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Cereal bars made from brewers' spent grain, apple and *Spirulina platensis*: antioxidant activity and antihyperglycaemic effects

Barras de cereais formuladas com bagaço de malte, maçã e *Spirulina platensis*: atividade antioxidante e efeito antiglicêmico

Barritas de cereales formuladas con bagazo de malta cebada, manzana y de *Spirulina platensis*: actividad antioxidante y efecto anti-glucémico

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Abstract
Increase in diabetes prevalence has led to the need to develop practical food that has fewer available carbohydrates and/or nutrients with positive effects on postprandial glycaemia. This study aimed at evaluating the feasibility of using brewers' spent grain (BSG) at different ratios and granulometry (1.44 and 1.09 mm), along with Spirulina platensis biomass and dehydrated apples (DA), to produce cereal bars. Acceptability of formulations that had the highest percentages of DA was about 70%. Not only in vitro antioxidant activity, but also in vivo glycaemic response, glycaemic index (GI) and glycaemic load (GL) of cereal bars made from BSG and DA and of the ones with *S. platensis* were evaluated and compared to commercial cereal bars (CB). Cereal bars with S. platensis exhibited the highest content of phenolic compounds (124.28± 10.67 mg GAE/ 100 g), besides 22.47± 0.39 g/100 g of dietary fibre, which helped to decrease glycaemic response.
Keywords: Dietary fibre; Acceptability; Phenolic compounds; Anti-diabetic effect.

Resumo
Em face da crescente incidência de diabetes se faz necessário desenvolver alimentos práticos que contenham menos carboidratos disponíveis e/ou nutrientes que apresentem efeito positivo sobre a glicemia pós prandial. O objetivo deste trabalho foi avaliar a viabilidade do bagaço de malte (BSG) em diferentes proporções e granulometrias, e a adição de *Spirulina platensis* na elaboração de barras de cereais com maçã desidratada. Através da análise da composição proximal, propriedades físicas e sensoriais, verificou-se que a adição de BSG (1.44 e 1.09 mm) proporcionou aumento na concentração de fibras alimentares totais, proteínas e cinzas nas barras de cereais. O índice de aceitabilidade foi superior a 70% para as formulações que continham maior percentual de maçã desidratada. Na sequência, foram avaliadas a atividade antioxidante in vitro, a resposta glicêmica (RG), o índice glicêmico (IG) e carga glicêmica (CG) *in vivo* de barras de cereais com bagaço de malte e maçã desidratada (G), barras de cereais com *S. platensis* (SP) comparando com barra de cereal comercial (BC). A SP apresentou maior conteúdo de compostos fenólicos (124,28 ±

10,67 mg GAE/ 100 g), além de 22.47±0.39 g/ 100gde fibras alimentares que auxiliaram na redução da resposta glicêmica.
Palavras-chave: Fibras alimentares; Aceitabilidade; Compostos fenólicos; Efeito antiglicêmico.

Resumen
El aumento de la prevalencia de la diabetes ha llevado a la necesidad de desarrollar alimentos prácticos que tengan menos carbohidratos y/o nutrientes disponibles con efectos positivos sobre la glucemia posprandial. Este estudio tuvo como objetivo evaluar la viabilidad de utilizar el grano usado de los cerveceros (BSG) en diferentes proporciones y granulometría (1,44 y 1,09 mm), junto con la biomasa de *Spirulina platensis* y manzanas deshidratadas (DA), para producir barras de cereales. La aceptabilidad de las formulaciones que tenían los porcentajes más altos de DA fue de aproximadamente el 70%. No solo se evaluó la actividad antioxidante in vitro, sino también la respuesta glucémica in vivo, el índice glucémico (IG) y la carga glucémica (GL) de las barras de cereales elaboradas a partir de BSG y DA y de las que tenían *S. platensis* y se compararon con las barras de cereales comerciales (CB). Las barras de cereales con *S. platensis* presentaron el mayor contenido de compuestos fenólicos (124,28 ± 10,67 mg GAE / 100 g), además de 22,47 ± 0,39 g / 100 g de fibras dietéticas, lo que ayudó a disminuir la respuesta glucémica.
Palabras clave: Fibras alimentarias; Aceptabilidad; Compuestos fenólicos; Efecto antidiabético.

1. Introduction

The world population had about 1.9 billion overweight adults and 650 million obese people in 2016. This scenario has been associated with increase in non-communicable diseases, which are avoidable when energy intake from total fat and sugar is limited (WHO 2020). Development of food products must consider healthfulness, practicality, sensory appeal and sustainability. Even though cereal bars have been known as healthy and practical food, most of them exhibit high percentages of available carbohydrates in their composition, a fact that increases glycaemic indexes (GI). Efforts should be made to help consumers choose food with carbohydrates that do not increase postprandial glycaemia, so as to decrease the incidence of Type 2 Diabetes Mellitus, an important disease whose prevalence has been steadily increasing all over the world. Data issued by the International Diabetes Federation (IDF) in 2019 estimated that 8.3% of the world population (463 million people) has got diabetes (IDF 2019).

Nutritional approaches that help to control glycaemia, such as food with low digestion and absorption rates, besides low GI, have been recommended. Intake of high-fibre food has played an important role in decrease in glucose absorbance (Augustin et al. 2015, Hefni, Thomsson et al 2021). Its consumption is related to delayed gastric emptying and decrease in the number of postprandial hyperglycaemia episodes (Goff et al. 2018).

Brewers' spent grain (BSG) is the main solid residue of the brewing industry. According to the Brazilian Association of Breweries, Brazil produced 14.1 billion L of beer in 2018 (CERVBRASIL 2020). Every 100 L of beer is estimated to yield from 14 to 29 kg of BSG on a dry weight basis (Santos and Ribeiro 2005). The nutritional component that is found at high amounts in BSG is dietary fibre. Its inclusion in the human diet is an alternative to aggregate nutritional value to processed products and mitigate environmental impact by using Circular Economy practices (Slorach et al. 2019).

Balisteiro et al. (2017) carried out an in vitro study and showed that phenolic compounds found in juices made from Brazilian native fruit led to better postprandial glycaemic response due to their capacity for inhibiting enzymes of carbohydrate metabolism, α-amylase and α-glycosidase. Industrial apples have reached high levels of productivity since cultivars have been adapted to climate conditions in Brazil. One of the main phenolic compounds found in apples is phlorizin, which is associated with delayed glucose uptake, by means of inhibition of the Na2+-dependent glucose transporter (SGLT-1) in the intestine, inhibition of renal reabsorption and increase in glucose uptake by the adipose tissue (Makarova et al. 2015)

The carbohydrate path in the gastrointestinal tract is regulated by several intrinsic and extrinsic factors and related to different compositions of food and to their interaction. Similar amounts of carbohydrate with different combinations of other macronutrients lead to different glycaemic responses (Iyer et al. 2007). Hu et al. (2019) stated that peptides found in S. platensis (SP) exhibited anti-diabetic activity by inhibiting the activity of enzymes α-amylase, α-glycosidase and dipeptidyl

peptidase-4. In addition, SP has shown its ability as a metabolic modulator, associated with contents of proteins, unsaturated fatty acids, antioxidant activity, vitamins and minerals (Iyer et al. 2007).

The use of BSG in the development of food products bestows technological and environmental potential. SP biomass has had its use acknowledged not only as a dietary supplement but also as a source of nutrients with functional properties in food development. Therefore, this study aimed at producing potentially functional cereal bars at different ratios and particle sizes (granulometry) of BSG, SP biomass and dehydrated apples (DA).

