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Research Article

# Effect of *Spirulina platensis* on Textural, Sensory and Some Physicochemical Characteristics in Gluten-free Functional Biscuit Production

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**ABSTRACT**

This study aims to investigate the impact of *Spiriluna platensis* powder (SPL), from microalgaes, on the technological, nutritional, and sensory properties of gluten-free functional biscuits. Biscuits were formulated with different rates of SPL powder (0%, 0.5%, 1%, and 5%) and evaluated for their physical, chemical, and sensory attributes. The inclusion of SPL significantly increased the protein, dietary fibre, phenolic, and antioxidant content of the biscuits. Biscuits with 5% SPL showed the highest levels of protein (8.01%), phenolics (27.8%), antioxidants (64.18%), and dietary fibre (2.1%). The addition of SPL also affected the hardness, brittleness and colour of the biscuits, resulting in darker and harder biscuits at higher SPL ratios. Sensory analysis revealed that biscuits with 0.5% SPL were most preferred, with higher SPL rates leading to a decrease in sensory scores.

**Keywords:** *Spiriluna platensis*, sensory properties, antioxidant, gluten-free, biscuits

# Glutensiz Fonksiyonel Bisküvi Üretiminde *Spirulina platensis*'in Tekstürel, Duyusal ve Bazı Fizikokimyasal Özellikler Üzerine Etkisi

**ÖZ**

Bu çalışma, mikroalglerden *Spiriluna Platensis* tozunun (SPL) glutensiz fonksiyonel bisküvilerin teknolojik, besinsel ve duyusal özellikleri üzerindeki etkisini araştırmayı amaçlamaktadır. Bisküviler farklı oranlarda SPL tozu (%0, %0,5, %1 ve %5) ile formüle edilmiş ve fiziksel, kimyasal ve duyusal özellikleri açısından değerlendirilmiştir. SPL'nin eklenmesi bisküvilerin protein, diyet lifi, fenolik ve antioksidan içeriğini önemli ölçüde arttırdı. %5 SPL'li bisküviler en yüksek düzeyde protein (%8,01), fenolikler (%27,8), antioksidanlar (%64,18) ve diyet lifi (%2,1) gösterdi. SPL ilavesi aynı zamanda bisküvilerin sertliğini, kırılganlığını ve rengini de etkileyerek daha yüksek SPL oranlarında daha koyu ve daha sert bisküvilerin oluşmasına neden olmuştur. Duyusal analiz, %0,5 SPL'li bisküvilerin en çok tercih edildiğini, daha yüksek SPL oranlarının duyusal puanlarda düşüşe yol açtığını ortaya çıkardı.

**Anahtar Kelimeler:** *Spiriluna platensis*, duyusal özellikler, antioksidan, glutensiz, bisküvi

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# Effect of *Spirulina platensis* on Textural, Sensory and Some Physicochemical Characteristics in Gluten-free Functional Biscuit Production

