Antioxidant and nutritional potential of cookies enriched with *Spirulina platensis* and sources of fibre

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Summary

The use of by-products is an important strategy to valorize agro-industrial productive chains. The production of low-cost functional foods is a concern of the food industry. The aim of this work was to investigate the chemical composition and the technological, microbiological, sensorial and antioxidant properties of four formulations of cookies, produced with the replacement of 20% of wheat flour by equal proportions of flours from by-products (cassava, soybean and peach palm) and with the addition of *Spirulina platensis* (2% or 5%, dry basis). The formulation containing composite flour and 5% of biomass, had an increase of 20% of protein, 30% of ash and 96% of fibre when compared to the control. Due to the high level of total phenolic compounds (12.2 g·kg^{-1}) and antioxidant capacity (mean value 40.3 mmol·kg^{-1}, as Trolox equivalent) of *S. platensis*, this formulation had an increase of 64% of phenolic compounds and 37% of antioxidant capacity in relation to the control. The nutritional enrichment showed that the formulations tested were viable, despite the decrease in the sensorial acceptance and the alterations in technological properties. The microbiological analysis showed that the formulated products were within the limits of the Brazilian legislation.

Keywords

Spirulina platensis; fibre; antioxidant potential; functional foods; by-products

The food industry has a challenge to produce low-cost, nutritive and convenient foods. The offer of functional foods has increased, since they have the potential to health promotion, due to the bioactive compounds that are present or added to traditional formulations [1]. In order to meet this demand, it is important to study sources of fibres and antioxidants compounds that are technologically viable, with positive environmental and economic impacts.

Among the compounds with functional properties, the dietary fibre is important because it can positively act on health by slowing down the hydrolysis, digestion and absorption in the small intestine, increasing the volume of stools and reducing the levels of glucose and cholesterol absorbed from the lumen [2, 3]. The by-products are an interesting source of fibres that have low cost. During the cassava starch production, large amounts of solid residues are generated that contain starch and fibres in various proportions. These are discarded or destined to animal feeding [4]. The production of soya milk and tofu generates an interesting by-product, okara, which has high protein and fibre contents, together with minerals and isoflavones [5]. Peach palm, which is processed by canning, produces, due to the lack of uniformity of the stem structure, high volumes of discards with a high content of fibre [6]. Thus, flours are produced from these by-products, which have good

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nutritional value. The safety for consumption of these flours was checked and they were found to meet the microbiological criteria established by legislation [7].

Substances known as antioxidants are bioactive compounds that are able to inactivate free radicals, which are instable molecules that may cause several deleterious effects to human health [8]. *Spirulina platensis*, a cyanobacterium that can be consumed by humans, has high antioxidant potential and several therapeutic properties, such as the ability to inhibit some viruses, cytostatic and cytotoxic action in cancer treatment, capacity to decrease blood pressure, lipids and glucose in blood, weight reduction, increase in the population of microorganisms in the intestinal flora, improvement in immunologic response, renal protection against heavy metals and medicines, besides showing radio-protective activity and being efficient against malnutrition [9]. The biomass of *S. platensis* has high protein content and it can be added to food products, once several studies have demonstrated that its consumption is safe and there are no risks of toxicity, being considered GRAS (Generally Recognized as Safe) [10–12].

Biscuits and cookies are highly consumed, but they have low nutritional value [13]. Thus, the association of flours with high fibre content with *S. platensis*, which is rich in antioxidant compounds and proteins, is interesting to the production of cookies with high nutritional value. However, the addition of these ingredients must be studied to develop technologies that allow their efficient use. While using sources of proteins and fibres, it is important to study the effects of their addition on technological parameters. The interaction between fibres and gluten proteins may decrease gas retention and the dough expansion, which may influence the acceptance of the products [2, 13].

The aim of this work was to investigate the addition of *S. platensis* biomass to cookies formulated with flours of cassava, soybean and peach palm by-products, in order to evaluate their antioxidant potential, nutritional enrichment and their technological, microbiological and sensorial properties.