2. Methodology

The following ingredients, which were bought at supermarkets in Ponta Grossa, Paraná (PR), Brazil, were used to produce cereal bars: oat flakes (Jasmine®), roasted unsalted granulated peanuts (Kuky®), honey (Primel®), glucose syrup (Yoki®), xanthan gum (Sabor Verde®) and Gala apples. *S. platensis* was purchased at BioMundo in Brasília, Distrito Federal, Brazil. Brewers' spent grain (BSG) was donated by the Koch Beer Brewery, located in Ponta Grossa, RS, Brazil. Enzymes α-amylase (StarMax TG120), protease (ProteMax 580L) and amyloglycosidade (StarMax GA 400) were donated by PROZYN®. Lancets (G-Tech®), strips Accu Check Active (Roche®) and a digital glucometer were bought in shops in Ponta Grossa, RS, Brazil. Anhydrous glucose (Gluc up 75, NewProv®) was also donated. All reagents had analytical grade.

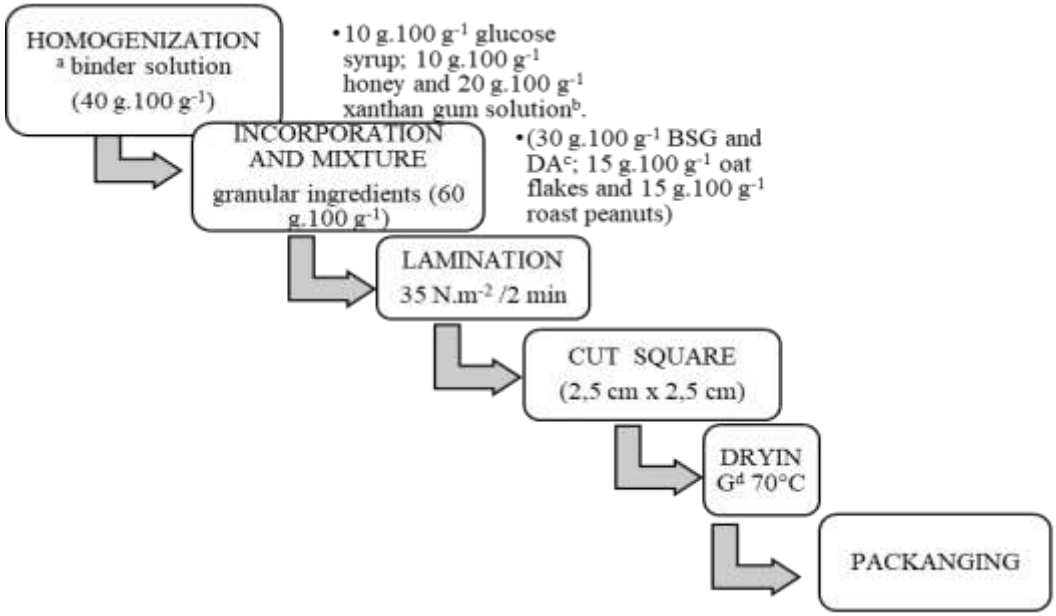
2.1 Raw material and experimental design

BSG was collected at the Koch Beer Brewery, in Ponta Grossa, PR, Brazil, and dried in a forced air oven (MA035; Marconi, Piracicaba) at 70 °C. It was standardized and characterized (g/100g): 3.42±0.06 moisture; 2.84±0.03 ash; 15.77±0.33 protein; 4.58± 0.46 lipid; 37.87±0.07 total fibre; 35.54±0.90 carbohydrate; 0.14±0.01 (g gallic acid equivalent/100 g sample) total phenolic compounds; and 0.01±0.001 (g gallic acid equivalent/100 g sample) antioxidant activity by the DPPH assay. Particle sizes (granulometry) of BSG were 1.09 and 1.44 mm when they were added to the cereal bars.

Gala apples were washed and sanitized with a chlorine solution (100 mg/L) and then rinsed under running water. They were cut lengthwise into strips (1 cm^2) and both ascorbic (200 mg/kg) and citric (200 mg/kg) acids were added to them so as to inhibit the enzymic browning process. Apple strips were dried in a forced air oven at 60 °C, up to ~8 g/100 g moisture. Dehydrated apples (DA) were characterized (g/100 g): 10.86± 0.39 moisture; 2.47±0.06 ash; 1.13±0.10 protein; 0.63±0.02 lipid; 8.06±0.09 total fibre; 76.85±0.54 carbohydrate; 0.17± 0.01 (mg GAE/ 100 g) total phenolic compounds; and 0.10±0.01 (mg AAE/100 g) antioxidant activity by the DPPH assay.

A simplex-centroid design was used to evaluate effects of BSG (1.09 and 1.44 mm), DA and interactions (binary and ternary mixtures) on proximate composition, physical properties, instrumental colour and sensory analysis of cereal bars (Tables 1, 2 and 3). In every cereal bar formula, BSG (1.09 and 1.44 mm) and DA represented 30 g.100 g-1, while ratio was determined by the simplex-centroid design, totalling 10 formulations. Ratios of BSG (1.09 mm), BSG (1.44 mm) and DA ranged from 5 to 30 g/100 g. Cereal bars at different ratios of BSG and DA were randomly produced, as shown in Table 1 and Figure 1. Addition of *S. platensis* biomass (2.5 g/100 g), as a partial substitution for peanuts, was restricted to only one formula, which had its phenolic composition, antioxidant activity and postprandial glycaemic response analysed.

Figure 1. Flow chart - cereal bars formulated with different proportions of BSG and DA.



[a]Stand mixer 400 W (BLACK DECKER). [b]xanthan gum solution prepared with water at 90 °C, homogenized and stored at 8 °C. [c]Simplex centroid design. [d]Kiln dryer model MA035 (Marconi, Piracicaba).
Source: Own authorships.

2.2 Proximate composition, physical properties, instrumental colour and sensory analysis

BSG and DA and cereal bars formulations were analysed in agreement with recommendations issued by the AOAC (Horwitz and Latimer 2005) regarding moisture (method 925.09), ash (method 923.03), protein (method 920.87) and lipid (method 920.85). Total carbohydrates were estimated by difference. The energy value was calculated by the summation of carbohydrates multiplied by 4 kcal, lipids by 9 kcal, proteins by 4 kcal and dietary fibres by 1.9 kcal (Menezes et al. 2016). In order to determine total dietary fibre, samples were defatted. Their quantification was found after enzymatic digestion and successive washes with water and ethanol at 78 and 96%, filtration and an enzymatic-gravimetric method (AOAC 985.29), with some adaptation.

The mechanical property of cereal bar hardness was measured by a TA-XT Plus Texture Analyser (Stable Micro Systems, Godalming, UK). Compressive strength needed to disrupt the sample was measured by an HDP/BSG probe in the following conditions: pre-test velocity N/A, test velocity 2.0 mm/s, post-test velocity 10.0 mm/s and penetration depth 15 mm/s. Water activity (Aw) was determined by a hygrometer (Aqualab Series 3TE, Decagon Devices, Inc.) at 25 °C in triplicate. Instrumental colour was determined by a colorimeter (Hunterlab®, Miniscan EZ, USA) and the Hue angle was calculated. Ten measurements of every formula were carried out.

Before the sensory analysis, *Bacillus cereus*, *Salmonella* sp, total and thermotolerant coliforms were analysed in raw materials, BSG, DA and cereal bar formulations, in compliance with the IN no. 60/2019 (Brasil 2019). Methodologies were applied as described by the American Public Health Association (APHA) (Vanderzant and Splittstoesser 1992).

The sensory analysis was carried out with 78 untrained judges (50 females and 28 males) whose ages ranged between 18 and 56 years old, after having been approved by the Ethics Committee (CLAE 65857017.4.0000.5215) at the Universidade Estadual de Ponta Grossa (UEPG), located in Ponta Grossa, PR, Brazil. Samples were monadically introduced to the judges. The 9-point hedonic scale (from 1-dislike extremely to 9-like extremely) was used to evaluate the following attributes: colour, aroma, texture, flavour and overall acceptance. Acceptability was calculated by the ratio between the resulting mean and the maximum grade given to overall acceptance, multiplied by 100 (ISO 2014).