## Introduction

Nowadays as a result of the current increase in diseases like celiac, obesity, diabetes and cancer, there is a higher trend toward functional foods beneficial to health in diets (Arab et al., 2019; Koh et al., 2020; Verbeke, 2005; Wirkijowska et al., 2018) Biscuits are snacks with high popularity, reaching a large consumer audience as a result of not going stale for long durations, being quick to eat, easily accessible and economic (Di Cairano et al., 2018; Sudha et al., 2007; Wesley et al., 2021). Biscuits sold in the market are generally based on cereals containing gluten (Rai et al., 2014; Wesley et al., 2021). However, as they may be formulated with high-value compounds and different flours, they have the possibility to be included in diets for different diseases by providing more choice and diversity. Individuals with celiac disease and other gluten-derived diseases (dermatitis herpetiformis, gluten ataxia and wheat allergy) (Di Cairano et al., 2018; Foschia et al., 2016) must eat a gluten-free diet for life. Most of these diets include products with high carbohydrate content, low protein content and nutritional deficiencies (Di Cairano et al., 2018; Fasano & Catassi, 2012; Fassano & Catassi, 2012; Jnawali et al., 2016; Leonard et al., 2017; Naqash et al., 2017). The use of cereals not containing gluten in bakery products causes problems with production and negative consumer approval (Di Cairano et al., 2018). Among gluten-free products, rice flour is easily digestible, involves low allergic reactions, is tasty according to consumers and can be produced from broken rice waste, which makes it indispensable as a resource in terms of raw material and sustainability (Kim & Shin, 2014). To overcome the nutritional deficiencies of functional compounds, the development of health and technological features appears to be an important tool (Abdo et al., 2021; Alongi et al., 2019; Pasqualone et al., 2015). In new research studies, they have referred to foods that have high nutritional values, as well as potential health benefits, and even reduce the risk of degenerative diseases by improving physical and emotional health, as superfoods (Gupta & Mishra, 2021). *Spirulina platensis* has been called a superfood (Jung et al., 2019) due to its superior protein (essential amino acids), essential fat acids, vitamin and mineral content, as well as its versatile use properties, such as anti-viral, anti-bacterial, antioxidant, anti-diabetic, anti-cancer and anti-inflammatory effects (Ahsan et al., 2008; Jung et al., 2019; Marles et al., 2011; Menegotto et al., 2019). Additionally, *Spirulina platensis* has been recognized as a nutritious food by the United Nations World Food Conference (Jung et al., 2019). *Spirulina platensis* contains many natural bioactive compounds with high value and antioxidant capacity and has been the target of studies on the production of new foods and functional food components (Batista et al., 2017; Batista et al., 2012; Gouveia et al., 2007; Singh et al., 2015). In recent years, microalgae have been applied as an additive in many vegan or gluten-free products such as biscuits, pasta, sauces and jelly desserts. It has been stated that in addition to increasing the nutritional properties of the food it is added to, it makes positive contributions to the textural, colour and sensory properties (Fradique et al., 2010; Liber et al., 2020; Ramírez-Rodrigues et al., 2021; Thirumdas et al., 2018). Additionally, microalgae have strong survival ability against negative conditions in nature and are natural economic resources (Chen et al., 2022) that can be produced with high yield (Chen et al., 2022; Levasseur et al., 2020). This study, aimed to provide gluten-free food consumers with alternative biscuits with improved functional, nutritional and quality properties from rice flour enriched with *Spirulina platensis* powder (SPL), which has high nutritional value. The effect of commercial *Spirulina platensis* powder added to rice flour at different rates (0%, 0.5%, 1% and 5%) on the technological, nutritional and sensory properties of biscuits will be investigated. The effects of SPL addition on protein, dietary fibre, phenolics, antioxidants, hardness, brittleness and colour were statistically analyzed.

## Material and Method

### Material

Rice flour (Sade Organik Ürünler San. Ve Tic. A.Ş., İstanbul), butter (Pervin, Seksüt Endüstrisi Kurumu A.Ş, Bursa), L-size eggs (Bili bili, Verim Gıda Ürünleri San. Ve Tic. A.Ş, Bursa), baking powder (Destan, Sena Muhtelif Ürün used for production of the biscuits. During production, a dough kneading machine (Öztiryakiler Gurmeaid), and an oven (Svabe Dahlen, Sweeden) were used.

### Experimental Design

Biscuit dough was made by adapting the method of Rahman et al. (2020). Biscuits were produced, with one set of biscuits having no supplementation (0% control sample series), while the other sets had 0.5%, 1% and 5% *Spirulina platensis* powder (SPL). It is made by replacing 100 g of rice flour with 0.5%, 1% and 5% SPL in biscuit production. It consists of 40 g of margarine, 36 g of sugar, 2 g of baking powder, 2 g of cinnamon, 10 mL of milk and 10 g of egg. Depending on the *Spirulina platensis* powder ratio, biscuits containing SPLC 100% rice flour, 0% *Spirulina platensis*, SPL1 0.5% *Spirulina platensis*, SPL2 1% *Spirulina platensis*, SPL3 5% *Spirulina platensis* were produced as a substitute for rice flour. The dough was shaped with the aid of a cookie cutter and then baked at an appropriate temperature and duration (165±5°C 17 min) with the aid of an

Paketleme Gıda San ve Tic. Ltd. Şti., İstanbul), cinnamon (Destan, Seğmen Kardeşler Gıda Üretim ve Ambalaj Sanayi A.Ş., Ankara), milk (Dost, Aynes Gıda, Denizli), powdered sugar, and *Spirulina* (Aktarix) were

oven (Svabe Dahlen, Sweden). For biscuit production, the most appropriate sugar, rice flour, cinnamon, *Spirulina platensis*, baking powder, egg and butter proportions were calculated and used.

### Biscuit Production

Rice flour, *spirulina* powder and baking powder were taken into a mixing bowl (Kitchen Aid, UK), then milk, egg, sugar and cinnamon were added and mixed first at speed 1 for approximately 200 seconds, then at speed 2 for 50 seconds to obtain the dough. The resulting dough was rested for approximately 5 minutes. The rested biscuit dough was placed on the marble counter and the rolling pin was opened to 4 mm. The rolled-out dough was made round using a 60 mm diameter mould. Baked in the oven (Svabe Dahlen, Sweeden) at 170±5 °C for 17 min. The baked biscuits were cooled for about 2 hours and packed in polypropylene bags. Sensory analysis was performed immediately after cooling.