MATERIAL AND METHODS

Flours and *Spirulina platensis* biomass production

The solid by-products of cassava, soybean and peach palm were collected from industries in the region of Umuarama (Paraná, Brazil). They were submitted to drying in an oven with forced air circulation at 60 °C for 36 h (MA 035, Marconi, Piracicaba, Brazil) and triturated in a cutting mill type Willye (Model SL-031, Solab, Piracicaba, Brazil). The particle size of flours was standardized using a set of sieves with particle separation from 150 mm to 600 mm, subjected to vibration for 10 min [14].

S. platensis UTEX 1926 was maintained in Schlösser medium [15] without agitation. The cells were inoculated in 500 ml Erlenmeyer flasks containing 200 ml of Schlösser medium and cultivated on a rotary shaker at 1.7 Hz, 30 °C, illuminated at 72 μmol·m^{-2}s^{-1} during 6–8 days. The cell suspension was filtered through membranes with 50 μm pore diameter and the cells were suspended in a fresh Schlösser medium. Such suspension was used as inoculum of cultivation in open pond, built with white PVC sheets, being 50 mg·l^{-1} (dry mass basis) the initial concentration in this photobioreactor. The batch cultivation was carried out at 30 °C, illumination 72 μmol·m^{-2}s^{-1}, with circulation of the culture provided by paddle shells at 0.3 Hz. The working volume was 5 l and the water lost by evaporation was daily replaced with distilled water [16]. After 14 days of cultivation, the cell concentration reached 1 410 mg·l^{-1} and the biomass was harvested, washed with distilled water and finally submitted to the drying process at 60 °C using a vacuum oven (HZV; Horyzont, Krakow, Poland) with air insufflation provided by an air pump (Seven Star, Guangdong, China). The resulting dried biomass was used in the formulation of cookies.

Formulation of cookies

The cookies were processed on a pilot scale with ingredients obtained from local market, following the basic formulation: 100 g wheat flour (Renata, Sumaré, Brazil), 35 g margarine (Delícia, São Paulo, Brazil), 1 egg (Harmonia, Umuarama, Brazil), 80 g chocolate chips (Arcor, Rio das Pedras, Brazil), 45 g brown sugar (Jasmine, Curitiba, Brazil), 4 ml vanilla essence (Dr. Oetker, São Paulo, Brazil), 5 g baker's powder (Royal Kraft Foods, Curitiba, Brazil) and milk (Latco, Francisco Beltrão, Brazil) in sufficient quantity [17].

The basic control was produced only with wheat flour (formulation 1). In formulation 2, a portion of 20% of wheat flour was replaced with equal proportion (20%) of composite flour containing cassava (6.7%), soybean (6.7%) and peach palm (6.7%) by-products. This replacement percentage was adopted because it was the best accepted in sensorial tests, and it contained a higher amount of fibre, according to previous studies (unpublished data). In order to check the increase in nutritional and antioxidant values in the cookies produced, 2 g or 5 g per 100 g of flours of *S. platensis* dehydrated biomass was added, that resulted in formulation 3 (20% composite flour + 2% of biomass) and formulation 4 (20% composite flour + 5% of biomass). The components of the four formulations were weighed and mixed until complete homogenization. Each portion of 20 g was manually modelled and baked in a pre-heated oven (Tedesco, FTT-300, Caxias do Sul, Brazil) at 180 °C for 20 min.

Proximate composition, antioxidants and microbiological quality of *S. platensis* and cookies

The cookies and *S. platensis* biomass were submitted to microbiological analysis: *Salmonella* spp. (absence or presence in 25 g); coliforms at 45 °C, with results expressed as most probable number (MPN) per gram; coagulase positive *Staphylococcus* and *Bacillus cereus*, with results expressed as colony forming units (CFU) per gram [18] and the results were compared with the Brazilian legislation [19]. They were also analysed for chemical composition: moisture (method 925.09), ash (method 923.03), protein (method 920.87), crude fibre (method 978.10) and fat (method 920.85) according to AOAC [20]. The content of saccharides was determined by difference after subtracting the moisture, protein, fat and ash content from the total matter. The energy value was calculated based on the centesimal composition, using Atwater conversion factors [21].

