı içeren buğday ekmekleri hazırlanmış ve ekmeklerin tekstürel, hacim, renk ve duyusal özellikleri araştırılmıştır. Ayrıca, ekmeklerin in vitro mide-bağırsak sindirim sırasında toplam fenolik madde içeriği (TFM), antioksidan aktivite (AOA) ve protein sindirilebilirliğindeki (PS) değişim belirlenmiştir. En yüksek sıkılık değerine sahip örnek kontrol

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örneğiydi (919.4 g, p≤0.05). SP ve SPE ilaveli ekmekler kontrol örneğinden daha yüksek hacim değerlerine sahipti (p≤0.05). SPE-0.25 örneği en düşük L* (52.2±1.0) ve b* (18.9±0.2) değerlerine sahipti (p≤0.05). Ekmeklerin AOA'leri (SP-0.125, SP-0.5 ve SPE-0.25) in vitro mide sindiriminden sonra artmıştır (p≤0.05). SPE-0.50 duyusal özellikler açısından en çok tercih edilen ekmek olmuştur. Ayrıca, SPE-0.125 ilaveli ekmek, en yüksek TFM içermiştir ve in vitro bağırsak sindirimi sonrası, tüm oranlarda SPE içeren ekmekler kontrol ve SP ilaveli ekmeklerden daha yüksek AOA'ye sahip olmuştur (p>0.05).

Anahtar kelimeler: *Spirulina platensis*, ekmek, zenginleştirme, tektürel özellikler, fenolik madde, duyusal özellikler.

INTRODUCTION

Spirulina platensis is a multicellular, symbiotic and filamentous blue-green microalgae belonging to the Oscillatoriaceae family (Estrada et al., 2001). It shows greater growth in salty waters (>30 g/L) with high pH values (8.5-11.0) and in areas with high solar radiation levels. In addition to being an important natural protein source with a protein content of 60-70% in dry weight (dw), *Spirulina platensis* also contains omega-3 and omega-6 polyunsaturated fatty acids, essential amino acids, minerals, vitamins, pigments having antioxidative activity, and polysaccharides (Wang et al., 2007; Bermejo et al., 2008; Chamorro-Cevallos et al., 2008; Gad et al., 2011; El-Tantawy, 2015; Pelizer et al., 2015; Vo et al., 2016). In addition, it contains vitamins B1 (thiamine), B2 (riboflavin), B3 (nicotinamide), B6 (pyridoxine), B9 (folic acid), B12 (cyanocobalamin), C, D and E, as well as minerals such as potassium, calcium, chromium, copper, iron magnesium, manganese, phosphorus, selenium, sodium and zinc (Ghaeni and Roomiani, 2016).

Food fortification is accomplished by adding functional food components such as protein or micronutrients to food products in order to improve their nutritional properties. At this point, it is known that *Spirulina platensis* can be used for the enrichment of various food products due to its high protein content (Lee et al., 2011; Ak et al., 2016). For instance, Lee et al. (2011) used *Spirulina platensis* to produce functional bread. In the study of Lee et al. (2011), bread samples enriched with 0.8% *Spirulina platensis* were evaluated as better than the control group. In a study of Abd El Baky et al. (2015), biscuit products were enriched with purified phycocyanin obtained from *Spirulina platensis*. In the work of Gün et al. (2022), incorporation of 4% *Spirulina platensis* to biscuit resulted in an enhancement of protein. Furthermore, the best sensory color scores observed in biscuit containing 4% *Spirulina platensis*. Raczyk et al. (2022) prepared pasta by mixing semolina flour with an addition of 3, 5, 7, and 10% (w/w) of *Spirulina platensis* powder. According to results of their study, supplemented pastas had a significantly better amino acid profile and higher total fiber content than the control sample. Shahbazizadeh et al. (2015) investigated the effect of antioxidant and organoleptic properties of cookies fortified with *Spirulina platensis* biomass at 0, 0.5, 1 and 1.5% w/w. They found that iron, protein and γ-linolenic acid content of fortified cookies increased as a result of *Spirulina platensis* incorporation, coupled antioxidant properties and organoleptic properties of the cookies containing 1-1.5% *S. platensis* received highest scores (Shahbazizadeh et al., 2015).

Bread which is widely consumed all over the world, and other bakery products are the leading products food enrichment studies using natural nutrients with high nutritional properties such as *Spirulina platensis* (Ak et al., 2016; Niccolai et al., 2019; Montevecchi et al., 2022). Therefore, tha aims of the present study were to investigate the textural, volume, color and sensory properties of wheat bread with *Spirulina platensis* (SP) and *Spirulina platensis* protein extracts (SPE) at levels (0.125, 0.25 and 0.50%) and determine changes in total phenolic content, antioxidant activity and % protein digestibility during in vitro gastro-intestinal digestion.

MATERIAL AND METHODS

Materials

Spirulina platensis was obtained from Akuatik Fisheries and Cosmetics Products Ltd. (Adana,

Turkey). Folin Ciocalteu's reagent, hydrochloric acid, sodium hydroxide, ethanol, acetic acid, chloroform, *n*-hexane, sodium carbonate, (±)-6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox), copper (II) chloride solution, neocuprine, 2, 2-diphenyl-1-picrylhydrazyl, α-amylase, pepsin and pancreatin were obtained from Sigma-Aldrich Chemie GmbH (Steinheim, Germany). In this study, Type 2 bread wheat flour produced in accordance with the Turkish Food Codex Wheat Flour Communiqué to be used in bread making was obtained from Çandaroğulları Derya Flour and Feed Industry Trade Limited Company (Manisa, Turkey). The iodized table salt was purchased from Billur Salt Industry Joint Stock Company (İzmir, Turkey) and fresh yeast from Pak Food Production and Marketing Joint Stock Company (Kocaeli, Turkey).

