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# An insight into the use of extruded brewers' spent grain as a healthy human snack ingredient. Effects on food structure, sensory quality, satiety and gastrointestinal tolerance

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ABSTRACT

Reduced in sugar biscuits made with fructooligosaccharides (15.2%) and 0%, 8% and 17% of extruded brewers' spent grain (EBSG) were formulated as a healthy snack. The investigation aimed to study the impact of the formulation on technological parameters, sensory attributes and gastrointestinal effects. Biscuits' hardness, color, water and oil holding capacity, starch gelatinization and microstructure were analyzed. Products were tested in a randomized blind cross-over design trial with 51 adults, determining sensory acceptance, satiety and gastrointestinal tolerance. Results showed an addition of 17% EBSG caused product hardening, darker color, and a more compact structure. These parameters were in line with consumers' product description and caused lower acceptability, while the acceptability of biscuits with 8% or 0% EBSG did not differ ($p > 0.05$). However, biscuits with 17% of EBSG were the only ones to exhibit a positive effect on short-term appetite control. Formulations showed good and similar gastrointestinal tolerance ($p > 0.05$).

## 1. Introduction

Nowadays different food market requirements are arising, which are making evident the need for nutrition security. COVID-19 pandemic has had a detrimental effect on diet quality and food insecurity (Picchioni, Goulao, & Roberfroid, 2021), registering an increase in snack consumption and comfort foods (Aguilar-Martínez et al., 2021; Bonaccio et al., 2021; Sánchez et al., 2021). In addition, war in Ukraine has shown the world resource scarcity as 22 million tons of 2021 year Ukraine crop cannot reach swathes of people highly dependent on the grain (Hyslop, 2022; Therefore, FAO 2030 Agenda is looking forward to ending hunger, achieving food security, improving nutrition and, promoting sustainable agriculture, consumption and production patterns (UN General Assembly, 2015). Besides, people have become more aware on health, resulting in an increase demand for healthy lifestyle products (Kerry, 2021). As a consequence, many governments have adopted food reformulation policies due to the increase in non-communicable diseases based on the reduction of sodium, trans-fatty acids and added sugars in foods (Gressier, Sassi, & Frost, 2020).

Dietary fiber confers a wide range of health effects which depend on the physicochemical properties of the fiber, the gut microflora in the human gastrointestinal tract (Stewart, Nikhanj, Timm, Thomas, & Slavin, 2010). Soluble dietary fibers are associated to increase the viscosity of the aqueous phase leading to the reduction of glycemic response and plasma cholesterol. Insoluble dietary fibers result in increased fecal bulk and decrease in intestinal transit (Mudgil, 2017). Thus, the bulking and viscosity properties of dietary fiber are predominantly responsible for their influence on satiety and satiation (Slavin & Green, 2007). Dietary fiber chemical structure also influences on its fermentability: soluble polysaccharides are fermented faster than insoluble saccharides, such as cellulose (Capuano, 2017).

Therefore, the incorporation of dietary fiber into food products may have some dose-related undesirable gastrointestinal effects due to their natural osmotic potential and excessive fermentation (Le Bourgot,

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Rigaudier, Juhel, Herpin, & Meunier, 2022), which also depends on the individual sensitivity factors and adaptation to chronic consumption (Marteau & Seksik, 2004). However, as indicated by Livesey (2001) the properties of dietary fiber that cause them to be indigestible and so contribute to abdominal discomfort are also responsible for their physiological benefits: risk and benefit are not always separable. In fact, few researchers have documented what people are willing to allow themselves to experience regarding the symptomatic response to dietary fiber. Additionally, many of the symptoms may have no impact on acceptability of the product containing dietary fiber, as long as, the product sensory acceptability is good (Livesey, 2001).

Moreover, foods formulated with by-products may present enhanced health promoting properties, while technological and sensory attributes such as color, texture and flavor, may be affected (Duizer, West, & Campanella, 2020). Therefore, the challenge behind product reformulation is improving the content of certain nutrients without increasing the energy content, maintaining food safety levels, flavor and texture so that the product continues to be accepted by consumers (AECOSAN, 2020).