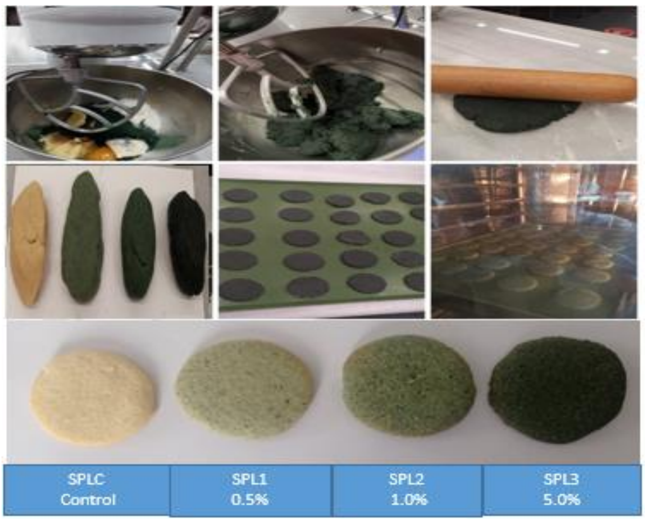


**Figure 1.** Control biscuit and biscuit samples with 0.5%, 1% and 5% (w/w) *Spirulina platensis*

**Physical Analyses**

The thickness of the gluten-free biscuit samples was measured with a digital calliper (0.001 mm Mitutoyo, Tokyo, Japan) according to the AACC Standard Method No: 10-54.01 principle, while the diameter was measured with a ruler. After measuring the diameter and thickness values (mm) of the biscuit samples, the spread rate of the biscuits was found by dividing the diameter by the thickness. The results for diameter, thickness and spread ratio were calculated by taking the average of 5 measurements (Committee, 2000).

**Chemical Analyses**

Humidity analysis of the biscuits was completed according to the method (Committee, 2000). The moisture analyzer (AND MX-50, Japan) was operated by selecting the 105 ℃ for 25 min. method. At the end of the duration, the % humidity result on the device was recorded. Ash analysis was completed according to AACC Method No: 08.01 (Committee, 2000). Ash amounts of biscuit samples were given as %ash. Protein amount detection was performed according to the Kjeldahl AACC Method No: 46-11A (AACC, 2000). After completing incineration, the Kjeldahl balloons were left to cool. The distillation process was completed for 10 min. in an Erlenmeyer flask with 3% boric acid and 33% NaOH solution. At the end of distillation, the solution in the Erlenmeyer flask was titrated with a burette filled with 0.1 N HCl solution. The results were inserted in the % raw protein formula to identify the protein proportion in samples. The nitrogen factor was calculated using 5.70.

**Fat Analyses**

The oil content analyses of the biscuit samples were performed using a Soxhlet extraction system (Extraction unit, E-816, Buchi, Flawil, Switzerland) in which hexane was used as a solvent. The oil content of the biscuit samples was expressed as % fat using the following equation.

$$\text{Fat}\ (\%) = (\text{m2} - \text{m1}) * 100/m2$$

m1: Weight of erlenmeyer flask
m2: Weight of the erlenmeyer flask +oil
m: Weight of samples

**Extraction of Biscuit Samples**

Biscuit samples were ground thoroughly with an electric grinder. Powdered biscuit samples were mixed with methanol solution (70%) (weight: volume 1:10) and homogenized by ultraturax for 5 min. Bioactive components of the samples were extracted by keeping them at room temperature and in the dark for 2 hours and centrifuged at 6000 rpm for 10 min. The supernatant was removed and stored at +4°C for analysis (Kaur & Kaur, 2018).

**Determination of Total Phenolic Content (TPC)**

After diluting 0.5 mL of sample extract with distilled water in appropriate proportions, it was mixed with 2.5 mL of Folin–Ciocalteu reagent (0.2N) and 2 mL of $Na_2CO$ (7%) and kept at 25 °C in the dark for 30 min. The TPC of the samples was calculated by reading the absorbances with a spectrophotometer (Shimadzu UV 1800; Kyoto, Japan) set at a wavelength of 760 nm. TPC results are expressed as mg gallic acid equivalents (GAE) per 100 grams of sample with a linear standard curve range ($r^2$ =0.994) (Singleton et al., 1999).