Methods

Chemical composition

The moisture, ash, protein and lipid content of wheat flour (control), *Spirulina platensis*, *Spirulina platensis* protein extracts and wheat flours with *Spirulina platensis* and *Spirulina platensis* protein extracts were determined using the relevant AOAC (Association of Official Analytical Chemists) methods (AOAC, 1990). Carbohydrate content of the samples was calculated by subtracting 100 from the sum of protein, lipid, ash and moisture values. All measurements were performed in 3 repetitions.

Production of bread samples

Protein extracts from *Spirulina platensis* were obtained in our previous study (Yılmaz and Yucetepe, 2021). The formulations of control bread (without SP and SPE) and breads with SP and SPE are given in Table 1. *Spirulina platensis* and protein extracts obtained from *Spirulina platensis* were added to wheat flour at three different levels (0.125%, 0.25 and 0.50%) based in our previous study (Yılmaz and Yucetepe, 2021). Based on dough weight, SP and SPE ratios were 0.08%, 0.15% and 0.3%, when based on flour weight SP and SPE ratios 0.125%, 0.25% and 0.50% (Table 1). A flour-based bread formulation was formed with 59.9% water (v/w), 1.3% salt (w/w) and 2.96% yeast (w/w). According to the results of farinograph analysis in our previous study (Yılmaz and Yucetepe, 2021), ideal water absorption of the flour varied between 56.5 and 57.1% and it was close to the value of 59.9% water used in bread formulation. The water absorption amount of the flour increased from the water absorption value of the flour obtained by farinograph analysis, which ranged from 56.5-57.1%, to 59.9% due to salt and yeast in the bread formulations. This value was very close to the flour-based water ratio of 60% (v/w) in the bread formulation with *Spirulina platensis* in study conducted by İlhan et al. (2020).

Table 1: Formulations of control bread and breads with *Spirulina platensis* and *Spirulina platensis* protein extracts.

Ingradients	Breads						
	Control (%)	The breads with SP and SPE					
		SP-0.125 (%)	SP-0.25 (%)	SP-0.50 (%)	SPE-0.125 (%)	SPE-0.25 (%)	SPE-0.50 (%)
Wheat flour	60.9	60.8	60.7	60.6	60.8	60.7	60.6
SP	-	0.08	0.15	0.3	-	-	-
SPE	-	-	-	-	~0.1	~0.2	0.3
Water	36.5	36.5	36.5	36.5	36.5	36.5	36.5
Salt	0.8	0.8	0.8	0.8	0.8	0.8	0.8
Yeast	1.8	1.8	1.8	1.8	1.8	1.8	1.8

SP: *Spirulina platensis*, SPE: *Spirulina platensis* protein extract. SP-0.125, SP-0.25 and SP-0.50: Bread with 0.125%, 0.25% and 0.50% SP, respectively. SPE-0.125, SPE-0.25 and SPE-0.50: Bread with 0.125%, 0.25% and 0.50% SPE, respectively.

In the present study, the bread production method of İlhan et al. (2020) was used with slightly modifications. The breads were manufactured in factory of Erke Adk Industry Trade Ltd. in Istanbul, Turkey. All ingredients were mixed in accordance with the ratios given in Table 1 and dough was formed. The mixture was kneaded until it had a smooth surface. After the kneading process, 15 min of resting was applied for mass fermentation, and at the end of the time, the dough was cut into 270 g pieces and rolled by hand to obtain small pieces of dough. After the rounded dough pieces were rested for another 15 min, covered with a damp cloth, they were shaped in a shaping machine and fermented for 70 min at 30 °C and 80% relative humidity. The fermented doughs were baked at 230 °C for 14 min. The bread samples, which were cooled to room temperature after baking, were packed with aluminum foil and stored at -20 °C until analysis.

Determination of texture, volume and color characteristics

Textural analysis

Textural properties including firmness and elasticity of the breads samples were investigated using a TA.XTplusC Texture Analyzer (Stable Micro Systems Ltd., London, United Kingdom) with 25 mm aluminum cylindrical probe according to AACC method 74-09 (AACC, 1986). Briefly, 2 cm thick slices from the bread samples were compressed to 50% of their original height. The highest compression force obtained was expressed in gram as bread firmness. Measurements were carried out in 3 repetitions.

Volume analysis

The volume of the bread samples was determined by the Stable Micro Systems (Stable Micro Systems Ltd., London, United Kingdom) according to the rapeseed displacement principle according to AACC method no:10-05 (AACC, 2008). Specific volume of the bread samples was expressed as mL/g.

Color analysis

The color analysis of the bread samples was performed using a colorimeter (CR 400, Minolta, Japan). Color parameters are described as L* (brightness), a* (redness-greenness), b* (yellow-blueness).

In vitro gastrointestinal digestion

In vitro gastro-intestinal digestion of the control sample and the breads prepared with SP and SPE was carried out according to the Infogest method (Minekus et al., 2014). The digested samples obtained after stomach and intestinal digestion were stored at -80 ºC until analysis. Total phenolic content (TPC), antioxidant activity (AOA) and in vitro protein digestibility (PD) of the samples were determined after in vitro digestion process.