2.3 Phenolic composition and antioxidant activity

In order to determine total phenolic composition and antioxidant activity, sample: solvent in the ratio of 1:50 (m/v) was used for BSG, cereal bar G and the cereal bar with 2.5 g/100 g SP. In the case of DA, the ratio was 1:20 (m/v). Bioactive compounds were extracted by a hydroalcoholic solution (ethanol:water in the ratio of 60:40 v/v). Concerning SP biomass, an aqueous extract at 1 g/L was used. All extracts were yielded with the help of an ultrasound device for 60 min at 25 °C. Then, they were centrifuged at 7.10^3 x g for 15 min at 25 °C (Himac CR21GII, Hitachi) and the supernatant was separated. Quantification of total phenolic compounds (TPC) was carried out by the Folin-Ciocalteau spectrophotometric method, in agreement with Singleton et al. (1999). Results were expressed as mg gallic acid equivalent/100 g sample (mg GAE/100 g). Antioxidant activity was based on the capture of the free radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) described by Brand-Williams, Cuvelier et al. (1995). Results were expressed as mg ascorbic acid equivalent/100 g sample (mg AAE/100 g).

2.4 Postprandial glycaemic response

In the analysis of glycaemic response, three cereal bars - a commercial one which leads the market and two formulated ones - were used. The commercial cereal bar (CB) was composed of glucose syrup, oat, rice and corn flakes, maltodextrin, BSG, salt, Brazil nuts, raisins, DA, brown sugar, inverted sugar, palm oil, polydextrose, cinnamon powder and corn oil, besides antioxidants soybean lecithin and mixed tocopherols. These ingredients were listed on its label. The other formulations under evaluation were: formulation G (Table 1) and formulation *SP*, which was similar to G, but had a smaller amount of peanuts (12.5 g/100 g) that was replaced with *SP* biomass (2.5 g/100 g).

Volunteers, whose ages ranged between 18 and 52 years old, were nondiabetic UEPG undergraduates, post-graduate students and employees. Mean body mass indexes (BMI) of the four women and six men were 24± 4 kg/m^2 and 25± 4 kg/m^2, respectively. The project was approved by the Ethics Committee (CLAE 80704717.6.0000.0105).

Glycaemic tests followed procedures described by FAO/WHO (1998). Participants fasted from 10 to 12 hours before the tests. A puncture on the pulp of a middle finger was performed to collect blood by a disposable lancet. Capillary glycaemia levels were determined by an Accu-Check® Active (ROCHE) digital glucometer 0 (before food consumption), 30, 60, 90 and 120 min after food consumption throughout the test. Four foods were tested to analyse glycaemic responses; the first intake was 100 mL glucose solution (Gluc up 75, NewProv®). In the second week, 35,38± 0,09 g - CB was consumed while, in the third week, 60,25± 0,35 g cereal bar with BSG (formulation G) was eaten and, in the fourth week (the last one), 60,26±0,30g cereal bar with BSG and *S. platensis* (formulation SP) was consumed. The amount of the reference food and cereal bars which were consumed corresponded to 25 g available carbohydrates.

Variables found by curves of glycaemic response followed methodologies proposed by Côrrea et al. (2007) and Balisteiro et al. (2017). They were:

- BG = basal glucose found at time 0, expressed as mg.dL^{-1};
- VPg = value of glucose peak, defined as the highest value above the basal one, found after food consumption, expressed as mg/dL;
- Dg = absolute glucose increase, defined as the absolute difference between the maximum glucose value found after the stimulus and expressed as mg/dL (Dg=VPg – BG);
- PIg = percentage increase in glucose (relation between absolute increase (Dg) and basal value (BG), expressed as percentage (PIg=Dg/BGx100);
- AUC = total area below the curve defined as the area below the glucose curve up to the x-axis, expressed as mg.(dL/min) ; VIg = velocity of increase in glucose, relation between absolute glucose increase (Dg) and time (min), when the peak value occurred, expressed as mg.(mL/min) (VIg=Dg/peak time).

GI of the three cereal bars under investigation was calculated by the ratio between the AUC of the food under analysis and the one of the reference food, expressed as percentage. Glycaemic load (GL) was calculated by multiplying GI by the amount of available carbohydrates in the portion of food under analysis, expressed as percentage.

2.5 Data analysis

All data were expressed as mean ± standard deviation. Homoscedasticity was checked by the Levene test. Differences among treatments were submitted to the one-way analysis of variance (ANOVA), followed by the Fisher's least significant difference test ($p \leq 0.05$) and the Student's t-test, which analysed TPC and antioxidant activity. The Welch's and the Kruskal-Wallis tests ($p \leq 0.05$ both) were applied to normally distributed data with unequal variance and samples without normal distribution, respectively. The non-parametric test Kruscal-Wallis was applied to data that exhibited $p<0.05$ for homoscedasticity by the Levene test. Isolated, binary and ternary effects of the different cereals bars on responses were evaluated by the Response Surface Methodology. The statistical quality of the proposed models was evaluated by the percentage of variability explained by the multiple linear regression equation (R^2), the coefficient of determination adjusted to the experimental data (R^2_{adj}), significance of the model and by the lack of fit of the model, $p \leq 0.05$ (Granato, Calado, et al. 2014). Residue normality was evaluated by the Shapiro-Wilk's test. Statistica v.13.2 software (Statsoft, USA) was used for the analyses. Graphapad Prism software (trial version) was used in Figure 3.

3. Results

3.1 Cereal bar characteristics under analysis

Proximate composition of cereal bars is shown in Table 1. Moisture contents ranged from 6.7±0.1 to 11.4±0.1 g/100 g ($p \leq 0.05$). Cereal bars with 10, 20 and 30 g/100 g DA exhibited the highest moisture contents when they were compared to formulations with and without BSG contents.

Ash contents ranged from 1.5± 0.1 to 2.0± 0.1 g/100 g ($p \leq 0.05$). The highest ash contents were found in formulations which had been added by 15 and 30 g/100 g BSG with 1.09 and 1.44 mm. Ktenioudaki et al. (2012) observed that, in grissini formulations, the higher the BSG concentrations (15, 25, 35 g/100 g), the higher the ash contents.

Protein contents ranged from 8.7±0.1 to 15.3±0.2 g/100 g ($p \leq 0.05$). The highest contents were found in formulations with 30 g/100 g BSG with 1.09 e 1.44 mm, while formulations with 15 and 20 g/100 g DA exhibited lower contents, in agreement with the amount of these macronutrients in added raw materials.

Lipid contents ranged from 10.8±0.1 to 15.2±0.3 g/100 g ($p \leq 0.05$) and showed significant influence of BSG addition. Fărcaş et al. (2015) carried out studies of BSG and found 6.6, 9.2 and 11 g/100 g lipids, respectively. Their studies also showed that the highest percentage of fatty acids consisted of linoleic, palmitic and oleic oils.

Total dietary fibre contents ranged from 8.7±0.1 to 23.2±0.8 g/100g ($p \leq 0.05$). The highest contents were found in formulations with 30 g/100 g BSG with 1.44 mm and 15 g/100 g BSG with 1.09 and 1.44 mm.

Carbohydrate contents ranged from 38.9±1.0 to 59.1±0.3 g/100 g ($p \leq 0.05$). The highest carbohydrate content was found in the formulation with 30 g/100 g DA, while formulations with no DA exhibited the lowest ones. Resulting energy values were similar to the ones reported by Menezes et al. (2016), who introduced two commercial cereal bars; one had 22 g/100g total fibre and 353 kcal/100g, while the other exhibited 4,6 g/100g total fibre and 389 kcal/100g.

Table 2 shows results of physical properties and instrumental colour of cereal bars. Hardness ranged from 143.28 to 237.14 N ($p \leq 0.05$); its highest value was found in the formulation with 30 g/100 g BSG with 1.44 mm.

Aw values of formulations were below 0.600 ($p \leq 0.05$), thus, they hindered microbial growth and enabled the product to be safe when it was stored in adequate packaging and at appropriate temperature.