The extraction of antioxidant compounds was conducted with ethanolic aqueous solution 80% (v/v). The quantification of total phenolic compounds (*TPC*) was realized by Folin-Ciocalteu method, as described by Swain and Hillis [22], the absorbance being measured at 760 nm (spectrophotometer 700 Plus, Femto, São Paulo, Brazil). For quantification, a standard curve was prepared with gallic acid and the results were expressed as grams of gallic acid equivalent per kilogram of sample.

The scavenging activity of DPPH$^{\bullet}$ radicals (2,2-diphenyl-1-picrylhydrazyl; Sigma-Aldrich Chemical, St Louis, Missouri, USA) was determined according to Brand-Williams et al. [23]. The ferric reduction power (FRAP) of the extract was assessed following the methodology described by Benzie and Strain [24]. The antioxidant capability of the extracts with ABTS$^{\bullet+}$ free radical (2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid; Sigma-Aldrich Chemical) was carried out according to Thaipong et al. [25]. Trolox (6-hydroxy-2,5,7,8-tetramethylchloromane-2-carboxylic acid; Sigma-Aldrich Chemical) was used to construct calibration curves and all the results were expressed as Trolox equivalent (in millimoles of Trolox per kilogram of sample).

Technological properties of cookies

The expansion coefficient was determined by the method of millet seeds dislocation and it was calculated using the equation 1 [26].

$$E_c = \frac{V_2 - V_1}{V_1} \times 100 \quad (1)$$

where E_c is the expansion coefficient (in percent), V_2 is the volume of the baked dough and V_1 is the volume of the raw dough (in cubic centimetres).

The colour of the samples was determined by reflectance spectrophotometer MiniScan XE Plus (Hunter Associated Laboratory, Naperville, Illinois, USA). The colour system used was CIE (Commission International de l'Eclairage) $L^*a^*b^*$ (L^* – luminosity; a^* – red-green tonality; b^* – blue-yellow tonality). The measurements were done in triplicates against a black background [27].

The determination of cookies hardness was made by Brookfield CT3 Texture Analyzer (Brookfield Engineering Laboratories, Middleboro, Massachusetts, USA). Each cookie sample was placed horizontally in a platform and cut in half, by means of a knife-shaped probe TA-SBA (texture analyzer shear blade accessory), with pretest speed of 2.0 mm·s^{-1}, test speed of 5.0 mm·s^{-1}, trigger force of 0.07 N and distance of 8.0 mm, thus determining the rupture force (hardness). Ten determinations were made for each formulation in samples randomly selected and the results were expressed in newtons [2, 27]. Water activity was determined by Aqualab (Decagon, Pullman, Washington, USA) at 25 °C.

Sensorial analysis of cookies

The cookies formulation acceptance test was realized by a team of 30 untrained panelists. The parameters analysed were: appearance, aroma, flavour, texture and overall impression. The methodology used a nine-point hedonic scale ranging from 1 (dislike extremely) to 9 (like extremely). Purchase intention was also evaluated with a 5-point scale, ranging from 5 (I would certainly buy it) to 1 (I would certainly not buy it) [28]. The project was approved by the Permanent Committee for Ethics in Research Involving Human Beings from Maringa State University (UEM, Maringá, Brazil).

Statistical Analysis

The analyses were carried out in three individual replicates and the results were expressed as mean ± standard deviation. The variance analysis

(ANOVA) applied to the data followed by Tukey's test ($p < 0.05$) were performed using the software Statistica version 7.0 (StatSoft, Tulsa, Oklahoma, USA), including the analysis of principal components.