In this context, reduced in sugar biscuits high in dietary fiber have been developed. Two different sources of dietary fiber were included to balance the potential physiological effects: fructooligosaccharides, as sugar substitute, and extruded brewers' spent grain. Brewers' spent grain (BSG) represents the major by-product from the brewers industry (Lynch, Steffen, & Arendt, 2016). Although, BSG is not considered a novel food by the European Union according to the Regulation (EU) 2015/2283 (European Commission (EC), 2024), it is mainly used as cattle feed or as source of energy in breweries (Lynch et al., 2016). Besides, this brewers by-product is source of insoluble dietary fiber mainly cellulose, lignin and hemicellulose, proteins and bioactive compounds (Lynch et al., 2016).

Under this context, by a circular economy approach leading to the revalorization of agro-industrial by products, a sustainable healthy snack was proposed. The aim of this study was to define whether formulated biscuits presented market potential. Thus, the effect of substituting reduced in sugar biscuits' digestible carbohydrate content with different contents of extruded brewers' spent grain has on products' technological properties, sensory attributes and gastrointestinal effects (satiety and gastrointestinal wellbeing) was determined.

## 2. Materials and methods

### 2.1. Food ingredient

Brewers' spent gain (BSG) was given by Fábricas Nacionales de Cerveza (Minas, Uruguay). It was obtained from the last 24 h of lager beer production made up of barley and maize. Brewers' spent grain nutritional characterization and microbiological quality were previously analyzed. These results were published in Gutiérrez-Barrutia, del Castillo, Arcia, and Cozzano (2022). Once received BSG was frozen and afterwards it was dried at 45 °C ± 2 °C in a convection oven and grounded in a laboratory mill (Retsch ZM 200) to a particle size of 0.5 mm. Based on Gutiérrez-Barrutia et al. (2022), BSG was extruded at 15.8% sample moisture content, 163.4 revolutions per minute screw speed, 122.5 °C barrel temperature and 100 revolutions per minute feeding rate. A single screw extruder Brabender Co Cordero E330 (Germany) was used with die diameter of 4 mm, screw diameter of 1.8 cm and screw length equal to 40 cm. Extrudates were grounded to 0.5 mm and kept at −20 °C.

### 2.2. Biscuit formulation

Based on a traditional sweet biscuit (TB) three different reduced in sugar biscuits were formulated according to European Legislation (CE N° 1924/2006); i.e. at least 30% of sugar reduction from a reference recipe. Two types of fructooligosaccharides (FOS) Orafti® P95 and Orafti® L95 (Orafti®GR, Beneo, Belgium) were used as sugar substitute and soluble fiber source. Wheat flour was substituted by extruded brewers' spent grain (EBSG), mainly as source of insoluble fiber, in three different levels 0 %, 14 % and 30 %. Thus, reduced in sugar biscuits were formulated with the addition 15.2 g/100g of FOS and different levels of EBSG: 0% (FOS-EBSG0), 8% (FOS-EBSG8) and 17% (FOS-EBSG17). Biscuits formulations are presented in Table 1.

After preparing the dough, it was left to stand at 7 °C for 24 h. Then it was manually stretched with a rolling pin to 0.3 cm and cut with a circular metallic cutter (6 cm diameter). Biscuits were baked in a convection oven for 15 min at 180 °C and left to cool at room temperature (24 °C). Finally, products were grounded and kept frozen (−20 °C) for chemical analysis.

### 2.3. Biscuits' techno-functional characterization

#### 2.3.1. Texture

Biscuit's hardness was measured by the peak force during penetration (Handa, Goomer, & Siddhu, 2012). Texture Profile Analyzer (Model: TA-XT Plus, Stable Micro Systems, Surrey, England) was used in a compression test mode. The biscuits were placed in the center of a circular support insert and were pressed by a 2 mm cylinder probe. Pre-test, test and post-test speeds were 6.0, 5.0 and 6.0 mm/s, respectively (Woody, 2003). The return distance was set at 60 mm. Ten replicates of each sample were analyzed.

#### 2.3.2. Physical analysis

Biscuits diameter was determined by measuring perimeter. Spread ratio was calculated as the coefficient between biscuits' diameter and thickness. Five replicates of each sample were analyzed.

#### 2.3.3. Color

Color was determined on grounded biscuits and EBSG using Specord 210 Plus (Analytik Jena, Germany) which defined each color from the CIE Lab color space: $L^*$ (luminosity), $a^*$ (red-green) and $b^*$ (yellow-blue), was determined. Experiments were performed in triplicate.