Instrumental colour parameters of formulations were influenced by different BSG and DA concentrations ($p \leq 0.05$). Formulations with the highest BSG percentages (20 and 30 g/100 g) led to darker products by comparison with formulations with the highest percentages of DA (15, 20 and 30 g/100 g). Values of the Hue angle (hº) showed that all formulations, despite the significant difference among them ($p \leq 0.05$), are close to 90º; thus, they tend to yellow.

Formulations agreed with guidelines of the Brazilian legislation regarding microbiological aspects under evaluation. Therefore, they were considered appropriate for consumption.

Grades given to the five attributes under evaluation exhibited significant difference ($p \leq 0.05$), as shown in Table 3. The comparison between results of colour in the sensory analysis and instrumental colour based on L* values showed that consumers attributed more points to formulations with 30 g/100 g DA, 15 g/100 g BSG with 1.44 mm and DA, and 5 g/100 g BSG (1.09 and 1.44 mm) and DA, which tended to lighter colours. Overall acceptance ranged 5.21 to 7.05 points, an appreciable value, considering that fibre is added to the product. Acceptability was above 70% in the cases of formulations C, F, G and J (78, 73, 72 and 75%, respectively).

Table 1. Proximal composition of cereal bars with brewers spent grain, dehydrated apple according to experimental design (coded and real values).

Assay	BSG[1] 1.09 mm (g/100 g)	BSG 1.44 mm (g/100 g)	DA[2] (g/100 g)	Moisture (g/ 100 g)	Ashes (g/ 100 g)	Proteins (g/ 100 g)	Lipids (g/ 100 g)	DF[3] (g/ 100 g)	CHO's[4] (g/ 100 g)	Energy Value (including DF) (kcal/ 100 g)
A	1 (30)	0	0	7.45 ^{f} ±0.10	1.94 ^{a} ±0.01	15.31 ^{a} ±0.21	15.18 ^{a} ±0.27	18.61 ^{e} ±1.03	41.51 ^{ef} ±0.94	399 ^{a} ±4
B	0 (0)	1 (30)	0	6.65 ^{g} ±0.13	1.98 ^{a} ±0.01	14.97 ^{ab} ±0.19	14.20 ^{b} ±0.27	23.24 ^{a} ±0.84	38.96 ^{g} ±1.06	388 ^{bc} ±3
C	0 (0)	0	1 (30)	11.25 ^{ab} ±0.14	1.45 ^{g} ±0.09	8.65 ^{f} ±0.11	10.83 ^{f} ±0.07	8.72 ^{i} ±0.03	59.10 ^{a} ±0.35	385 ^{bcd} ±1
D	0.5 (15)	0.5 (15)	0	8.35 ^{e} ±0.30	1.90 ^{ab} ±0.02	14.76 ^{b} ±0.22	13.20 ^{c} ±0.07	22.55 ^{ab} ±0.56	39.23 ^{g} ±0.32	378 ^{ef} ±2
E	0.5 (15)	0	0.5 (15)	10.56 ^{cd} ±0.45	1.48 ^{fg} ±0.07	11.62 ^{e} ±0.82	11.35 ^{e} ±0.03	13.28 ^{g} ±0.37	51.72 ^{b} ±0.98	381 ^{de} ±3
F	0	0.5 (15)	0.5 (15)	10.14 ^{d} ±0.50	1.58 ^{de} ±0.06	11.52 ^{e} ±0.10	12.57 ^{d} ±0.06	15.67 ^{f} ±0.37	48.53 ^{c} ±0.74	383 ^{cd} ±2
G	0.33 (10)	0.33 (10)	0.33 (10)	11.13 ^{ab} ±0.58	1.64 ^{d} ±0.05	12.61 ^{d} ±0.22	11.20 ^{ef} ±0.03	21.62 ^{bc} ±0.72	41.80 ^{e} ±1.24	360 ^{i} ±4
H	0.67 (20)	0.17 (5)	0.17 (5)	10.81 ^{bc} ±0.39	1.75 ^{c} ±0.03	13.70 ^{c} ±0.01	13.18 ^{c} ±0.05	20.28 ^{d} ±0.79	40.28 ^{g} ±0.78	373 ^{fg} ±2
I	0.17 (5)	0.67 (20)	0.17 (5)	8.67 ^{e} ±0.15	1.86 ^{b} ±0.06	13.74 ^{c} ±0.24	11.36 ^{e} ±0.12	20.87 ^{cd} ±0.29	43.50 ^{d} ±0.27	371 ^{g} ±1
J	0.17 (5)	0.17 (5)	0.67 (20)	11.45 ^{a} ±0.12	1.54 ^{ef} ±0.03	12.26 ^{d} ±0.10	13.23 ^{c} ±0.71	12.15 ^{h} ±0.36	49.37 ^{c} ±0.46	389 ^{b} ±4
p-Value (homoscedasticity)				0.022	0.212	0.001	0.036	0.095	0.1374	0.3693
p-Value (*one-way* ANOVA)				≤0.001	≤0.001	≤0.001	≤0.001	≤0.001	≤0.001	≤0.001

[1]Brewers Spent Grain. [2]Dehydrated Apple. [3]Total Dietary Fibre. [4]Carbohydrates.
Preparation and analysis of the formulations were carried out at random; different letters in the same column represent statistically significant results ($p < 0.05$).
Source: Own authorships.

Table 2. Physical properties and instrumental colour of cereal bars manufactured with BSG e DA.

Assay	BSG[1] 1.09 mm (g.100 g^{-1})	BSG 1.44 mm (g.100 g^{-1})	DA[2] (g.100 g^{-1})	Hardness (N)	Aw	L*	a*	b*	h°
A	1 (30)	0	0	205.88^{b} ± 67.19	0.431^{e} ± 0.013	48.67^{c} ±1,40	5.90^{e} ±0.51	20.49^{ef}±1.03	73.96^{ab} ±0.88
B	0 (0)	1 (30)	0	237.14^{a} ± 51.11	0.510^{b} ± 0.001	49.03^{bc}±1.12	6.31^{de} ±0.41	22.14^{c} ±1.15	74.09^{a} ±0.48
C	0 (0)	0	1 (30)	143.28^{c} ± 40.36	0.428^{e} ± 0.002	51.42^{a} ±1.93	9.39^{a} ±0.70	27.99^{a} ±1.00	71.48^{d} ±0.88
D	0.5 (15)	0.5 (15)	0	151.82^{c} ± 50.73	0.534^{a} ± 0.006	48.00^{c} ±2.09	5.89^{e} ±0.56	20.96^{def}±1.28	74.32^{a} ±1.07
E	0.5 (15)	0	0.5 (15)	156.09^{c} ± 31.57	0.425^{e} ± 0.004	48.54^{c} ±1.08	6.80^{c} ±0.44	21.94^{cd}±0.72	72.78^{c} ±1.01
F	0	0.5 (15)	0.5 (15)	169.18^{c} ± 28.33	0.538^{a} ± 0.001	50.89^{a} ±1.78	6.75^{cd} ±0.67	22.43^{c} ±1.46	73.27^{cd} ±1.01
G	0.33 (10)	0.33 (10)	0.33 (10)	154.85^{c} ± 34.72	0.537^{a} ± 0.001	49.04^{bc}±1.69	6.33^{de} ±0.42	21.47^{cde}±1.03	73.58^{ab} ±0.57
H	0.67 (20)	0.17 (5)	0.17 (5)	158.31^{c} ± 43.67	0.541^{a} ± 0.006	48.07^{c} ±1.10	6.29^{de} ±0.21	20.34^{f} ±0.91	72.80^{c} ±0.59
I	0.17 (5)	0.67 (20)	0.17 (5)	159.08^{c} ± 55.15	0.491^{c} ± 0.002	48.30^{c} ±1.16	6.60^{cd} ±0.44	22.42^{c} ±0.97	73.60^{ab} ±0.56
J	0.17 (5)	0.17 (5)	0.67 (20)	148.41^{c} ± 37.43	0.476^{d} ± 0.005	50.25^{ab}±2.22	7.49^{b} ±0.68	23.91^{b} ±1.45	72.63^{c} ±1.02
p-Value (homoscedasticity)				≤0.001	0.002	0.523	0.067	0.418	0.477
p-Value (*one-way* ANOVA)				≤0.001	≤0.001	0.000	≤0.001	≤0.001	≤0.001

[1]Brewers Spent Grain.[2]dehydrated apple.
Different letters in the same column represent statistically significant results ($p < 0.05$).
Source: Own authorships.