RESULTS AND DISCUSSION

Proximate composition

The chemical composition of the dried *S. platensis* biomass and of the cookies formulated is presented in Tab. 1. The biomass contained high amounts of proteins of satisfactory digestibility and a profile of essential amino acids that was close to FAO recommendation [10, 16, 29], Therefore, its addition to food products seemed interesting. The high protein content in the biomass (614.2 g·kg^{-1}) and the presence of lipids (61.7 g·kg^{-1}) with a high portion of essential fatty acids could be important to improve the nutritional value of the cookies. A great variety of photosynthetic microorganisms are known to contain sufficient amounts of high added value components, especially polyunsaturated fatty acids, such as linoleic acid [18:2 (*n*-6); IUPAC name: *cis,cis*-9,12-octadecadienoic acid] and γ-linoleic acid [18:3 (*n*-6); IUPAC name: all-*cis*-6,9,12-octadecatrienoic acid], the latter being a widely recognized food supplement [30]. The biomass had high amount of B vitamins, carotenoids and minerals. Studies showed favourable toxicological aspects and its applicability to the reversion of child malnutrition. Furthermore, the content of nucleic acids is low, when compared to other microbial biomasses [9, 10].

The replacement of a part of wheat flour by flours from agro-industrial by-products (formulation 2) decreased by 9.2% the protein content of the cookies in relation to the control (formulation 1), likely because to the high fibre content of the composite flour added [7]. On the other hand, there was an increase of 20% in protein content of samples added with 5% of biomass (formulation 4), when compared to formulation 1. The commercial cookies had low protein content, which highlights the importance of using alternative sources of protein enrichment. The consumption of 100 g of cookies added with biomass (2% or 5%) may contribute with about 24% of daily protein requirement, since the recommended daily intake is 50 g for adults [31].

The addition of *S. platensis* to the formulations 3 and 4 promoted an increase in ash content ($p < 0.05$), and the highest content of biomass utilized (5%) increased significantly the lipid content. The use of flours from by-products and biomass in the formulations 2, 3 and 4 provided higher fibre content than the control. The flours from by-products used in this study stood out because of their high fibre contents, satisfactory physical properties and low contents of phytic acid [9]. Furthermore, US Food and Drug Administration allows foods to be labeled as 'a good source of fibre' or 'high fibre' if they contain more than 2.5 g or 5.0 g of dietary fibre per serving, respectively [31]. Although the dietary fibre content of the samples was not determined, the values obtained by the crude fibre analysis indicated that the formulations 2, 3 and 4 could be considered fibre-rich products. The crude fibre analysis was based on acid and alkaline hydrolysis, and it did not estimate the water-soluble compounds, so the values obtained by this methodology were generally lower than those obtained by the dietary fibre test, which involves enzymatic hydrolysis of non-fibre components.

According to Bilgiçli et al. [32], several sources of fibre can be used in cookies production in order to improve their texture, colour, flavour and decrease the energy value. Fibre intake has a positive impact on human health, as it can act by slowing down hydrolysis, digestion and absorption in the small intestine, increasing the volume

Tab. 1. Proximate composition of *S. platensis* and of cookies formulated with flours from by-products and biomass.

Component	Moisture[1] [g·kg^{-1}]	Ash [g·kg^{-1}]	Proteins[2] [g·kg^{-1}]	Lipids [g·kg^{-1}]	Crude fibre [g·kg^{-1}]	Saccharides [g·kg^{-1}]	Energy value [kJ·g^{-1}]
S. platensis	101.2 ± 3.2	24.2 ± 2.2	614.2 ± 2.1	61.7 ± 3.2	53.1 ± 7.8	145.6	1471.4
Formulation 1	106.8 ± 3.2^{b}	15.2 ± 1.0^{b}	106.7 ± 9.7^{b}	315.5 ± 2.1^{b}	34.7 ± 2.9^{c}	421.1	2072.6
Formulation 2	125.5 ± 1.3^{a}	14.9 ± 0.0^{b}	96.8 ± 1.2^{c}	332.3 ± 8.0^{b}	57.7 ± 1.3^{b}	372.8	2037.5
Formulation 3	116.8 ± 4.6^{b}	18.8 ± 0.0^{a}	111.5 ± 2.0^{ab}	326.4 ± 1.3^{b}	63.3 ± 2.3^{a}	363.2	2024.6
Formulation 4	100.9 ± 8.4^{b}	19.7 ± 2.1^{a}	128.4 ± 2.2^{a}	356.5 ± 9.2^{a}	68.1 ± 3.4^{a}	326.4	2104.3