**Antioxidant activity assays**

**DPPH (1,1-Diphenyl-2-picryl-hydrazyl) Methods**

0.1 mL of sample was mixed with 4.9 mL of DPPH solution (0.1 µM in methanol) and kept in the dark at 27°C for 20 min. Its absorbance was then determined using a UV spectrophotometer at a wavelength of 517 nm (Brand-Williams et al., 1995). DPPH calculation was made according to the following formula:

$$DPPH\ Radical\ Inhibition(\%) = \frac{\text{Ac}-\text{As}}{\text{Ac}} * 100$$

$A_C$: Absorbance of control, As: Absorbance of sample

**FRAP (Ferric Reducing Antioxidant Power) Assay**

For the study FRAP reagent, 300 mM acetate buffer (pH 3.6), 10 mM TPTZ (2,4,6-tripyridyl-s-triazine) and 20 mM FeCl3.6H2O and C, called solutions A, B, were prepared respectively. These solutions were then mixed in a ratio of A:B:C to 10:1:1. After 100 µL sample extract, 900 µL distilled water and 2 mL working FRAP reagent were thoroughly mixed and kept in the dark for 30 min., the absorbances were read at 593 nm wavelength. FRAP results of the extracts were expressed as mg Trolox equivalent antioxidant capacity (TEAC)/100g sample ($r^2$ =0.992) (Benzie & Strain, 1996).

**Dietary Fibre**

Total dietary fibre (TDF) was determined according to AOAC Method 985.29 (Dodevska et al., 2013) on duplicate samples of dried and defatted (if fat content >10%) material. Samples were fired at 100 °C; incubated with protease (to solubilize and depolymerize proteins) and amyloglucosidase (to hydrolyze starch fragments to glucose) at 60 °C; and 4 volumes of ethanol were added to precipitate soluble fibre. The residue is filtered; washed with 78% ethanol, 95% ethanol, and acetone; dried and weighed. One copy is analyzed for protein and the other is incubated at 525 °C to determine ash. TDF was obtained by subtracting the weight of protein and ash from the weight of the filtered and dried residue (Dodevska et al., 2013).

**Sensory Analysis**

To determine the sensory features and assessing the consumability of the produced biscuits, a 20-person panel comprising lecturers and undergraduate students at İstanbul Aydin University completed a sensory analysis. The biscuit samples were randomly named with 3-letter codes and presented to the panellists for analysis. The panellists were requested to assess the biscuits in terms of taste, smell, colour, crunchiness, crispness, texture and general approval. They were asked to give points on the rating form for the results of these criteria. Panelists were presented with the control sample and biscuit samples with different proportions of *spirulina* with codes. Panelists were given no information about the contents of the biscuits to ensure an objective assessment. Scales were rated on a nine hedonic scale of 1: very bad, 2: bad, 3: moderate, 4: not good or bad, 5: good, and 6: very good. Average points of 5 or more were assessed as liked.

**Statistical Analysis**

One-way ANOVA was performed for statistical analysis and Tukey's test was chosen to measure the means of the results with significant differences ($P<0.05$). Data analysis was conducted using the software package (JMP 9) (Nakov et al., 2018).

**Findings and Discussion**

**Physical Properties**

The degree of spread was identified in line with diameter and thickness measurements for gluten-free biscuit samples. The mean diameter, thickness and spread rates for gluten-free biscuit samples containing rice flour enriched with SPL are shown in Table 1. Thickness varied from 0.5-0.76, diameter from 5.44-6.06 and spread rate was 8.01-10.9. The SPL content affected thickness, diameter and spread. The lowest thickness was observed in SPLC biscuits and the highest was determined in the SPL3 sample($p<0.05$). In SPL1 and SPL2 samples, the thickness was found to be statistically similar ($p>0.05$) and the 0.5% increase did not affect the thickness. The presence of SPL increased the diameter compared with the control. However, the proportional increase in SPL was not found to be statistically significant in diameter in SPL1, SPL2 and SPL3 samples ($p>0.05$). SPL was found to be statistically significant on spread in biscuits ($p<0.05$). The highest spreading rate was determined in control biscuits SPLC and the lowest was determined in SPL3 biscuits. A high SPL ratio increased the thickness and decreased the spreading rate. According to the study by Awan et al. (1995), the increase in protein in biscuits lowered the diameter and spread factors and increased the thickness of biscuits (Awan et al., 1995).

**Table 1.** Physical characteristics of biscuit samples

| Biscuit Samples | SPL Ratio | Thickness of biscuit (mm) | Diameter of biscuit (cm) | Spread ratio |
|---|---|---|---|---|
| **SPLC** | Control | 0.5±0.00$^{c}$ | 5.44±0.17$^{b}$ | 10.9±0.17$^{a}$ |
| **SPL1** | 0.50% | 0.68±0.07$^{b}$ | 6.14±0.38$^{a}$ | 9.08±0.57$^{b}$ |
| **SPL2** | 1% | 0.7±0.00$^{b}$ | 5.88±0.20$^{ab}$ | 8.40±0.23$^{c}$ |
| **SPL3** | 5% | 0.76±0.12$^{a}$ | 6.06±0.12$^{a}$ | 8.01±0.59$^{c}$ |

Mean of 3 replicates ± SD, Means in a column not sharing the same superscript are significantly different at $p \leq 0.05$.