Biscuits' melanoidin content was measured as an indicator of the development of Maillard reaction responsible for the browning of baked products. Melanoidins were extracted by an aqueous extraction proposed by Patrignani and González-Forte (2020) using Amicon® Ultra-15 Centrifugal Filter Units with a 10 kDa nominal molecular mass cutoff membrane. The high molecular weight (HMW) fraction absorbance was measured at 360 nm. Caramel was used as a standard. Experiments were performed in triplicate and the results were expressed in mg of caramel melanoidins equivalent per gram of sample.

#### 2.3.4. Water and oil holding capacity

Water and oil holding capacity was measured for EBSG and biscuits

**Table 1**
–Biscuits formulation.

| Ingredients | FOS-EBSG0 (g/100g) | FOS-EBSG8 (g/100g) | FOS-EBSG17 (g/100g) |
|---|---|---|---|
| Wheat flour | 57.0 | 49.0 | 40.0 |
| EBSG | 0.0 | 8.0 | 17.0 |
| Brown sugar | 3.8 | 3.8 | 3.8 |
| FOS (Orafti® P95) | 10.2 | 10.2 | 10.2 |
| FOS (Orafti® L95) | 5.0 | 5.0 | 5.0 |
| Baking powder | 1.0 | 1.0 | 1.0 |
| Butter | 11.0 | 11.0 | 11.0 |
| Egg | 12.0 | 12.0 | 12.0 |
| Water (mL) | 3 | 3 | 3 |

FOS-EBSG0: Biscuit with 15.2% FOS and no EBSG. FOS-EBSG8: Biscuit with15.2% of FOS and 8% of EBSG. FOS-EBSG17: Biscuit with15.2% of FOS and 17% EBSG. FOS: Fructooligosaccharides. EBSG: Extruded brewers' spent grain.

based on Wang et al. (2015). Samples were mixed with water (1:20 w/v) for 24 h or with olive oil (1:10 w/v) for 1 h. Finally, they were centrifuged at 7500 g for 15 min. Results were obtained as follows:

$$WHC\ or\ OHC = \frac{W_1}{W} \quad (1)$$

Where, WHC means water holding capacity, OHC is oil holding capacity, $W_1$ is the weight of water or oil absorbed and $W$ is the sample initial weight. Experiments were done in duplicate.

#### 2.3.5. Differential scan calorimetry (DSC)

Based on Slade, Levine, and Wang (1996), samples for DSC analysis were prepared by grounding biscuits and adding equal amounts of distilled water (1:1 w/w). Powdered biscuits and water were homogenized by hand with spatula till obtaining a consistency of homogenized hydrated slurry. Samples were filled into stainless steel DSC pans which were hermetically sealed and weight. DSC samples weights were between 11 and 37 mg. Slurry was heated on a DSC (Q-1000 TA Instruments) from 10 °C to 130 °C at 5 °C/min. Transition enthalpy per gram of sample, onset temperature, peak temperature and completion temperature were determined. Gelatinization enthalpy was calculated as the transition enthalpy per gram of starch in the sample (Schuchardt et al., 2016). Biscuits starch content is shown in Supplementary Material (Table S1). Experiments were done in duplicate.

#### 2.3.6. Scanning electron microscopy

To determine the microstructure of tested biscuits, they were analyzed using scanning electron microscopy (SEM). Samples without any further treatment were grounded and mounted on the carbon-metallic stabs and sputtered with 12 nm of gold. All treatments were viewed and captured using a Hitachi SEM (model S-3000N) at an accelerating voltage of 15 kV.

### 2.4. Nutritional and sensory study

#### 2.4.1. Study design and protocol

The experiment was based on Iriondo-Dehond et al. (2020). Fifty-one volunteers were recruited by on-line and oral communication from Universidad Católica del Uruguay, Latitud-LATU Foundation, and surroundings. Exclusion criteria were pregnancy or breastfeeding, gluten and lactose intolerance, milk protein allergy, and diagnosed gastrointestinal disorders. There were 19 males and 32 female whose average age was 41 years old. Regarding their body mass index (BMI), 2% of the participants were under weight (BMI $<$18.5), 94% of the participants presented normal weight (18.5 $<$ BMI $<$25) while 4% presented obesity (BMI $>$30).

A blind crossover design was done. It included three treatments consisting of the different biscuits formulated in the present study (FOS-EBSG0, FOS-EBSG8, FOS-EBSG17), shown in Table 1, which were identified by a three-digit random numbers. Three sequences of intervention groups were used following a balanced Latin square design, to avoid first-order-carryover. Participants received one treatment per week and were asked not to change their physical and dietary habits for the time the trial was taking place. Volunteers chose the starting hour between 7 a.m. and 10.30 a.m., which was maintained on each treatment. Participants could choose to do the experiment as home-test or at their workplace facilities and were provided with specific instructions of the procedure and a telephone number for any doubts. To avoid omissions or errors in filling out the forms, participants were tracked of its answers and constantly reminded of the procedures.