Table 3. Sensory date of cereal bars manufactured with different proportions of BSG and DA.

Assay	BSG[1] 1.09 mm (g.100g^{-1})	BSG 1.44 mm (g.100 g^{-1})	DA[2] (g.100 g^{-1})	Colour	Aroma	Texture	Flavour	Overall acceptance	Acceptability (%)
A	1 (30)	0	0	5.95 ^{cde} ±1.49	5.99 ^{bc} ±1.72	4.91 ^{bc} ±2.03	5.23 ^{bc} ±1.84	5.33 ^{cd} ±1.54	67
B	0 (0)	1 (30)	0	5.62 ^{de} ±1.78	5.73 ^{bc} ±1.65	4.88 ^{bc} ±2.10	5.17 ^{c} ±1.84	5.38 ^{cd} ±1.74	60
C	0 (0)	0	1 (30)	8.18±0.99 ^{a}	7.15 ^{a} ±1.59	5.79 ^{ab} ±1.92	6.90 ^{a} ±1.66	7.05 ^{a} ±1.14	78
D	0.5 (15)	0.5 (15)	0	5.54 ^{e} ±1.77	5.65 ^{c} ±1.53	4.96 ^{bc} ±1.85	4.99 ^{c} ±1.88	5.21 ^{d} ±1.61	65
E	0.5 (15)	0	0.5 (15)	5.74 ^{cde} ±2.27	6.49 ^{ac} ±1.76	6.05 ^{a} ±2.12	6.35 ^{a} ±2.08	6.15 ^{bc} ±1.68	68
F	0	0.5 (15)	0.5 (15)	6.60 ^{bc} ±1.68	6.54 ^{ab} ±1.63	5.35 ^{abc} ±1.93	6.73 ^{a} ±1.57	6.53 ^{ab} ±1.36	73
G	0.33 (10)	0.33 (10)	0.33 (10)	6.58 ^{cd} ±1.44	6.29 ^{bc} ±1.35	5.83 ^{ab} ±1.60	6.64 ^{a} ±1.69	6.45 ^{ab} ±1.53	72
H	0.67 (20)	0.17 (5)	0.17 (5)	6.06 ^{cde} ±1.65	6.06 ^{bc} ±1.61	5.73 ^{ab} ±1.85	6.26 ^{ab} ±1.69	6.19 ^{bc} ±1.49	69
I	0.17 (5)	0.67 (20)	0.17 (5)	5.45^{e}±1.89	5.68 ^{c} ±1.69	4.51 ^{c} ±1.93	5.14 ^{c} ±1.76	5.26 ^{cd} ±1.69	66
J	0.17 (5)	0.17 (5)	0.67 (20)	7.54 ^{ab} ±1.40	7.13 ^{a} ±1.60	5.65 ^{ab} ±2.13	6.83 ^{a} ±1.84	6.77 ^{ab} ±1.23	75
p-Value (Test normality, K-S*)				<0.010	<0.010	<0.010	<0.010	<0.010	
p-Value (Kruskal-Wallis ANOVA)				≤0.001	≤0.001	≤0.001	≤0.001	≤0.001	

*K-S: Kolmogorov-Smirnov; different letters in the same column represent statistically significant results ($p \leq 0.05$), checked by Kruscal-Wallis.
Source: Own authorships.

3.2 Response surface modelling

The Response Surface Methodology was applied to evaluate effects of BSG/1.09 mm, BSG/1.44 mm and DA, besides binary and ternary mixtures in relation to proximate composition, physical properties and sensory analysis. These data were mathematically modelled and results are shown in Table 4. All multiple regression models were significant (p<0.05) and explained about 77% data variability (R^2adj>0.77), while residues followed normal distribution. However, all models exhibited lack of adjustment (p<0,05), i. e., since models shown by this study were not predictive, the methodology of optimization could not be applied. As a result, four criteria were used to choose the most adequate formulation to study glycaemic response: fibre content, hardness, acceptability and overall acceptance. The selected formulation was the ternary mixture of the central point with 10 g/100 g BSG/1.09 mm, 10 g/100 g BSG/1.44 mm and 10 g/100 g DA, which had an appreciable number of points for overall acceptance (6.45± 1.53) and hardness (154.85± 34.72). Besides, it had relevant dietary fibre content (about 22 g/100 g) and its acceptability was 72%.

Contour curves were used to visualize effects of BSG/1.09 mm, BSG/1.44 mm and DA on response variables (p<0.05) (Figure 2). The ternary mixture contributed to increase total dietary fibre contents, a fact that may be observed in the formulation with 10 g/100 g BSG (1.09 and 1.44 mm) and DA (Figure 2A). Single addition of 30 g/100 g BSG (1.44 mm) showed increase in hardness of cereal bar formulations (Figure 2B), while in the binary mixture at different ratios of BSG with 1.09 and 1.44 mm, there was decrease in hardness, a positive effect on sensory quality of cereal bars. Single addition of 30 g/100 g DA led to the lowest value of hardness. Figure 2C shows that overall acceptance was positively influenced by the addition of high DA contents to cereal bar formulations. However, they had low fibre contents and high carbohydrate ones, a fact that impacts directly on glucose absorption.

Table 4. Regression coefficients obtained by response surface methodology to explain the effects of BSG 1.09 mm and 1.44 mm and DA on the proximate composition, hardness, instrumental colour and sensory of cereal bars.

Effects	Regression coefficients	Standard error	t-Value	p-Value	-95% confidence	+95% confidence
			Moisture			
(A) BSG[1] 1.09 mm (g/100g)	8.44	0.61	13.90	< 0.001	6.96	9.93
(B) BSG 1.44 mm (g/100g)	7.06	0.61	11.61	< 0.001	5.57	8.54
(C) DA[2] (g/100g)	11.79	0.61	19.40	< 0.001	10.30	13.27
ABC	59.52	19.96	2.98	0.025	10.67	108.37
R^2	0.863					
R^2 adj	0.794					
p-Value (model)	0.025					
p-Value (lack of fit)	0.005					
p-Value (residuals)	0.596					
			Ash			
(A) BSG[1]	1.92	0.04	43.05	< 0.001	1.81	2.034

1.09 mm (g/100g)						
(B) BSG 1.44 mm (g/100g)	1.93	0.04	50.07	< 0.001	1.84	2.027
(C) DA[2] (g/100g)	1.42	0.04	31.73	< 0.001	1.31	1.528
AC	-0.77	0.23	-3.30	0.016	-1.33	-0.199
R^2	0.954					
R^2 adj	0.932					
p-Value (model)	0.016					
p-Value (lack of fit)	0.001					
p-Value (residuals)	0.138					
Proteins						
(A) BSG[1] 1.09 mm (g/100g)	14.99	0.43	34.53	< 0.001	13.97	16.02
(B) BSG 1.44 mm (g/100g)	14.75	0.43	33.97	< 0.001	13.73	15.78
(C) DA[2] (g/100g)	9.00	0.43	20.72	< 0.001	7.97	10.02
R^2	0.935					
R^2 adj	0.916					
p-Value (model)	<0.001					
p-Value (lack of fit)	0.001					
p-Value (residuals)	0.003					
Total Dietary fibre						
(A) BSG[1] 1.09 mm (g/100g)	19.31	0.97	19.92	< 0.001	16.94	21.68
(B) BSG 1.44 mm (g/100g)	23.39	0.97	24.13	< 0.001	21.02	25.76
(C) DA[2] (g/100g)	7.71	0.97	7.96	< 0.001	0.001	10.08
ABC	96.62	31.85	3.03	0.023	18.68	174.57
R^2	0.958					
R^2 adj	0.937					
p-Value (model)	0.023					
p-Value (lack	0.001					