In another study, it was determined that wheat flour biscuits containing 0.5, 1, 1.5, 2 and 2.5% *Spirulina* powder affected the diameter and thickness very little compared to the control biscuits, and at the same time, increasing the addition of *Spirulina* powder decreased the spreading rate and volume of the biscuits (Ziena et al., 2020). It has been determined that *Spirulina* powder added to whole grain wheat, oat and barley flour at the rates of 0, 1, 2 and 3% reduces the cookie spreading rate. It has been explained that this is due to the fibre content reducing the spreading rate of the cookies (Gupta et al., 2011; Onacik-Gür et al., 2018).

**Chemical Properties**

Data in Table 2 shows the chemical analysis findings for different biscuit samples made with different amounts of SPL powder. The humidity content of the samples was between 3.45% and 5.08%. Humidity contents increased depending on the increase in SPL. As stated in Table 2, no statistically significant difference was observed in fat contents ($p>0.05$). Fat analysis in studies in the literature supports the result (Egea et al., 2014; Pop, 2022; Prakash & Pandey, 2023)

**Table 2.** Proximate chemical composition of biscuit samples

| Parameters | SPLC(Control) | SPL1 (0.50%) | SPL2 (1%) | SPL3 (5%) |
|---|---|---|---|---|
| **Humidity (%)** | 3.45±0.28$^{b}$ | 3.62±0.13$^{b}$ | 4.8±0.29$^{a}$ | 5.08±0.18$^{a}$ |
| **Ash (%)** | 0.80±0.02$^{b}$ | 0.81±0.02$^{b}$ | 0.82±0.03$^{b}$ | 1.25±0.01$^{a}$ |
| **Fat (%)** | 29.86±0.56$^{a}$ | 29.60±0.39$^{a}$ | 29.58±0.74$^{a}$ | 29.10±0.60$^{a}$ |
| **Protein (%)** | 7.28±0.02$^{c}$ | 8.23±0.01$^{b}$ | 8.35±0.01$^{b}$ | 10.60±0.02$^{a}$ |
| **TPC (mg GAE/100g)** | 33.13±0.40$^{d}$ | 42.4±4.36$^{c}$ | 59.28±0.60$^{b}$ | 64.18±0.10$^{a}$ |
| **Antioxidant (mg TE/100g)** | 108.31±6.18$^{b}$ | 121.80±1.12$^{b}$ | 215.94±5.6$^{a}$ | 251.86±3.43$^{a}$ |
| **FRAP (mg TEAC/100g)** | 36.27±0.40$^{c}$ | 37.27±0.0$^{c}$ | 54.74±1.0$^{b}$ | 60.66±0.30$^{a}$ |
| **Dietary Fibre (%)** | 0.91±0.01$^{d}$ | 1.22±0.02$^{c}$ | 1.95±0.03$^{b}$ | 2.72±0.01$^{a}$ |

Mean of 3 replicates ± SD, Means in a column not sharing the same superscript are significantly different at $p \leq 0.05$.

When the SPL proportion changed, the raw protein, ash, and dietary fibre contents of all biscuit samples significantly increased compared to the control sample (SPLC) . Protein values from 7.28% to 10.60%, ash from 0.8% to 1.25%, dietary fibre from 0.91% to 2.72%. The highest protein content was determined in the SPL3 sample and the lowest SPLC sample. While it was determined that there were statistically significant differences dietary fibre values ($p<0.05$). A similar study adding certain proportions of *Spirulina* powder to wheat flour found that the moisture proportion of the biscuits did not change, while protein and ash proportions increased linked to the increase in *Spirulina* powder (Ziena et al., 2020). Another study of biscuits supplemented with 0%, 2.5% and 4.0% of *S. platensis* grown in laboratory conditions found the %moisture was 2.1-2.91%, while crackers with 0%, 2.5% and 4.0% supplementation had %moisture 1.54-1.8% (Gün, 2019). Studies have emphasized that the protein content of *S. platensis* can reach up to 70% (da Rosa et al., 2015). It has been determined that *S. platensis* added to many products such as pasta, biscuits, yoghurt, chocolate, etc. increases the protein content (da Silva et al., 2019; Fradique et al., 2010; Rodríguez De Marco et al., 2014; Şahin, 2019). It has been stated that 0.25, 0.5 and 1% of *S. platensis* added to the ayran (a traditional Turkish drink) increased the protein in ayran from 17.17% to 22.61% (Çelekli et al., 2019). It has been determined that 2% *S. platensis* increases the protein values in homemade chocolates and biscuits (Şahin, 2019). It has been determined that 5, 10 and 20 g/100 g *spirulina* biomass used instead of wheat flour increases the protein level of pasta (Özyurt et al., 2015). It has been determined that there is no difference in protein between raw and cooked pasta and that proteins do not pass into the cooking water $100g^{-1}$(Şahin, 2019). It was stated that *S. platensis* added to biscuits and crackers at 0%, 2.5% and 4% increased the protein content by 59.92% in biscuits and 43.47% in crackers (Gün, 2019). It has been determined that dietary fibre content increases due to the increase in SPL. As seen in Table 3, dietary fibre content increased depending on the SPL increase. In their study, (Raczyk et al., 2022) found that the total dietary fibre of *spirulina* (8.45%) was significantly higher than that of semolina (4.44%). *S. platensis* powder evaluated by other authors has stated that they have various fibre contents such as 3%, 7.93% and 14.98% (Michalak & Chojnacka, 2015; Nicoletti, 2016; Ramírez-Rodrigues et al., 2021). Similar to literature studies on *S.platensis*, it is seen that it enriches the protein and dietary fibre content in rice flour biscuits.