On each test day, volunteers first completed appetite rating prior to having breakfast, i.e. in fasting state. Afterwards, participants were provided with a standardized breakfast: 40 g of white bread, with 5 g of butter, an apple and 250 mL of tea of coffee, which were supposed to eat in 15 min. Volunteers who did it in the home-test mode were provided with a test kit which included breakfast components, tested food and instructions the day before. Questions on appetite ratings were answered every 30 min until the consumption of the biscuits. Two hours after finishing breakfast, volunteers ate the tested biscuits (30 g) in 15 min and answered a sensory acceptance questionnaire of the product. After that, participants continued to answer the appetite rating questionnaire for two more hours every 30 min. The whole test lasted 4 h and a half. When they finished, participants were asked to fill in an on-line gastrointestinal symptom questionnaire, which was also answered in the 24 h after biscuits ingestion. Questionnaires used are available in Supplementary Material.

#### 2.4.2. Sensory analysis

Participant's acceptance on tested biscuits was evaluated using a nine-point hedonic scale that ranged from: "1-dislike extremely" to "9-like extremely". Volunteers were asked on their purchase intention on a seven-point scale meaning "1-I would definitely don't buy it" and "7-I would definitely buy it". Three attributes were evaluated on a seven point Just-About-Right (JAR) scale: sweetness (too little sweet – very sweet), texture (too soft-too hard) and color (too light – too dark). Finally, a check-all-that-apply (CATA) questionnaire was done to describe the product. The attributes were: homemade, strange taste, delicious, intense flavor, healthy, caked, dry, sandy, ugly, stringy, tasty, wetty, soft, bitter, crispy, aftertaste, unpleasant, little taste and salty.

#### 2.4.3. Appetite ratings

Subjective appetite ratings were answered on a 10 cm Visual Analogue Scale (VAS) scale. Participants were asked to set alarms on their phones at the indicated time. The appetite rating profile included measures of: hunger, satiety, fullness, willingness to eat, willingness to eat fatty, salty and sweet products and thirst. The scale was anchored at 0 cm ("nothing at all") and at 10 cm ("a large amount"). Results were expressed as the difference between the appetite score obtained and the one obtained right before biscuit's ingestion. Curves for appetite ratings among the 4.5 h of the test are presented in Supplementary Material.

#### 2.4.4. Gastrointestinal wellbeing measurements

Gastrointestinal symptoms were evaluated 2- and 24-h after tested biscuits intake. Volunteers were asked to compare their symptoms to how they usually felt in a scale from 0 to 3: "0-no or habitual occurrence of symptom", "1-slightly more than usual", "2-much more than usual" and "3-exceptionally more than usual" (Koutsou, Storey, & Ba, 1999). The symptoms evaluated were: bloating, nausea, abdominal pain, flatulence, diarrhea, constipation and gastrointestinal (GI) rumbling. Symptom gastrointestinal score was calculated as the mean of all the individual answers, which could go from 0 to 3. The total gastrointestinal score for each tested biscuit was given as the sum of all symptoms gastrointestinal scores.

### 2.5. Statistical analysis

One way analysis of variance (ANOVA) applying Tukey test, were used for determining significant differences between samples ($p \leq 0.05$) techno-functional characterization. Significant differences for biscuits' sensory acceptance and willingness to purchase were analyzed with Kruskal-Wallis test ($\alpha \leq 0.05$), as data was not normally distributed. JAR results were used to determine the drop in overall liking by a penalty analysis. The frequency of mentioned of each CATA attribute was identified and Cochran's Q test was performed on the raw binary CATA data to determine significant differences between samples for each attribute ($p \leq 0.05$). Appetite rating results were analyzed with ANOVA applying Tukey test ($\alpha \leq 0.05$). Significant differences in gastrointestinal wellbeing answers were establish by a Chi-square test used to compare the frequency of each symptom associated to the ingestion of biscuits with different levels of EBSG to those without EBSG. The software used was XLSTAT Version 2022.2.1 (Addinsoft New York, USA), including its sensory plug in.