Determination of the total phenolic content

The TPC of the control sample and the bread samples before and after in vitro digestion was determined by the Folin-Ciocalteu method (Toor and Savage, 2006). In a summary, 200 µL of the sample was mixed with 1.5 mL of 10-fold diluted Folin-Ciocalteu reagent and 1.2 mL of 7.5% Na_2CO_3 solution, and its absorbance at 765 nm was read after 90 min of incubation. Results were expressed as mg gallic acid equivalents (GAE)/100 g dry weight (dw) (Toor and Savage, 2006).

Determination of antioxidant activity

Antioxidant activity of the samples were determined by 2,2-diphenyl-1-picrylhydrazil radical (DPPH) radical scavenging and copper (II) ion reduction antioxidant capacity (CUPRAC) methods before and after in vitro digestion. In the DPPH method, 2 mL of 0.1 mM DPPH (in methanol) was added to 100 µL of the sample (Kumaran et al., 2006). In the CUPRAC method, 1 mL of copper (II) chloride solution, 1 mL of neocuprine solution (in ethanol), 1 mL of ammonium acetate buffer solution (pH 7.0) and 1 mL of distilled water were added on 100 µL sample. The results obtained with the DPPH and CUPRAC methods were expressed as mg Trolox equivalents (TE)/100 g dw (Apak et al., 2004).

Determination of in vitro protein digestibility (%)

Protein content of the bread samples was determined spectrophotometrically by Lowry method (Lowry et al., 1951) after in vitro

digestion. Bovine serum albumin was used as a standard. The in vitro protein digestibility (%) of the digested samples was calculated using the following equation:

$$PD\ (\%) = \frac{P_r}{P_t} \times 100$$

PD: Protein digestibility, P_t: Total protein content and P_r: Protein content after in vitro digestion.

Sensory analysis

Sensory analysis of the bread samples were carried out by 11 trained panelists at Erke Adk Industry Trade Ltd. The samples were scored using a hedonic scale between 1-5 (5: very good, 4: good, 3: acceptable, 2: acceptable with difficulty, and 1: unacceptable) in terms of odor, crust color, crumb color, crumb pore size, bread pore homogenity, shape, volume, acceptability and preferability. Analysis were performed in triplicate.

Statistical analysis

The experimental results were analyzed using the Minitab Statistics Program (Minitab, Version 17, Minitab Inc., State College, Pennsylvania, USA). The differences between mean values were compared using Tukey test. Differences at $p \leq 0.05$ were considered to be significant.

RESULTS AND DISCUSSION

Chemical composition

Chemical compositons of wheat flour (control), *Spirulina platensis*, *Spirulina platensis* protein extracts and wheat flours with *Spirulina platensis* and *Spirulina platensis* protein extracts were given in Table 2. Moisture, protein, lipid, ash and carbohydrate content of wheat flour used in this work were calculated as 11.60±0.0%, 11.80±0.10%, 1.29±0.0%, 0.71±0.0% and 74.60±0.10%, respectively (Table 2). Similarly, Cansız (2020) found moisture, ash and protein content of wheat flour used in bread manufacture as 13.0%, 0.92% and 11.30%, respectively. Keskin and Evlice (2015) determined protein, ash, lipid and carbohydrate content of wheat flour as 10.5%, 1.8%, 2.6% and 78.6%, respectively.

Table 2: Chemical compositons of wheat flour (control), *Spirulina platensis*, *Spirulina platensis* protein extracts and wheat flours with *Spirulina platensis* and *Spirulina platensis* protein extracts.

Sample	Moisture (%)	Protein (%)	Lipid (%)	Ash (%)	Carbohydrate (%)
Control	11.60±0.0^{b}	11.80±0.10^{b}	1.29±0.0^{b}	0.71±0.0^{b}	74.60±0.10^{a}
SP	8.95±1.15^{c}	53.10±0.20^{a}	2.73±0.0^{a}	0.82±0.0^{a}	34.40±1.34^{b}
SPE	17.30±0.20^{a}	53.40±0.40^{a}	0.89±0.0^{c}	0.42±0.0^{c}	27.99±0.60^{c}
SP-0.125	12.30±0.20^{b}	12.10±0.50^{b}	1.30±0.0^{b}	0.71±0,0^{b}	73.59±0.70^{a}
SP-0.25	11.80±0.40^{b}	12.20±0.40^{b}	1.33±0.0^{b}	0.70±0.0^{b}	73.97±0.80^{a}
SP-0.50	12.00±0.0^{b}	12.00±0.0^{b}	1.34±0.0^{b}	0.71±0.0^{b}	74.75±0.00^{a}
SPE-0.125	11.20±0.0^{bc}	11.90±0.0^{b}	1.30±0.0^{b}	0.71±0.0^{b}	74.89±0.00^{a}
SPE-0.25	11.70±0.2^{b}	12.20±0.0^{b}	1.31±0.0^{b}	0.71±0.0^{b}	74.28±0.00^{a}
SPE-0.50	11.10±0.4^{bc}	12.00±0.20^{b}	1.30±0.0^{b}	0.71±0.0^{b}	74.89±0.60^{a}

Control: Wheat flour without SP or SPE. SP: *Spirulina platensis*, SPE: *Spirulina platensis* protein extract. SP-0.125, SP-0.25 and SP-0.50: Wheat flour including 0.125%, 0.25% and 0.50% SP, respectively. SPE-0.125, SPE-0.25 and SPE-0.50: Wheat flour including 0.125%, 0.25% and 0.50% SPE, respectively. Each value is given as mean±standard deviation (n=3). Different letters in the column indicate statistically ($p \leq 0.05$) difference by Tukey test.