Average values in the same line followed by the same letter are not significantly different by Tukey's test ($p < 0.05$).
Formulation 1 – 0% biomass; Formulation 2 – 20% composite flour; Formulation 3 – 20% composite flour + 2% biomass; Formulation 4 – 20% composite flour + 5% biomass. 1 – dried at 105 °C, 2 – conversion factor 6.25.

Tab. 2. Evaluation of the technological properties of cookies formulated with flours from by-products and biomass.

Sample	Expansion coefficient	Hardness [N]	Water activity	Colour		
				L^*	a^*	b^*
Formulation 1	50.0 ± 0.0^{b}	18.0 ± 3.2^{b}	0.712 ± 0.0^{b}	46.4 ± 5.1^{a}	13.6 ± 1.8^{a}	29.9 ± 4.4^{a}
Formulation 2	55.0 ± 2.7^{a}	18.2 ± 3.6^{b}	0.813 ± 0.0^{a}	41.9 ± 2.4^{ab}	15.9 ± 0.9^{a}	28.7 ± 1.9^{a}
Formulation 3	50.0 ± 0.0^{b}	59.1 ± 8.2^{a}	0.621 ± 0.0^{b}	38.1 ± 2.5^{b}	10.9 ± 0.8^{b}	23.8 ± 1.9^{ab}
Formulation 4	50.0 ± 0.0^{b}	62.5 ± 10.4^{a}	0.631 ± 0.0^{b}	34.5 ± 2.1^{b}	9.5 ± 0.9^{b}	21.0 ± 1.9^{b}

Average values in the same column followed by the same letter are not significantly different by Tukey's test ($p < 0.05$).

of stools and reducing levels of glucose and cholesterol absorbed from the lumen [3]. In Brazil, the addition of *S. platensis* in food products is authorized by ANVISA (National Health Surveillance Agency), the intake being limited to 1.6 g per individual per day [11, 33]. The consumption of 100 g of cookies per day containing 2% or 5% of *S. platensis* is consistent with the limit recommended.

Technological properties and microbiological parameters

The results for technological evaluations are shown in Tab. 2. The cookies expansion index was used to predict their quality, as the alterations in such values bring standardization problems to the industry. The cookies produced only with wheat flour (formulation 1) showed expansion coefficients equivalent to the ones formulated with composite flour and *S. platensis* (formulations 3 and 4). However, formulation 2 (20% composite flour) had a greater expansion, due to the high content of fibres and low content of proteins. Cookies expansion during baking is a physical phenomenon that is controlled by the ability to absorb water from the dough components. Fibres show better capacity to retain water than starch, the main component of wheat flour, and this causes a competition for free water, increasing the expansion. The addition of proteins, such as *S. platensis* biomass, makes the dough stronger, which may hamper the extensibility in cases of higher levels of replacement of wheat flour [14, 27].

Regarding hardness, no significant differences were found between the formulations 1 and 2 ($p < 0.05$). However, the formulations 3 and 4 containing *S. platensis,* showed the highest hardness. The cookies showed a heterogeneous structure, considering shape and size of ingredient particles, as well as variations in thickness throughout their structure. In general, formulations with high fibre content resulted in stiffer and denser products, and the high protein content (from *S. platensis* biomass) was also responsible for the hardness of bakery products, interfering in the elastic net [2, 27, 34].

The luminosity (L^*) of formulations 1 and 2 was similar. Other authors found that the addition of by-products to formulations resulted in darkening [27]. The addition of biomass (formulations 3 and 4) decreased luminosity, when compared to the control, i.e. it caused darkening of the product. It was also noted in these formulations, that the values for a^* and b^* decreased upon the addition of *S. platensis*, being more expressive in formulations containing 5% of biomass (formulation 4). This was due to the green colour of *S. platensis* biomass, which brought to the cookies a dark brown coloration.