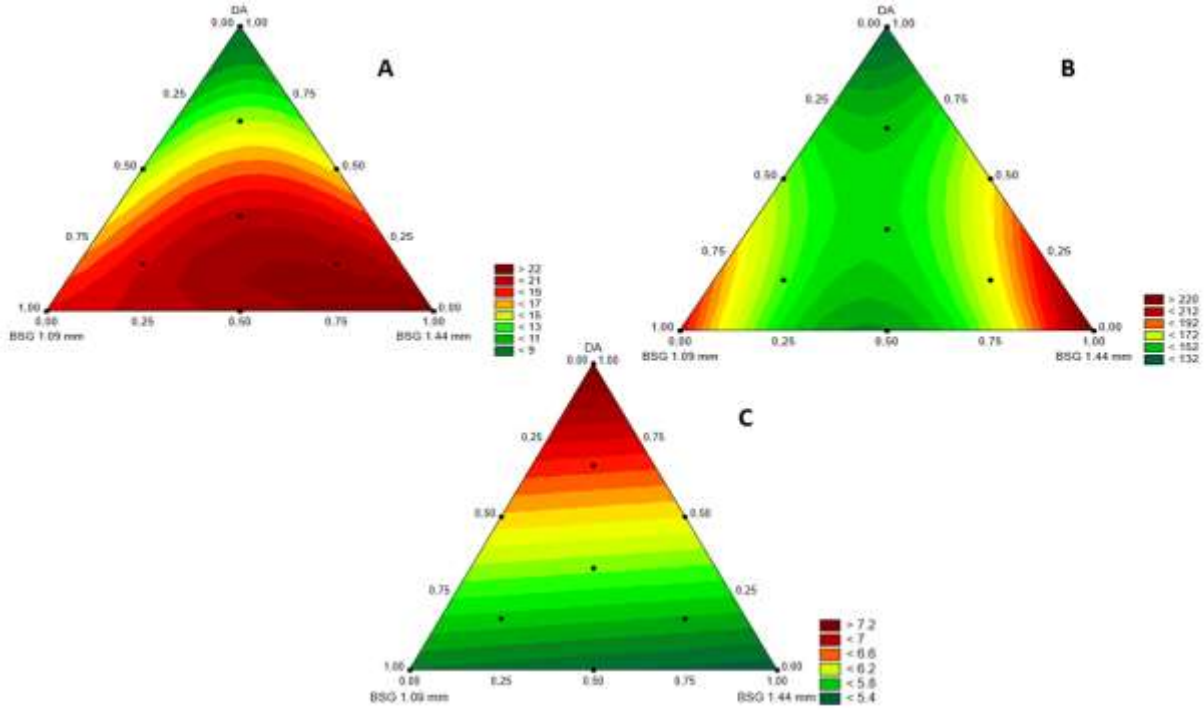
of fit)						
p-Value (residuals)	0.671					
Carbohydrates						
(A) BSG[1] 1.09 mm (g/100g)	41.15	1.24	33.15	≤ 0.001	38.11	44.19
(B) BSG 1.44 mm (g/100g)	39.46	1.24	31.79	≤ 0.001	36.42	42.49
(C) DA[2] (g/100g)	59.01	1.24	47.53	≤ 0.001	55.97	62.05
ABC	-122.90	40.80	-3.01	0.024	-222.72	-23.07
R^2	0.961					
R^2 adj	0.941					
p-Value (model)	0.024					
p-Value (lack of fit)	0.001					
p-Value (residuals)	0.098					
Hardness						
(A) BSG[1] 1.09 mm (g/100g)	200.42	10.03	19.98	< 0.001	175.87	224.97
(B) BSG 1.44 mm (g/100g)	225.88	10.03	22.51	< 0.001	201.33	250.43
(C) DA[2] (g/100g)	130.13	8.66	15.02	< 0.001	108.93	151.33
AB	-279.35	52.03	-5.37	0.002	-406.66	-152.05
R^2	0.897					
R^2 adj	0.845					
p-Value (model)	0.002					
p-Value (lack of fit)	0.002					
p-Value (residuals)	0.797					
Colour						
(A) BSG[1] 1.09 mm (g/100g)	5.88	0.15	38.25	< 0.001	5.39	6.37
(B) BSG 1.44 mm (g/100g)	5.46	0.14	38.38	< 0.001.	5.00	5.91
(C) DA[2] (g/100g)	8.09	0.15	52.63	< 0.001.	7.60	8.58
AC	-4.98	0.81	-6.15	0.009	-7.56	-2.41

ABC	16.89	4.61	3.66	0.035	2.21	31.57
AB(A-B)	12.06	2.75	4.38	0.022	3.30	20.81
AC(A-C)	-9.35	2.74	-3.42	0.042	-18.06	-0.64
R^2	0.988					
R^2 adj	0.965					
p-Value (model)	0.044					
p-Value (lack of fit)	0.005					
p-Value (residuals)	0.808					
Aroma						
(A) BSG[1] 1.09 mm (g/100g)	5.90	0.08	77.72	< 0.001.	5.71	6.10
(B) BSG 1.44 mm (g/100g)	5.72	0.08	75.29	< 0.001.	5.52	5.91
(C) DA[2](g/100g)	7.19	0.08	94.66	< 0.001.	7.00	7.39
AB(A-B)	6.03	1.54	3.92	0.011	2.08	9.98
AC(A-C)	-6.80	1.54	-4.42	0.007	-10.75	-2.85
R^2	0.984					
R^2 adj	0.970					
p-Value (model)	0.013					
p-Value (lack of fit)	0.001					
p-Value (residuals)	0.109					
Texture						
(A) BSG[1] 1.09 mm (g.100 g^{-1})	4.91	0.16	30.96	< 0.001.	4.50	5.32
(B) BSG 1.44 mm (g.100 g^{-1})	4.90	0.14	34.89	< 0.001.	4.54	5.26
(C) DA[2] (g.100 g^{-1})	5.77	0.15	37.45	< 0.001.	5.37	6.16
BC	2.88	0.80	3.60	0.016	0.82	4.94
AB(A-B)	8.74	2.51	3.48	0.018	2.28	15.19
R^2	0.933					
R^2 adj	0.880					
p-Value (model)	0.018					
p-Value (lack of fit)	0.004					
p-Value (residuals)	0.031					

Flavour						
(A) BSG[1] 1.09 mm (g/100g)	5.32	0.206	25.78	< 0.001.	4.79	5.85
(B) BSG 1.44 mm (g/100g)	5.08	0.233	21.79	< 0.001.	4.48	5.68
(C) DA[2] (g/100g)	7.04	0.226	31.10	< 0.001.	6.46	7.62
BC	3.42	1.178	2.90	0.034	0.39	6.45
AB(A-B)	11.53	3.695	3.12	0.026	2.03	21.02
R^2	0.939					
R^2 adj	0.890					
p-Value (model)	0.026					
p-Value (lack of fit)	0.003					
p-Value (residuals)	0.188					
Overall acceptance						
(A) BSG[1] 1.09 mm (g/100g)	5.491	0.246	22.362	< 0.001.	4.910	6.071
(B) BSG 1.44 mm (g/100g)	5.337	0.246	21.735	< 0.001.	4.756	5.918
(C) DA[2] (g/100g)	7.269	0.246	29.602	< 0.001.	6.688	7.849
R^2	0.817					
R^2 adj	0.765					
p-Value (model)	0.003					
p-Value (lack of fit)	0.003					
p-Value (residuals)	0.404					

[1]Brewers Spent Grain.[2]dehydrated apple.
Source: Own authorships.