In the present study, protein, carbohydrate, lipid and ash content of SP were 53.10±0.20%, 34.40±1.34%, 2.73±0.0% and 0.82±0.0%, respectively. Similarly, in the study conducted by Kargın-Yılmaz and Duru (2011), protein, carbohydrate, lipid, ash, and moisture content of SP were found 55-70%, 15-25%, 6-8%, 7-13% and 3-7%, respectively. In the present study, protein content of SP and SPE was found to be statistically higher than the protein content of other samples ($p \leq 0.05$). On the other hand, moisture content of SPE was higher than those of the other samples (17.3%, $p \leq 0.05$) because of water used as a solvent in the applied extraction

process. The sample with the highest lipid content (2.73%) was SP ($p \leq 0.05$). The ash content of the samples varied between 0.42% and 0.82%, and SP had higher ash content than the other samples (Table 2, $p \leq 0.05$).

Textural properties

The firmness and elasticity properties of the bread samples given in Table 3. The firmness values of the samples varied between 676.2 and 919.4 g ($p \leq 0.05$). The sample with the highest firmness value was the control sample (919.4 g, $p \leq 0.05$), while the samples with the lowest were SP-0.25 (676.2 g), SP-0.5 (696.2 g) and SPE-0.25 (714.0 g) ($p \leq 0.05$). It was observed that the bread samples to which SP and SPE were added had a less firm structure than the control samples ($p \leq 0.05$). Similar to these findings, Różyło et al. (2017) also found that as the amount of brown algae added to the breads increased, the firmness value of the breads decreased. On the other hand, the control sample had the highest elasticity value (49.6±0.0%), whereas the bread including 0.5% SP was the sample with lowest elasticity (47.7±0.1%) ($p > 0.05$). Likewise, in the study of Sanjari et al. (2018), elasticity property decreased significantly in samples with *Spirulina* compared to the control sample. However, in the study conducted by Różyło et al. (2017), compared with the control bread (0% algae), a larger elasticity was obtained while even using 2%, 4%, and 6% of brown algae. In our study, the effect of SP and SPE on the elasticity of the bread samples was not statistically significant ($p > 0.05$).

Table 3: The firmness, elasticity, volume and color values of the control and the bread samples with *Spirulina platensis* and *Spirulina platensis* protein extracts at levels of 0.125%, 0.25% and 0.50%.

Sample	Firmness (g)	Elasticitiy (%)	Volume (mL/g)	Color		
				L*	a*	b*
Control	919.4±34.7^{a}	49.6±0.0^{a}	5.2±0.1^{g}	60.8±0.4^{a}	4.4±0.5^{f}	20.8±0.2^{a}
SP-0.125	836.3±4.6^{ab}	48.1±0.3^{a}	5.3±0.0^{f}	57.5±1.4^{abc}	6.2±0.7^{b}	20.8±0.0^{a}
SP-0.25	676.2±33.4^{b}	47.9±1.5^{a}	5.7±0.1^{c}	60.5±1.7^{ab}	3.9±1.5^{g}	20.0±0.7^{ab}
SP-0.50	696.2±1.9^{b}	47.7±0,1^{a}	5.5±0.0^{e}	53.3±0.1^{bc}	4.5±0.3^{e}	19.0±0.0^{ab}
SPE-0.125	782.4±44.9^{ab}	47.8±0.2^{a}	5.8±0.0^{b}	55.3±3.1^{abc}	7.1±1.8^{a}	20.7±0.3^{ab}
SPE-0.25	714.0±51.1^{b}	47.8±0.4^{a}	5.5±0.1^{d}	52.2±1.0^{c}	5.2±0.4^{c}	18.9±0.2^{b}
SPE-0.50	764.6±36.7^{ab}	48.2±0.1^{a}	5.9±0.1^{a}	52.2±1.8^{abc}	5.1±1.2^{d}	19.1±0.0^{ab}

SP: *Spirulina platensis*, SPE: *Spirulina platensis* protein extract. SP-0.125, SP-0.25 and SP-0.50: Bread with 0.125%, 0.25% and 0.50% SP, respectively. SPE-0.125, SPE-0.25 and SPE-0.50: Bread with 0.125%, 0.25% and 0.50% SPE, respectively. Each value is given as mean±standard deviation (n=3). Different letters in the column indicate statistically ($p \leq 0.05$) difference by Tukey test.

Volume characteristics

As seen in Table 3, volume values of control and SP and SPE added breads samples varied between 5.2-5.9 g/mL. The breads with SP and SPE had higher volume values than the control samples (Table 2, $p \leq 0.05$). When the density and volume properties of the samples were evaluated together, it was seen that the samples with a firmer structure had a lower volume. For example, it was determined that the firmer control samples also had the lowest volume value among all samples (Table 3). Based on in our previous study (Yılmaz and Yucetepe, 2021), it can be said that the water holding capacity of the samples generally increased with the addition of SP and SPE, therefore the enriched bread samples had higher volume values. Similarly, compared to the control bread, a larger volume was obtained using 4% of brown algae *Ascophyllum nodosum* powder in gluten-free bread (Różyło et al., 2017). Unlike our study, Mamat et al. (2014) determined that bread volume decreased as the amount of added macroalgae increased in bread samples with macroalgae.