## 3. Results and discussion

BSG improves the nutritional value of the end products. However, it also changes the processing, technological, mechanical and physicochemical properties, including visual appearances, such as color and compact texture, in addition to acceptability (Naibaho, 2021).

### 3.1. Techno-functional characterization

Table 2 shows the results obtained for the techno-functional properties analyzed. The substitution of wheat flour with different levels of EBSG caused an increase in biscuits' hardness ($p < 0.05$). However, no significant differences ($p > 0.05$) were found for the hardness of biscuits having 8% or 17% of EBSG. Previous authors have also reported an increased in biscuits' hardness as BSG was incorporated into biscuits when BSG substituted up to 20% of wheat flour (Heredia-Sandoval et al., 2019). Fiber composition is responsible for textural properties: IDF enhances rigidness (Naibaho, 2021). It was found that an increase in insoluble dietary fiber causes an increase in food product hardness when water dosage remains the same (Föste, Verheyen, Jekle, & Becker, 2020). BSG has high amounts of proteins and fiber which absorb water, what causes biscuits' hardening (Petrović, Pajin, Tanackov, Pejin, & Aleksandar, 2015).

Moreover, EBSG incorporation to biscuits caused a significant reduction ($p < 0.05$) on their luminosity, measured by $L^*$, and on $b^*$ CIE Lab color space coordinate. However, no clear trend was found regarding the effect of EBSG on $a^*$ CIE Lab color space coordinate of biscuits. Color parameters registered for EBSG are in the same order as the ones reported by Farcas et al. (2021) for different types of BSGs. Yet, slight differences were found in how EBSG addition affected the product color. It has been well documented that the addition of BSG results in a decrease of luminosity ($L^*$) in baked products (Farcas et al., 2021; Heredia-Sandoval et al., 2019; Ktenioudaki, Chaurin, Reis, & Gallagher, 2013; Petrović et al., 2015). BSG has a dark brown color, which significantly darkens food products and the Maillard reaction could be another reason for the reduction of brightness (Naibaho, 2021). However, the tested biscuits melanoidin content was significantly higher ($p < 0.05$) for FOS-EBSG0 (5.779 ± 0.287 mg of Caramel melanoidins/g) than for FOS-EBSG8 (2.258 ± 0.145 mg of Caramel melanoidins/g) and FOS-EBSG17(3.150 ± 0.148 mg of Caramel melanoidins/g), showing Maillard reaction may have been further developed in biscuit without EBSG addition. Furthermore, EBSG addition caused a decreased in b* of biscuits as registered in previous studies (Farcas et al., 2021; Heredia-Sandoval et al., 2019; Petrović et al., 2015). However, these studies suggested an increase in biscuits' $a^*$ with BSG addition, which was not reflected in the present study tested foods.

Table 2
Biscuits' techno-functional properties.

| | | EBSG | FOS-EBSG0 | FOS-EBSG8 | FOS-EBSG17 |
|---|---|---|---|---|---|
| Texture | Hardness (N) | n.d | 13.230 ± 0.710 [a] | 26.585 ± 1.400 [b] | 27.382 ± 1.419 [b] |
| Diameter (cm) | | n.d | 6.226 ± 0.207 [b] | 6.083 ± 0.133 [b] | 5.910 ± 0.01 [a] |
| Thickness (cm) | | n.d | 0.433 ± 0.107 [b] | 0.370 ± 0.082 [a,b] | 0.266 ± 0.057 [a] |
| Spread ratio | | n.d | 15.018 ± 3.329 [a] | 18.449 ± 2.807 [a] | 20.000 ± 0.075 [a] |
| Color | $L^*$ | 55.234 ± 0.812 [a] | 65.846 ± 0.422 [d] | 63.044 ± 0.427 [c] | 59.903 ± 0.343 [b] |
| | $a^*$ | 6.362 ± 0.085 [a] | 7.828 ± 0.412 [c] | 7.192 ± 0.198 [b] | 7.377 ± 0.313 [b,c] |
| | $b^*$ | 15.981 ± 0.895 [a] | 19.004 ± 0.979 [b] | 16.944 ± 0.744 [a] | 16.560 ± 0.730 [a] |
| Water holding capacity (%) | | 3.656 ± 0.151[c] | 1.181 ± 0.045 [a] | 1.240 ± 0.121 [a] | 2.050 ± 0.122 [b] |
| Oil holding capacity (%) | | 1.545 ± 0.016 [b] | 1.384 ± 0.018 [a] | 1.308 ± 0.066 [a] | 1.271 ± 0.001 [a] |
| Gelatinization enthalpy (J/g of starch) | | n.d | 1.240 ± 4.248 [a] | 3.970 ± 1.046 [a] | 26.397 ± 3.860 |
| Onset temperature (°C) | | n.d | 53.030 ± 4.030 [a] | 64.855 ± 2.877 [a] | 55.620 ± 0.579 [a] |
| Peak temperature (°C) | | n.d | 70.040 ± 0.480 [a] | 75.060 ± 1.937 [a] | 84.362 ± 1.007 [b] |
| Completion temperature (°C) | | n.d | 85.600 ± 11.596[a] | 87.355 ± 0.488[a] | 111.110 ± 13.987[b] |