Figure 2. The contour plots of response surface methodology: A Total Dietary fibres (g/ 100). B mechanical property – hardness (N). C Overall acceptance (scores).



Source: Own authorships.

3.3 Postprandial glycaemic response

In order to study the effect of cereal bar consumption on postprandial glycaemia, the CB was compared with formulation G (10 g/100 g BSG/1.09 mm; 10 g/100 g BSG/1.44 mm and 10 g/100 g DA), found in the mixture design, and with formulation *SP* with 2.5 g/100 g *S. platensis*.

Proximate composition is shown in Table 5. The cereal bar *SP* was found to exhibit the highest ash and protein contents ($p<0.05$). Replacement of peanuts with 2.5 g/100 g *S. platensis* decreased the lipid content significantly ($p<0.05$). The energy value of the CB was higher than the one of formulated bars.

Formulation SP exhibited the highest mean value ($p<0.05$) of TPC content and antioxidant activity by DPPH (Table 5). *S. platensis* addition with 1222±45 mg GAE/100g TPC content was found to be a viable alternative to increase antioxidant activity of cereal bars. Likewise, Bolanho et al. (2014) added biomass to cookies and found TPC content from 170 to 230 mg GAE/100g. Antioxidant content may modulate metabolism positively. Iyer et al. (2007) observed decrease in the glycaemic index of carbohydrate-based meals with the addition of 2.5 g *S. platensis* biomass.

Table 5. Proximate composition cereal bars (G, SP and BC) used to evaluation of the glycaemic response.

Assay	Moisture (g. 100 g^{-1})	Ash (g. 100 g^{-1})	Proteins (g. 100 g^{-1})	Lipids (g. 100 g^{-1})	Total fibre (g. 100 g^{-1})	CHO's[4] (g. 100 g^{-1})	Energy Value (including DF[4]) (kcal)	TPC[5] (mg GAE/100g)	DPPH (mg AAE/100 g))[6]
G[1]	8.15 ^{b}±0.20	1.66 ^{b}±0.01	13.29 ^{b}±0.19	12.50 ^{a}±0.11	21.96 ^{a}±0.38	42.44 ^{b}±0.28	377	95.87±2.15	16.19±2.01
SP[2]	8.2 ^{b}±0.26	1.77 ^{a}±0.03	14.52 ^{a}±0.19	10.97 ^{b}±0.06	22.47 ^{a}±0.39	42.00 ^{b}±0.25	368	124.28±10.67	21.88±1.69
CB[3]	10.71 ^{a}±0.13	0.97 ^{c}±0.02	5.47 ^{c}±0.10	4.69 ^{c}±0.18	3.23 ^{b}±1.21	74.93 ^{a}±1.37	390	ND*	ND*
p-Value (homoscedasticity)	0.360	0.256	0.622	0.453	0.287	0.107	-	-4.52**	-3.76**
p-Value (ANOVA)	≤0.001	≤0.001	≤0.001	≤0.001	≤0.001	≤0.001	-	0.011	0.020

[1]cereal bar with 20g/ 100g of BSG and 10g/100g of DA. [2]cereal bar with 2.5 g/100g *S. platensis*. [3]comercial cereal bar. [4]carbohydrats. [5]total phenolic content. Galic Acid Equivalent (GAE). Ascorbic Acid Equivalent (AAE). *not determined. ** Student's t-test.
Different letters in the same column represent statistically significant results ($p \leq 0.05$).
Source: Own authorships.

Values of basal glycaemia (Table 6) found before the intake of all food under investigation did not show any significant difference (p>0.05). The American Diabetes Association establishes that adults' fasting glycaemia values must be below 100 mg/dL (American Diabetes Association, 2020). Thus, volunteers that took part in this study were considered healthy individuals (89±7 mg/dL).

Increase in glycaemia was found after food consumption. The highest glycaemic peaks were found after intake of anhydrous glucose (reference food) and for the CB, whose values were 145±22 and 147±27 mg/dL, respectively. Formulations G and *SP* had similar behaviour and the lowest glycaemic peaks (107±11 and 109±9 mg/dL, respectively). Figure 3 shows the comparison among all food under investigation.

The comparison between both formulations under study (G and *SP*) showed similar behaviour of the glycaemic curve (p>0.05). There was increase in serum glucose levels in the first 30 minutes, followed by their decrease (97±22 and 95±16 mg/dL, respectively) 60 minutes after intake. Values close to the basal one were reached after 90 minutes (Figures 3 C and D). Neither increase nor abrupt decrease in postprandial glycaemia was observed, by comparison with the reference food and the CB. According to Augustin et al. (2015), fluctuations in glycaemia induce high oxidative stress, a fact that may not only cause deleterious effect on cells but also worsen the prognosis of Type 2 Diabetes, thus, increasing mortality rate.

Dietary fibre retards glucose absorption and control serum levels (Goff, et al. 2018; Augustin, et al. 2015). It was observed in glycaemic responses given to formulations G and *SP* and shows their potential as food that can maintain health.

Regarding the other variables under analysis (Table 6), significant differences (p<0,05) were found among formulations under study and the commercial one in terms of absolute glucose increase (DG), percentage increase in glucose (PIg) and velocity of increase in glucose (VIg), i. e., both formulations G and *SP* exhibited the lowest values.

However, the area under curve (AUC) did not show any significant difference among samples (p>0.05). Food whose carbohydrates are digested, absorbed and metabolized fast are the ones that have high glycaemic index (GI) and glycaemic load (GL) (GI values >70 and GL values >20) (Augustin et al. 2015). Thus, even cereal bars with high fibre and micronutrient contents with antioxidant capacity did not yield products with low GI and GL.

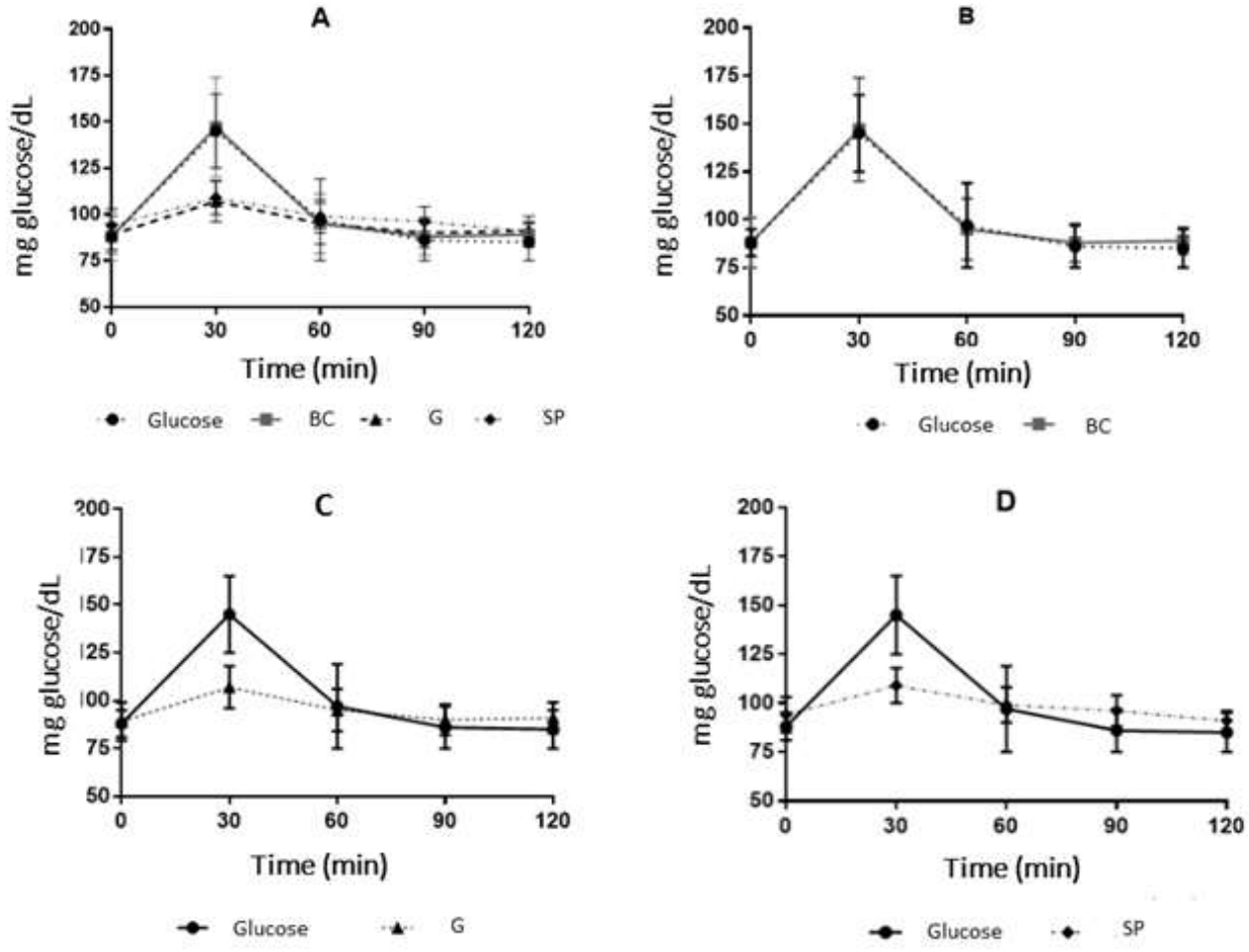
Considering that cereal bars were made with agglutinant agents composed of glucose and fructose, blood glucose increased. However, cereal bars G and SP exhibited low velocity of increase in glucose (VIg), as shown in Table 6. Therefore, monosaccharides in formulations G and *SP* were found to be gradually absorbed by bodies.