Color characteristics

As seen in Table 3, L*, a* and b* values of the samples differed with the addition of SP and SPE ($p \leq 0.05$). It was seen that L* values of the samples varied between 52.2 and 60.8 ($p \leq 0.05$). "Brightness" feature expressed by the L* value decreased with the addition of SP and SPE ($p \leq 0.05$). Similarly, Mamat et al. (2014) and Różyło et al. (2017) also found that the brightness value decreased with the addition of macroalgae to breads. The b* (yellow-blueness) value of the samples varied between 18.9-20.8 (Table 3). The L* (52.2±1.0) and b* (18.9±0.2) values were the lowest for the bread with SPE-0.25 ($p \leq 0.05$). The a* value of the samples, "redness-greenness", increased with the addition of SP and SPE, as expected, except for one sample (SP-0.25), due to the addition of green-colored SP and SPE ($p \leq 0.05$). Różylo et al. (2017) also reported an increase in a* value as the amount of brown algae added to the breads increased.

In vitro gastro-intestinal digestion of the breads

Changes in total phenolic content

The TPC of the samples before in vitro digestion ranged from 26.06±1.29mg GAE/100 g dw (SPE-0.50) to 31.69±0.09 mg GAE/100 g dw (SP-0.25) ($p > 0.05$, Table 4). The difference between TPC values of the bread samples with SP and SPE was insignificant ($p > 0.05$). Conversely, in the work of Egea et al. (2014), cookies enriched with *Spirulina platensis* had an increase of 64% of phenolic compounds in relation to the control. In the study of Fradinho et al. (2020), *S. platensis* provided a significant supplementation of phenolic compounds to the gluten-free pastas when compared to control (without *Spirulina platensis*). Furthermore, pasta with *Spirulina* exhibited high phenolic compounds content compared to control pasta without *Spirulina* (De Marco et al., 2014). In the study of Saharan and Jood (2017), the fortified breads with *Spirulina* powder at 6% had higher total phenolic contents. In our study, the differences in TPC between the control sample and the breads with SP and SPE were insignificant ($p > 0.05$) because SP and SPE were used at lower amount (0.125%, 0.25% and 0.50%) in bread formula compared to these studies in the literature (De Marco et al., 2014; Saharan and Jood, 2017).

Table 4: Changes in total phenolic content and antioxidant activity during in vitro gastrointestinal digestion of the control and the breads including *Spirulina platensis* and *Spirulina platensis* protein extracts at levels of 0.125%, 0.25% and 0.5%.

Sample	Total phenolic content (mg GAE/100 g dw)			CUPRAC (mg TE/100 g dw)		
	In vitro pre-digestion	Stomach phase	Intestinal phase	In vitro pre-digestion	Stomach phase	Intestinal phase
Control	30.96±0.65^{b,x*}	457.27±13.98^{a,x}	480.51±10.15^{a,x}	51.78±7.95^{a,x}	94.94±6.16^{a,xy}	110.75±14.30^{a,x}
SP-0.125	30.03±1.57^{b,x}	466.96±16.75^{a,x}	433.47±79.11^{a,x}	41.92±6.30^{c,x}	82.20±0.00^{b,y}	110.25±3.95^{a,x}
SP-0.25	31.69±0.09^{b,x}	493.95±44.85^{a,x}	445.33±45.15^{a,x}	43.84±0.00^{b,x}	114.25±5.75^{a,x}	73.51±7.81^{b,x}
SP-0.50	30.40±1.75^{b,x}	490.91±39.87^{a,x}	449.45±27.08^{a,x}	45.76±0.27^{b,x}	102.75±5.75^{a,xy}	90.53±2.80^{a,x}
SPE-0.125	29.20±1.11^{b,x}	438.99±11.77^{a,x}	551.11±71.89^{a,x}	36.17±2.19^{b,x}	114.25±0.82^{a,x}	114.47±10.82^{a,x}
SPE-0.25	29.02±1.48^{b,x}	409.92±53.58^{a,x}	505.43±59.93^{a,x}	40.00±0.55^{b,x}	101.92±6.58^{a,xy}	128.99±38.76^{a,x}
SPE-0.50	26.06±1.29^{b,x}	483.02±15.37^{a,x}	488.60±8.45^{a,x}	37.81±0.82^{b,x}	116.72±4.80^{a,x}	128.01±17.21^{a,x}

SP: *Spirulina platensis*, SPE: *Spirulina platensis* protein extract. SP-0.125, SP-0.25 and SP-0.50: Bread with 0.125%, 0.25% and 0.50% SP, respectively. SPE-0.125, SPE-0.25 and SPE-0.50: Bread with 0.125%, 0.25% and 0.50% SPE, respectively. Each value is given as mean±standard deviation (n=3). *: Means with the same letter within a line (a,b,c) and column (x,y,z) are significantly different by Tukey test ($p \leq 0.05$).

In the present study, TPC of all samples was significantly higher after in vitro gastric and intestinal digestions than pre-digestion samples ($p \leq 0.05$). Similar to our study, in a study of Chen et al. (2014), while TPC and antioxidant activity of some of 33 different fruits decreased after in vitro digestion, some increased. Moreover, SPE-0.125 had the highest TPC (551.11±71.89 mg

GAE/100 g dw), whereas SP-0.125 was the bread sample including the lowest TPC (433.47±79.11 mg GAE/100 g dw). Many literature data (e.g. Abdelhamid et al., 2018, Drevelegka and Goula, 2020; Rebollo-Hernanz at al., 2021) on food polyphenols relate only to compounds soluble in aqueous organic extracts, but especially polyphenols with a high degree of polymerization and associated with high molecular weight compounds can not be extracted with the standard extraction methods and solvents used. Therefore, this may be the reason why the total phenolic content was low in vitro pre-digestion. In the present study, TPC increased during in vitro digestion because these polyphenolic fractions may become bioactive after release from the food matrix by digestive enzymes in the small intestine and degradation in the large intestine (Jenner et al., 2005).