The lowest values of water activity (a_W) in the products were detected in cookies supplemented with *S. platensis*, i.e. formulations 3 and 4. The values of a_W found in this work ranged from 0.6 to 0.8, which reduced the possibility of microbial proliferation. The a_W affects the shelf life of the products. This parameter associated with the high temperature of baking, the high percentage of sugar added, and the packaging system that protects the product from moisture, light and oxygen, act as barriers to provide stability to the cookies during the storage [35].

The fact that the cookies did not promote microbial growth, suggested by low a_W values, was confirmed by microbiological analysis. The *S. platensis* biomass and the formulated cookies did not contain *Salmonella* spp. (absence in 25 g), and low levels of coagulase positive *Staphylococcus* (< 10 CFU·g^{-1}) and *Bacillus cereus* (< 10 CFU·g^{-1}) were observed in these samples. The biomass contained a low level of coliforms at 45 °C (< 0.3 MPN·g^{-1}) and a content of 0.4×10^{1} MPN·g^{-1} was detected in the cookies. These results were in concordance with the limits established by Brazilian legislation, being considered safe for consumption [19].

Tab. 3. Total phenolic compounds content and antioxidant capacity by methods DPPH•, ABTS•+ and FRAP in *S. platensis* and in the formulated cookies.

Samples	Total phenolic compounds [g·kg^{-1}]	Antioxidant Capacity		
		DPPH• [mmol·kg^{-1}]	ABTS•+ [mmol·kg^{-1}]	FRAP [mmol·kg^{-1}]
S. platensis	12.2 ± 0.1	74.9 ± 0.3	4.5 ± 0.1	41.4 ± 0.2
Formulation 1	1.4 ± 0.1^{c}	31.6 ± 0.5^{bc}	0.7 ± 0.0^{b}	12.1 ± 0.3^{c}
Formulation 2	1.7 ± 0.1^{b}	31.1 ± 0.6^{c}	0.9 ± 0.0^{a}	12.2 ± 0.1^{c}
Formulation 3	1.8 ± 0.0^{b}	33.1 ± 0.4^{b}	1.0 ± 0.0^{a}	15.2 ± 0.1^{b}
Formulation 4	2.3 ± 0.1^{a}	36.5 ± 0.9^{a}	1.1 ± 0.1^{a}	17.0 ± 0.0^{a}

Average values in the same column followed by the same letter are not significantly different by Tukey's test ($p < 0.05$).
Total phenolic compounds are expressed as gallic acid equivalents. Antioxidant capacity is expressed as Trolox equivalents.

Total phenolic compounds and antioxidant capacity

According to Tab. 3, the *S. platensis* biomass used for cookies formulation showed high content of phenolic compounds. COLLA et al. [36] studied different conditions of *S. platensis* cultivation, finding the content of phenolic compounds to range from 2.4 g·kg^{-1} to 5.0 g·kg^{-1}. Phenolic compounds are known to have antioxidant capacity and to interact with free radicals, without compromising their stability. In addition, they are able to inhibit lipid peroxidation in vitro by means of their ability to sequester free radicals and act as metal chelators [8, 9].

The addition of *S. platensis* contributed to the high content of phenolic compounds found in the cookies. Formulation 4 (20% composite flour + 5% biomass) had the highest content of *TPC*, while the lowest content was found in the control. The formulations 2 and 3 had similar content of *TPC* (1.8 g·kg^{-1}). AJILA et al. [37] found phenolic contents to range from 0.5 g·kg^{-1} to 4.5 g·kg^{-1} in cookies supplemented with 5–20% of mango peel in powder. Moreover, the authors noticed a significant increase in the antioxidant potential towards the DPPH• free radical.