Table 6. Postprandial glycaemic response: Absolute glucose increase (Dg), percentage increase in glucose (PIg), velocity of increase in glucose (VIg) and area under curve (AUC).

Parameters	Glucose[1]	CB[2]	G[3]	SP[4]	p-Value (homoscedasticity)	p-Value ANOVA/ Welch
GB (mg glucose/dL)	89 ± 7	88 ± 13	89 ± 10	94 ± 9	0.424	0.610
VPG mg glucose/ dL	145^{a} ± 22	147^{a} ± 27	107^{b} ±11	109^{b} ± 9	0.002	≤0.001
Dg (mg/dL)	56^{a} ± 21	58^{a} ± 24	18^{b} ± 8	17^{b} ± 6	≤0.001	≤0.001
PIg (%)	63^{a} ± 23	68^{a} ± 30	21^{b} ± 10	18^{b} ± 8	≤0.001	≤0.001
VIg (mg/mL.min)	1.88^{a} ± 0.70	1.94^{a} ± 0.80	0.58^{b} ± 0.28	0.50^{b} ± 0.26	≤0.001	≤0.001
AUC	12414 ± 1338	12633 ± 1365	11444 ± 1004	11897 ± 712	0.547	0.104
IG (%)	--	102 ± 8	93 ±10	96 ± 7	0.480	0.069
CG (%)	--	26 ± 2	23 ±3	24 ± 2	0.480	0.069

[1]as control. [2]comercial cereal bar. [3]cereal bar with 20g/100g of BSG and 10g/100g of DA. [4]cereal bar with 2.5 g/100g *S. platensis*. Different letters in the same column represent statistically significant results (p ≤0.05).
Source: Own authorships.

Figure 3 A, B, C and d. Postprandial blood glucose of healthy individuals at 0, 30, 60, 90 and 120 min after test meals: glucose (as control); BC (commercial cereal bar); G (cereal bar with 20g/ 100g BSG and 10g/ 100g DA); SP (cereal bar with 2.5 g/ 100 g *S. platensis*).



Source: Own authorships.

4. Discussion

Concerning proximate composition, this study inferred that macronutrients of every raw material affected results of formulations under study. The ingredient that contributed to increase lipid concentration in formulations was peanuts (addition of 15 g/100 g). Resolution of the Collegiate Board (RCB) no. 359, issued on December 23rd, 2003, establishes that cereal bars with more than 10 g/100 g lipids must be sold as 20 g portions (Brasil, 2003).

In agreement with RCB no. 54, issued on November 12th, 2012, products that exhibit at least 2.5 g fibre per portion may use the attribute "source of fibre". When they exhibit 5 g fibre per portion, they can claim to be food with "high content" of fibre (Brasil 2012). Thus, resulting formulations may be considered food with high fibre content (formulations B and D) and sources of fibre (formulations A, E, F, G, H, I and J). The only formulation that cannot claim to have nutritional property is formulation C, which does not contain BSG.

The Food and Drug Administration (FDA 2020) assumes that the daily value (% DV) of dietary fibre in a 2,000-calorie diet is 28 g. Products whose DV is equal or above 20% may claim the label "high content of dietary fibre" (FDA 2020).

According to the FDA, they can be sold in 40 g portions (FDA 2018). Therefore, formulations developed by this study may claim to be "high fibre" ones, except formulations C, E and J.

Regarding hardness, data showed that working with different particle sizes of BSG in cereal bar production led to decrease in hardness, a factor that favours product acceptance. Results of hardness of cereal bars reported in the literature show that there is gradual increase when high percentages of agro-industrial by-products are added, since they have considerable values of dietary fibre (Damasceno et al. 2016).

The sensory analysis showed that these bars had satisfactory acceptance (Damasceno et al. 2016) and provided an opportunity to value BSG in the human diet.

Limits of *S. platensis* incorporation into formulations are the ratio to be added and its cost, since it should provide a certain quantity of bioactive compounds, such as antioxidants, without affecting sensory acceptance. In the current legislation, there is a limit for microbial biomass incorporation due to the problem caused by accumulation of uric acid in the organism (Hu et al. 2019).

In order to understand why there was no significant difference in decrease in glucose absorption between cereal bars G and *SP*, two hypotheses may be considered. One of them says that polyphenol concentration found in cereal bar *SP* was not enough to influence activity of digestive enzymes. The phenolic compound content found by Balisteiro et al. (2017) was higher than the ones found in the study reported by this paper. These authors showed that phenolic compounds inhibited the activity of enzymes α-amylase and α-glycosidase of healthy individuals and stated that the polyphenol content decreased postprandial glycaemic response.

Digestive enzymes α-amylase and α-glycosidase may only hydrolyse glycosidic bonds α-1,4 and α-1,6 (Balistiero et al. 2017). The other hypothesis is related to the digestible carbohydrate content (with chains of two or more monomers), which may have been very low in cereal bars G and *S*, and to the fact that enzymes α-amylase and α-glycosidase did not have any substrate to react to. Based on it, decrease in glycaemic response found in cereal bars G and *SP* was associated with the fibre content.

These data are relevant because food industries can produce healthy food that affect glycaemic control positively and are sustainable.

5. Conclusion

The formulation with the ternary mixture of the central point, G, with 10 g/100 g of every dependent variable got an appreciable number of points for overall acceptance (6.4±1.5) and acceptability rate around 70%. Considering that this product exhibits 22 g/100 g dietary fibre, it was chosen as the best formulation to be used in the study of the effect of glycaemic response.

After intake of cereal bars G and *SP*, similar and gradual release of glucose in blood was observed. This effect on decrease in postprandial glycaemic response was associated with the total fibre content. The incorporated *S. platensis* amount was insufficient to increase the antioxidant capacity of cereal bars significantly. Decreasing hyperglycaemic peaks is fundamental to mitigate health disorders, such as Type 2 Diabetes Mellitus and insulin resistance. Besides, high fibre and phenolic compound contents help to prevent several no communicable chronic diseases.

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