Changes in antioxidant activity

Before in vitro digestion, AOA of the samples by CUPRAC method changed from 36.17±2.19 TE/100 g dw (SPE-0.125) to 51.78±7.95 mg TE/100 g dw (control) and addition of SP and SPE to breads did not cause a significant change in antioxidant activity of the bread samples because of the use of SP and SPE at low concentrations in bread formulations ($p>0.05$, Table 4). On the other hand, in the work of Zlateva et al. (2020), with an increased *S. platensis* concentration in wheat bread, significant changes were noted in antioxidant activity. In the present study, after in vitro gastric digestion, AOA of SP-0.25 (114.25±5.75 mg TE/100 g), SPE-0.125 (114.25±0.82 mg TE/100 g) and SPE-0.50 (116.72±4.80) was higher than other samples ($p\leq0.05$). Moreover, antioxidant activity of all bread samples with SP and SPE, except control sample, increased after in vitro gastric digestion ($p\leq0.05$). The breads including SPE at all levels had higher AOA than the control and the bread with SP after in vitro intestinal digestion ($p>0.05$). On the other hand, compared to undigested samples, AOA of the bread samples, except bread with SP-0.125 and the control sample, increased after in vitro intestinal digestion ($p\leq0.05$). Similarly, Chen et al. (2014 and 2015) also reported an increase in the antioxidant activity of some fruits after in vitro digestion.

In the present study, AOA of the samples by DPPH method could not be determined because the samples did not respond to DPPH radical. According Apak et al. (2007) and Çekiç et al. (2009), DPPH is more suitable for lipophilic antioxidants in organic solvent media. Moreover, in the study of Chen et al. (2015) and Puangkam et al. (2017), DPPH value of some vegetables decreased after in vitro gastrointestinal digestion, and it was stated that the reason for this decrease was the decrease in the biological reactivity of compounds with antioxidant activity under the intestinal digestive conditions where the pH value is approximately 7.4.

Change in protein digestibility (%)

The amount of protein before in vitro gastro-intestinal digestion of the samples was changed between 1.81 mg/g dw and 2.19 mg/g dw ($p>0.05$, Figure 1). After in vitro gastro-intestinal digestion, protein amounts of the samples varied between 11.86 mg/g dw and 12.30 mg/g dw ($p>0.05$, Figure 1). Similar to the findings obtained in the study, S´wieca et al. (2013) revealed that in vitro pre-digestion protein content of bread samples enriched with onion skin was found to be 13.30 mg/g in the lowest for control sample. In the same study, the lowest protein content in the bread samples after in vitro digestion was determined as 2.88 mg/g protein (S´wieca et al., 2013). In our study, protein digestibility (%) of the samples varied between 81.86% and 85.02% ($p>0.05$, Figure 1). On the other hand, these values were higher than protein digestibility (%) values (55.00-78.35%) of the bread samples enriched with onion skin investigated by S´wieca et al. (2013). Conversely, in the study of Saharan and Jood (2017), the fortified breads with *Spirulina* powder at 6% had higher in vitro protein digestibility. According to De Marco et al. (2014), protein content of bread wheat pasta incorporated with *Spirulina* resulted increased while its protein digestibility was reduced as microalgae content increased.

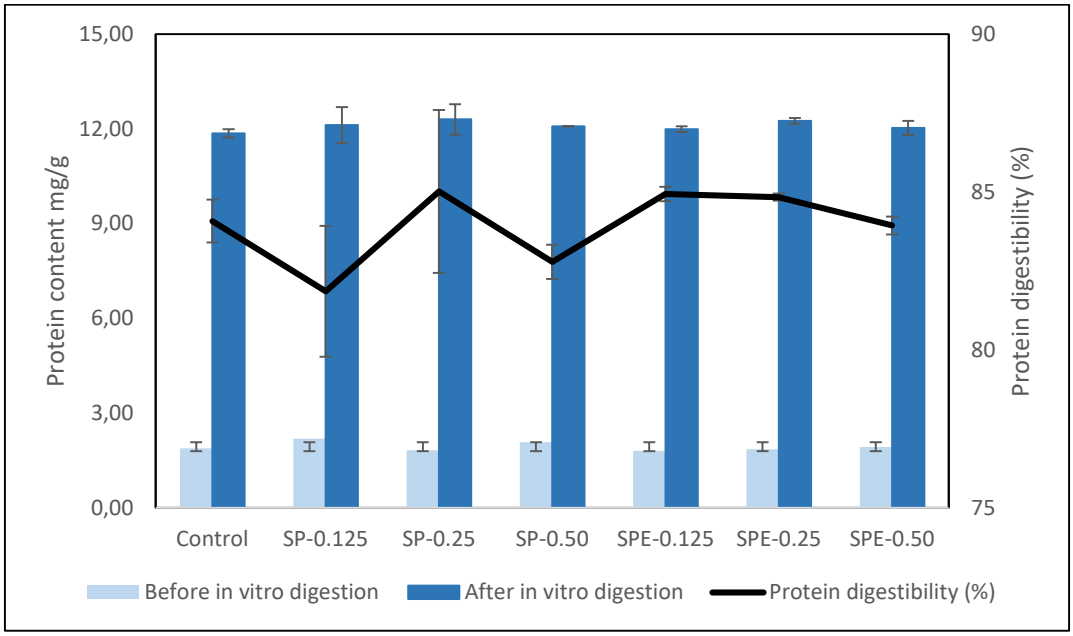

Figure 1: Change in protein content and % protein digestibility during in vitro digestion of the breads in which control and *Spirulina platensis* and *Spirulina platensis* protein extracts.