Using the methods for antioxidant potential determination based on DPPH•, ABTS•+ and FRAP, *S. platensis* showed high antioxidant capacity of 74.9 mmol·kg^{-1}, 4.5 mmol·kg^{-1} and 41.4 mmol·kg^{-1} (expressed in millimoles of Trolox per kilogram of sample), respectively. The antioxidant capacity of *Spirulina* sp. has been extensively studied. Alcoholic extract of the biomass inhibited the lipidic peroxidation more significantly (65% of inhibition) than chemical antioxidants, such as α-tocopherol (35% of inhibition), β-carotene (48% of inhibition) or butylated hydroxyanisole (45% of inhibition) [9]. Lipid peroxidation mediated by reactive oxygen species is an important cause of destruction and damage to cell membranes [8].

Based on antioxidant data obtained by ABTS•+ method, there was no significant difference between the formulations 2, 3 and 4, whose values were greater than control. Data obtained by DDPH• and FRAP methods showed that formulation 4 (20% composite flour + 5% biomass) had the highest antioxidant potential, followed by formulation 3 (20% composite flour + 2% biomass). Formulations 1 and 2 had the lowest antioxidant capacity in both tests, revealing the importance of supplementation of cookies with *S. platensis* biomass in order to increase their antioxidant potential.

Cookies sensorial analysis

The results for sensorial acceptance (Tab. 4) showed that formulation 1 had high values, reach-

Tab. 4. Sensorial features of cookies formulated with flours from by-products and biomass.

Sample	Appearance	Aroma	Flavour	Texture	Overall impression	Purchase intention
Formulation 1	7.1^{a}	7.5^{a}	7.9^{a}	7.2^{a}	7.8^{a}	4.0^{a}
Formulation 2	6.9^{a}	7.1^{a}	6.8^{b}	6.1^{ab}	6.7^{b}	3.2^{b}
Formulation 3	6.6^{a}	7.0^{a}	6.7^{b}	6.3^{ab}	6.9^{b}	3.2^{ab}
Formulation 4	5.2^{b}	5.9^{b}	6.5^{b}	5.8^{b}	6.3^{b}	2.7^{b}

Average values are presented; values in the same column followed by the same letter are not significantly different by Tukey's test ($p < 0.05$).

ing an average above 7.0 for the evaluated features. Formulation 4 (20% composite flour + 5% of biomass) showed low averages for all sensorial features. The cookies supplemented with 20% of composite flour and 2% of biomass (formulation 3) had averages that did not differ significantly from formulation 2, that had no biomass addition. So, the addition of flours from by-products and biomass decreased the sensorial acceptance when compared to formulation 1, with values between 5 and 6 that, in the hedonic scale, corresponded to "like slightly" and "neither like nor dislike". The integral products and the ones supplemented with fibre had a slight rejection from most consumers [30, 32, 37]. Kohajdová et al. [2] developed cookies with 5%, 10% and 15% of orange and lemon fibres, and stated that the general acceptance was inversely proportional to the amount added. Furthermore, several authors reported the making of foodstuffs with the addition of 1–5% of micro-weed biomass and they obtained similar sensorial results [10, 29, 38].

Colour and stiffness are two of the main characteristics that the consumer observes and they affect the acceptance of the products [2, 38]. It was possible to observe that the darker the cookies (low L^* values), the lower rating was given by the evaluators to appearance. In this study, these cookies also had a lower acceptance in the sensorial analysis.

The purchase intention results followed the same pattern as the sensorial features, i.e. the highest average was given to formulation 1 (4.0). The other formulations did not differ statistically, with an average of 3.0 ("maybe I would buy it/maybe I wouldn't buy it"). However, the use of efficient marketing strategies, like packaging design and functional food claims, can improve the sale of the products.

Principal component analysis

The analysis of principal components illustrated in Fig. 1 showed that the four formulations had different characteristics in relation to the parameters evaluated. The two principal components PC1 and PC2 in the graph coordinates explained 92% of total variance.

Formulation 1 stood out due to the high content of saccharides and the best sensorial acceptance. This sample also had high values of colour parameters L^* and b^*, while formulation 2 showed higher values of a^*, moisture and, consequently, water activity. It was possible to observe that the addition of *S. platensis* to the cookies (formulations 3 and 4) afforded high content of proteins, lipids, ash, energy, as well as total phenolic compounds, antioxidant capacity (DPPH$^{\bullet}$, ABTS$^{\bullet +}$ and FRAP) and hardness, in particular in formulation 4 (20% composite flour + 5% of biomass).