**Total Phenolic and Antioxidant Capacity**

Phenolic compounds synthesized as secondary metabolites (El-Baky et al., 2009) are accepted as one of the most important classes of natural antioxidants and have received increasing interest from consumers of healthy foods (Machu et al., 2015). A study reported that *Spirulina* powder was a good raw material for healthy nutrition due to containing total phenolic and total flavonoids (Ziena et al., 2020). The phenolic and antioxidant content of biscuit samples in the study are shown in Table 2. Phenolic content was 33.13-64.18 mg GAE/100 g and antioxidants were 108.31-251.86 mg TE/100 g. It was observed that the addition of dried SPL to the biscuits had a statistically significant effect ($p<0.05$) on the phenolic and antioxidant content when compared to the control samples. Biscuits containing SPL had higher phenolic and antioxidant content than SPLC, with the highest identified in SPL3 biscuits containing 5% SPL3 ($p<0.05$). The potential use of *S. platensis* powder for gluten-free pasta was investigated by Fradinho et al. (2020). The results of the study detected that the contents of phenolic compounds, chlorophylls and carotenoids increased (Fradinho et al., 2020). It has been stated that the increase in the microalgae rate of *Arthrospira platensis* in biscuits from 2% to 6% leads to an increase in polyphenols and antioxidant capacity (Batista et al., 2017). Studies have shown that microalgal biomass increases the content of phenolic compounds, chlorophylls and carotenoids after its inclusion in recipes. Lupine-based protein meat analogues containing *S. platensis* powder at a concentration of 15, 30 or 50% have been found to increase total phenolic and flavonoid content (Palanisamy et al., 2019). It has been determined that *Spirulina* added to dairy products increases the total phenolic, flavonoid and antioxidant contents of these products (Barkallah et al., 2017; Boyanova et al., 2022; Nazir et al., 2022). It was determined that the total phenolic content of *S. platensis* added to ice cream at the rates of .5%, 1% and 1.5% increased from 43.7 to -140.2 mg GAE/100g compared to the control sample (Boyanova et al., 2023). It was determined that bread to which 6% *Spirulina* platensis powder was added exhibited higher antioxidant activity than the control groups (Saharan & Jood, 2021).

**Textural Characteristics**

Textural properties of control biscuits and SPL added biscuits on days 1, 3, 5 and 7 day during 7 . days of storage are given in Figure 2. Generally, hardness and brittleness values are determined when identifying the textural quality of biscuits (Banerjee et al., 2014; González et al., 2018). The hardness and brittleness values for biscuits with SPL added to the recipe were affected at statistically significant levels ($p<0.05$). As the proportion of SPL in the biscuits increased, increases were observed in the hardness and brittleness values of the biscuits. During T7 day storage, the highest hardness and brittleness were determined in SPL3 biscuits and the lowest were determined in SPLC biscuits, which are control samples. This situation may be explained by the high fibre content in SPL creating a more compact biscuit texture. Similar results related to the change in textural features of biscuits were obtained in studies of biscuits enriched with foods containing high fibre content (Kulkarni & Joshi, 2013; Singh et al., 2012)