SP: *Spirulina platensis*, SPE: *Spirulina platensis* protein extract. SP-0.125, SP-0.25 and SP-0.50: Bread with 0.125%, 0.25% and 0.50% SP, respectively. SPE-0.125, SPE-0.25 and SPE-0.50: Bread with 0.125%, 0.25% and 0.50% SPE, respectively. Each value is given as mean±standard deviation (n=3).

Sensory analysis

The results of sensory analysis of the control and the bread samples including SP and SPE in terms of odour, crust and inner color, crumb pore size and homogenity, shape, volume, acceptability and preferability characteristics are shown in Table 5. Visual appearances of the bread samples are shown in Figure 2. The SP-0.50 was scored by the panelists with the lowest odor value (2.8±0.2) and the difference between the odor characteristics of the samples was statistically significant ($p \leq 0.05$). The highest odour score among the samples was 3.6 for the control sample, SPE-0.125 and 3.5 for SPE-25 and SP-0.125. The sensory properties and consumption of algae are restricted due to their fishy odor. As an interesting result in this study, all bread samples with SP and SPE, except SP-0.50, were evaluated to have odour characteristics close to the control sample by the panelists. While the difference between the crumb colors of the samples was not significant ($p > 0.05$), SPE-0.50 had a better crust color than the other samples ($p \leq 0.05$). Pore size values of bread crumb ranged from 2.3 to 3.8, and the highest score was given to SPE-0.50 ($p \leq 0.05$). Similarly, crumb pore homogenity of SPE-0.50 (3.8), crumb pore homogenity (3.6), bread shape (4.2), bread volume-swelling (4.4) scores were statistically significantly higher than the other bread samples ($p \leq 0.05$). The acceptability scores of the samples were lowest for SP-0.50 with 2.7, and the highest for SPE-0.50 with 3.9 ($p \leq 0.05$). When the control and the bread samples including SP and SPE were evaluated together with all the sensory properties, the most preferred bread sample by the panelists was SPE-0.50% with a score of 3.6±0.1. In the study of İlhan et al., (2020), according to the sensory analysis results of the bread samples with *Spirulina platensis* added in the ratios of 0.1%, 0.5%, 1.0%, 3.0%, it was determined that SP-0.1% and 0.5% added samples found to be as acceptable.

The bread sample with SPE-0.50 had higher scores in terms of crust color (3.9±0.1), crumb color (3.4±0.1), crumb pore size (3.8±0.2), crumb pore homogenity (3.6±0.0), bread shape (4.2±0.2), bread volume (4.4±0.0), acceptibility (3.6±0.1) and preferability (3.9±0.1) ($p \leq 0.05$, Table 5). In our study, *Spirulina platensis* and

protein extracts from *Spirulina platensis* did not cause any negative effect on sensory properties of the breads since SP and SPE were used at low concentration compared to the literature (De Marco et al., 2014; Saharan and Jood, 2017; Sanjari et al., 2018). On the other hand, Sanjari et al. (2018) reported that samples with *Spirulina* powder received the lowest sensory properties.

Table 5: Sensory analysis of control and bread samples with *Spirulina platensis* and *Spirulina platensis* protein extracts in terms of odour, crust and crumb color, crumb pore size and homogenity, shape, volume, acceptability and preferability.

Sample	Odour	Color		Crumb pore size	Crumb pore homogenity	Bread shape	Bread volume	Acceptibility	Preferability
		Crust	Crumb						
Control	3.6±0.0^{a}	2.5±0.1^{b}	3.1±0.1^{a}	2.3±0.2^{b}	2.4±0.1^{b}	3.1±0.2^{b}	2.3±0.2^{c}	3.1±0.2^{ab}	2.5±0.1^{d}
SP-0.125	3.5±0.0^{a}	2.6±0.0^{b}	3.1±0.2^{a}	2.7±0.1^{b}	2.6±0.0^{b}	3.3±0.1^{b}	2.6±0.1^{c}	3.3±0.1^{ab}	2.8±0.0^{cd}
SP-0.25	3.3±0.2^{ab}	3.1±0.2^{ab}	3.2±0.3^{a}	2.9±0.1^{b}	2.8±0.2^{b}	3.4±0.0^{ab}	2.8±0.1^{c}	3.1±0.2^{ab}	2.8±0.1^{cd}
SP-0.50	2.8±0.2^{b}	3.4±0.1^{ab}	3.0±0.1^{a}	2.7±0.1^{b}	2.7±0.1^{b}	3.1±0.1^{b}	3.0±0.2^{bc}	2.7±0.2^{b}	2.4±0.0^{d}
SPE-0.125	3.6±0.1^{a}	3.0±0.2^{ab}	3.4±0.1^{a}	2.7±0.1^{b}	2.7±0.2^{b}	3.3±0.1^{b}	2.8±0.0^{c}	3.2±0.0^{ab}	3.1±0.1^{bc}
SPE-0.25	3.5±0.0^{a}	3.4±0.3^{ab}	3.4±0.1^{a}	3.2±0.1^{ab}	3.1±0.2^{ab}	3.7±0.2^{ab}	3.7±0.2^{ab}	3.5±0.1^{ab}	3.4±0.0^{ab}
SPE-0.50	3.4±0.1^{ab}	3.9±0.1^{a}	3.4±0.1^{a}	3.8±0.2^{a}	3.6±0.0^{a}	4.2±0.2^{a}	4.4±0.0^{a}	3.9±0.1^{a}	3.6±0.1^{a}

SP: *Spirulina platensis*, SPE: *Spirulina platensis* protein extract. SP-0.125, SP-0.25 and SP-0.50: Bread with 0.125%, 0.25% and 0.50% SP, respectively. SPE-0.125, SPE-0.25 and SPE-0.50: Bread with 0.125%, 0.25% and 0.50% SPE, respectively. Each value is given as mean±standard deviation (n=3). Different letters in the column indicate statistically difference by Tukey test ($p \leq 0.05$).