The association of flours from by-products with *S. platensis* brought a nutrient and antioxidant increment to the products. However, there were

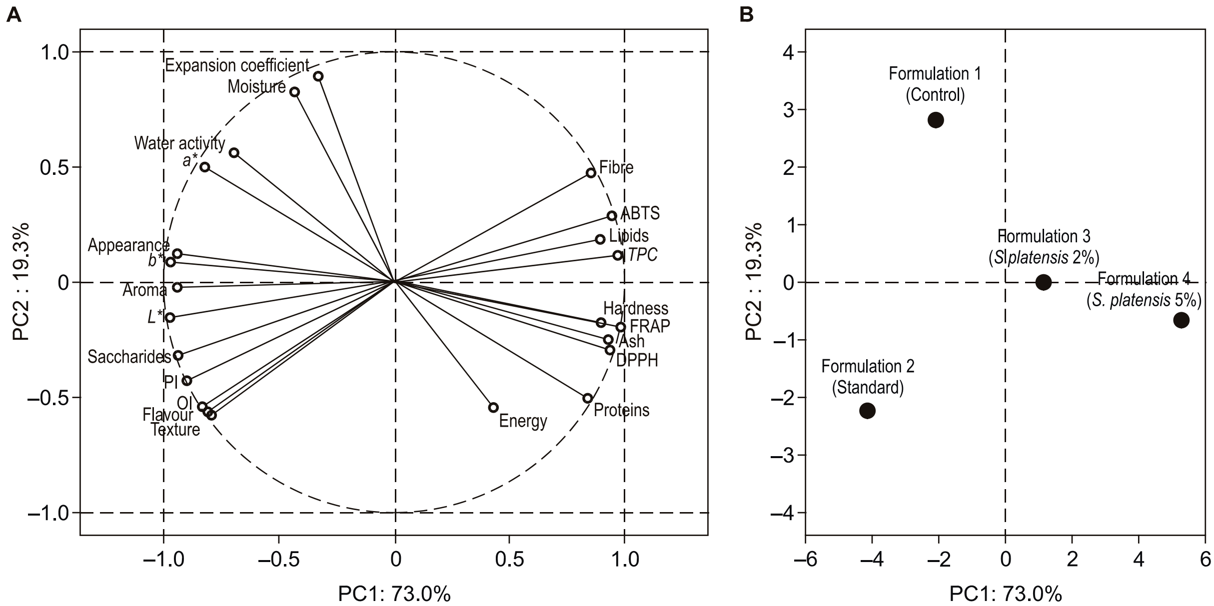

Fig. 1. Analysis of the principal components of 4 different cookies formulations.

A – analysis projection, B – sample graph.
PI – purchase intention, OI – overall impression, *TPC* – total phenolic content.

changes in sensorial acceptance, colour and hardness of the cookies formulated. Due to the nutritional value and the low cost of the ingredients added, the formulations studied can be a potential commercial product. Thus, this technology can be considered a promising alternative for the development of functional foods with positive impacts on nutritional, environmental and social aspects. As an improvement strategy, we suggest the addition of powder chocolate and chocolate chips to improve the dispersion of this component in the dough and to enhance the consumer acceptability.

CONCLUSIONS

The formulations developed with composite flour and *S. platensis* had nutritional enrichment related to fibre, protein and ash content, as well as high level of total phenolic compounds and high antioxidant capacity. Thus, it is viable to add these ingredients to the formulation of functional cookies. Although the expansion coefficient was not changed in formulations 3 and 4, there were alterations in colour and hardness, as well as lower sensorial acceptance when compared to the control. The microbiological analysis indicated that the products were safe for consumption. Therefore, the functional cookies developed can be considered a promising alternative for the application of new ingredients, from the economic, nutritional, technological and environmental points of view.

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