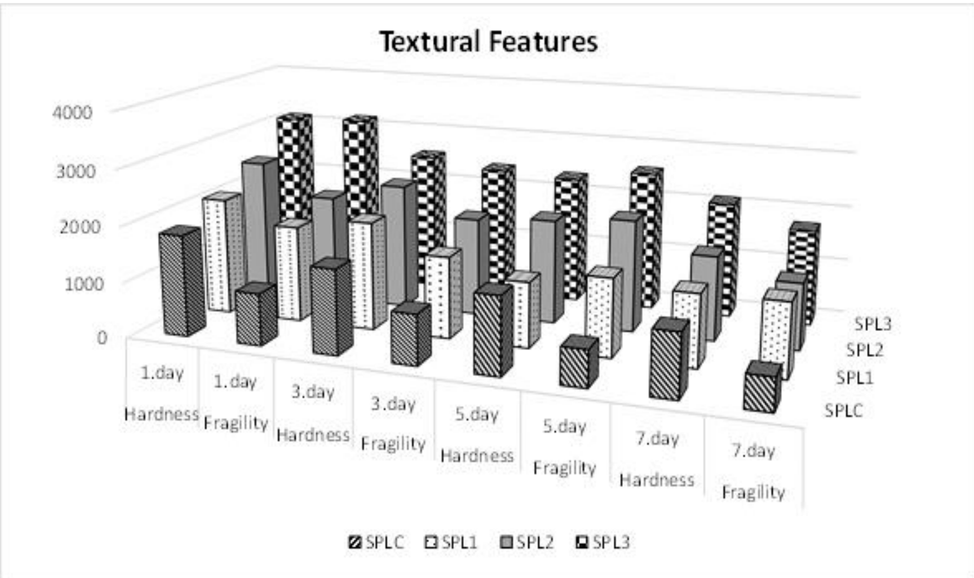


**Figure 2.** Textural properties of biscuits stored on days 1, 3, 5 and 7

Studies have shown that *S. platensis* provides positive contributions to products that exhibit hardness properties. It was determined that a functional Ricotta cheese enriched with different amounts of *spirulina* powder (0.25, 0.5, 0.75 and 1.0) g/100 g improved textural properties (Ismail et al., 2023). In a similar study, after *S. platensis* was added to yoghurt at four different concentrations (0.25, 0.5, 0.75 and 1%), its textural properties were examined and it was found that yoghurts containing 25% *S. platensis* were similar to the control sample (Barkallah et al., 2017). *S. platensis* extracts have been found to affect the texture of cassava flour noodles, making them more chewy, dense and not easily broken (Al-Baarri et al., 2021). These studies found that *S. platensis* added to cookies at 1%, 2% and 3% increased hardness due to moisture decrease(Onacik-Gür et al., 2018). It was determined that as the algae level and storage time (on the 1st, 5th and 10th day) of sponge cakes containing 0, 0.5, 1 and 1.5% *S. platensis* powder prepared with wheat flour increased, the textural parameters of the sponge cake (hardness, stickiness and cohesiveness) increased (Zangeneh et al., 2020). It was determined that the hardness of wheat flour biscuits containing 0.5, 1 and 1.5% *S. platensis* decreased with increasing microalgal biomass content. It has been stated that this situation results in the formation of weak gels when *S. platensis* is added to the dough. (Shahbazizadeh et al., 2015).

**Colour Analysis**

Table 3 shows the effect of SPL supplementation on the colour of the biscuit surface. All biscuits containing SPL had dark colors from 76.88 to 43.18 and darker colours had significantly lower L* readings. Among the colour values of the biscuit samples, the L*, a* and b* values of wheat flour statistically ($p<0.05$) decreased with the addition of SPL determined. Generally, as the dried SPL in the biscuits increased, the L* values were significantly reduced. The a* value was 1.33 to -4.3, while the b* value was 25.27 to 6.25 and significantly reduced with the increase in SPL. Greener, darker biscuits were obtained with the addition of SPL

**Table 3.** Colour characteristics of biscuit samples

| Biscuit Samples | SPL Ratio | L* | a* | b* |
|---|---|---|---|---|
| SPLC | Control | 76.88±0.61[a] | 1.33±0.20[c] | 25.27±0.67[a] |
| SPL1 | 0.50% | 61.85±0.46[b] | 3.69±0.20[b] | 15.40±0.27[b] |
| SPL2 | 1% | 62.01±0.34[b] | 4.92±0.21[a] | 16.47±0.19[b] |
| SPL3 | 5% | 43.18±0.31[c] | 4.30±0.31[a] | 6.25±0.05[c] |

The mean of 3 replicates ± SD, Means in a column not sharing the same superscript are significantly different at $p \leq 0.05$

In a study, the effects of various concentrations of *S.platensis* microalgae on the drying kinetics and colour change of kiwi pastille were investigated. Concentrations of S. platensis did not affect the drying process. It has been found that it reduces a* and L* values (Khazaei Pool et al., 2016). It was determined that the a * values of shortbread biscuits with the addition of 0, 1, 3 and 6% *S. platensis* powder during storage became less dense with increasing storage time, while the b* values increased (Marcinkowska-Lesiak et al., 2018). It was determined that the colour of yoghurt samples enriched with 1.5% *S. platensis* powder and 0.5% milk protein changed from yellow to greenish (low a* and b*). It was determined that the amount of chlorophyll in *S. platensis* powder caused colour change (Mesbah et al., 2022).