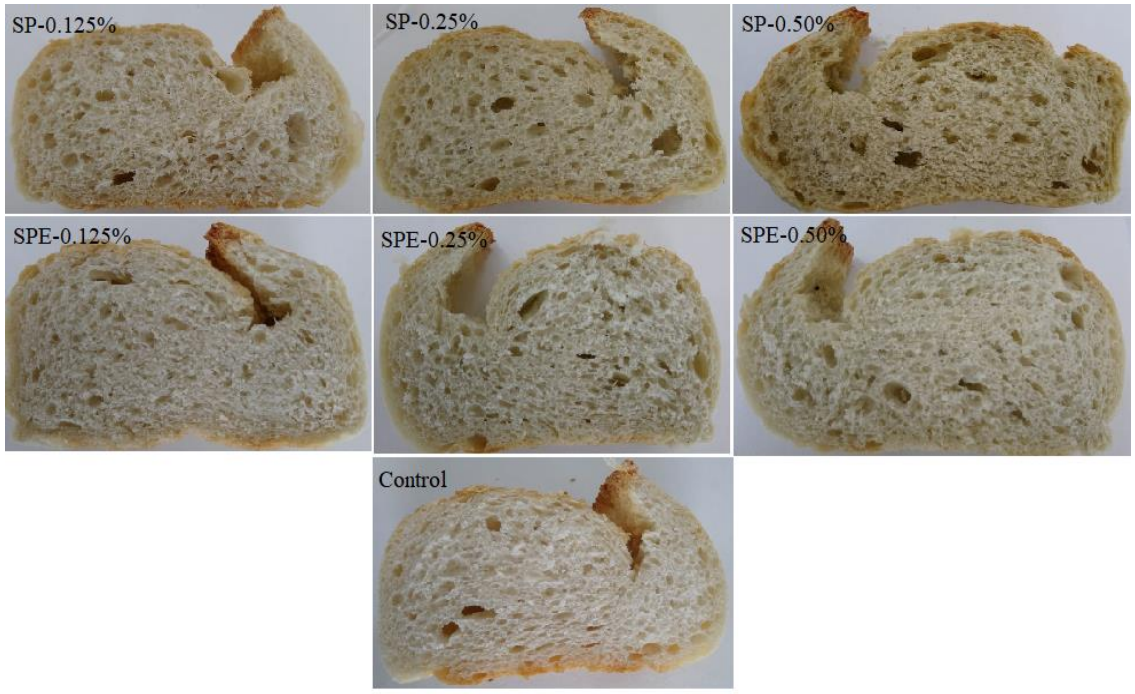

Figure 2: Visual appearances of the breads in which control and *Spirulina platensis* and *Spirulina platensis* protein extracts.

SP: *Spirulina platensis*, SPE: *Spirulina platensis* protein extract. SP-0.125, SP-0.25 and SP-0.50: Bread with 0.125%, 0.25% and 0.50% SP, respectively. SPE-0.125, SPE-0.25 and SPE-0.50: Bread with 0.125%, 0.25% and 0.50% SPE, respectively.

CONCLUSION

Spirulina platensis microalgae is an important food and food ingredients source due to its high protein content. In this study, firstly, the textural, volume, sensory and color properties of the bread samples with *Spirulina platensis* and *Spirulina platensis* protein extracts, as well as the change in total phenolic content, antioxidant activity and % protein digestibility during in vitro digestion were investigated. According to the results of the texture analysis, it was determined that the bread samples with SP and SPE had less firm structure and higher volume value than the control sample. In addition, the bread samples with SPE were found to be more preferable in terms of sensory properties than other bread samples. The L* and b* values were the lowest for the bread with SPE-0.25. Moreover, SPE-0.125 had the highest TPC and the breads including SPE at all levels had higher AOA than the control and the bread with SP after in vitro intestinal digestion. On the other hand, addition of *Spirulina platensis* or protein extracts obtained from *Spirulina platensis* to the bread samples did not statistically cause any change on total phenolic content, antioxidant activity and % protein digestibility of the samples. Therefore, in future studies, it may be aimed to improve the rheological, techno-functional and sensory properties of the bread samples prepared with the addition of *Spirulina platensis* and *Spirulina platensis* protein extracts at different rates, as well as to increase antioxidant activity in particular.

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CONTRIBUTIONS OF ALL AUTHORS

Aysun Yucetepe designed the study and critically reviewed the manuscript. All analysis were carried out by Meltem Yılmaz. The authors analyzed the data, wrote the manuscript and approved the final manuscript. This study is part of Meltem Yılmaz's master's thesis enitled "*Spirulina platensis* protein ekstraktları ile zenginleştirilmiş buğday unundan üretilen hamur ve ekmeğin reolojik ve bazı biyoaktif özellikleri"

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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