**Sensory Properties**

The gluten-free biscuit samples using rice flour and enriched with SPL were rated by 20 panellists for 6 categories of flavour, odor, colour, crispness, texture and general approval with objective approval of 1 (very bad), 2 (bad), 3 (moderate), 4 (neither good nor bad), 5 (good) and 6 (very good). Generally, which biscuit was chosen most and which were not chosen were determined. The mean scores for the 6 categories for the biscuits are presented in the sensory assessment graph in Figure 3.

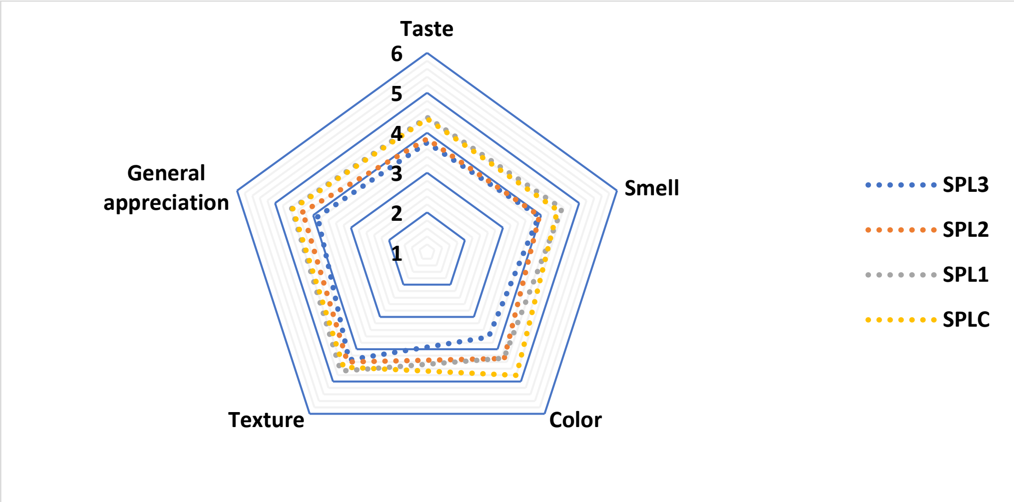


**Figure 3**. Sensory evaluation of biscuit samples

For assessment in terms of flavour, the 4 different biscuit samples had scores varying from 3.75 to 4.39. In terms of flavour, the most liked biscuit with 4.39 points contained 0.5% SPL1. Assessment in terms of odour for gluten-free biscuit samples found mean score varied from 3.94-4.55. The biscuit most liked in terms of odour contained 0.5% SPL1 and received 4.55 points. The least-liked biscuit contained 5% *S. platensis* and received 3.94 points. Assessment in terms of colour for gluten-free biscuit samples found mean scores were 3.62-4.81. The picture showing the colours of the biscuits can be seen in Figure 1. When biscuits are assessed in terms of colour, the most liked biscuit received 4.81 points and was the control sample. The biscuit samples containing 0.5% SPL1 and 1% SPL2 received equal points. When gluten-free biscuit samples were assessed in terms of crispness, scores varied from 4.86 to 5.25. The most liked biscuit in terms of crispness contained 0.5% SPL1 and received 5.25 points. The least liked biscuit contained 1% SPL and received 4.86 points. According to these results, the addition of a very small amount of SPL was identified to enhance the crispness of the biscuit. Assessment in terms of texture for gluten-free biscuit samples found mean scores varied from 4.33 to 4.68. In terms of texture, the most liked biscuit received 4.68 points and contained 0.5% SPL1. The least liked biscuit received 4.33 points and contained 5% SPL3. In terms of general approval of gluten-free biscuit samples, the highest points were for the control biscuit and the biscuit with 0.5% SPL1 added. When examined in a general sense, the lowest point among the categories was 3.75. In other words, even the least chosen biscuit was identified to receive a moderate rating in terms of biscuit features. When examined in terms of category, it appears the best value of 5.25 points is equivalent to a good rating.

## Conclusion

This study focused on the potential of *Spirulina platensis* powder as a functional ingredient in gluten-free biscuit production, highlighting its positive impact on nutritional, sensory, and physicochemical characteristics. The inclusion of *Spirulina platensis* powder significantly enhanced the protein, dietary fibre, phenolic content, and antioxidant properties of the biscuits, contributing to their health benefits. While higher rates of *Spirulina platensis* powder improved nutritional values, sensory analysis revealed a preference for biscuits with lower *Spirulina platensis* content, indicating a balance between nutritional enhancement and consumer acceptance is crucial. The findings suggest that *Spirulina platensis* powder can be effectively utilized in the development of gluten-free functional biscuits, offering a nutritious alternative for consumers seeking healthier snack options. Future research could focus on optimizing the formulation to further improve the sensory appeal and nutritional profile of *Spirulina platensis* powder-enriched gluten-free biscuits.

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