Results shown as mean ± standard deviation. Different letters within the same line show significant differences ($p < 0.05$). FOS-EBSG0: Biscuit with 15.2% FOS and no EBSG. FOS-EBSG8: Biscuit with15.2% of FOS and 8% of EBSG. FOS-EBSG17: Biscuit with15.2% of FOS and 17% EBSG. FOS: Fructooligosaccharides. EBSG: Extruded Brewers' Spent Grain. n.d means not determined.

Additionally, EBSG showed a significantly higher ($p < 0.05$) water and oil holding capacity than tested biscuits. No significant differences ($p > 0.05$) for biscuits oil holding capacity were found, while biscuits with 17% of EBSG presented a significantly higher ($p < 0.05$) water holding capacity than the rest. This may be due to the higher content of dietary fiber of FOS-EBSG17 (Supplementary Material Table S1). Besides, similar trends were found for biscuits whose wheat flour content was partially substituted by oat flour (Lee & Kang, 2018). The addition of BSG increases the amount of dietary fiber, which contains high hydroxyl groups thus leading to an increase in the ability to absorb water (Naibaho, 2021). Moreover, BSG water holding capacity may be linked to biscuits' hardening (Petrović et al., 2015).

Regarding the DSC analysis, FOS-EBSG17 presented a higher gelatinization enthalpy per gram of starch present in the sample ($p < 0.05$). A higher peak temperature for biscuits with 17% of EBSG was registered ($p < 0.05$). No significant differences in these parameters between biscuits without EBSG and biscuits with 8% EBSG were found ($p > 0.05$). The lower the gelatinization temperature, the lower the degree of starch gelatinization (Schuchardt et al., 2016). Furthermore, a higher peak temperature might be the result of a higher heating rate needed to achieve starch gelatinization (Malumba, Doran, Danthine, Blecker, & Béra, 2018). Thus, biscuits with 17% EBSG apart from presenting the lowest ($p < 0.05$) content of starch (Supplementary Material Table S1), presented the lowest degree of starch gelatinization. BSG has a low starch content, combined with a high fiber content, thus lowering the gelatinization of starch (Naibaho, 2021). Dietary fiber content may compete with starch for water, limiting starch gelatinization during baking (Guven & Sensoy, 2019). Biscuits with 17% EBSG were the ones with highest WHC, indeed. Gelatinized starch is more susceptible to amylolysis (Grundy et al., 2016), what may result in lower bioaccessible glucose levels after digestion.

Furthermore, differences in biscuits' microstructure can be elucidated from SEM images shown in Fig. 1. In the case of biscuits without EBSG (Fig. 1a and b) a developed protein network embedded with starch granules can be observed, as reported by Herken, Simsek, Ohm, and Yurdunuseven (2016). During biscuits' dough formation the protein and the other components do not seem to form a viscoelastic network surrounding and enveloping starch granules. Instead, starch granules seem only partly gelatinized, some maintained their spherical form while others lost their integrity (Herken et al., 2016). At the same time, similar to what Nawaz et al. (2021) described, biscuits without EBSG showed voids and scattered structure indicating more interstitial spaces within the matrix, what may account for a softer texture and higher ($p < 0.05$) expansion observed as a higher biscuit height and diameter compared to FOS-EBSG17 (Table 2). However, as EBSG was added to the biscuits a more tighten structure was appreciated (Fig. 1). BSG forms a closed structure diminishing gluten development. The addition of BSG disrupts or dilutes the formation of the protein network and generates a compact and harder texture which impairs $CO_2$ retention (Naibaho, 2021). The structure of BSG flour consists of husks and fiber filaments (Ktenioudaki et al., 2013), that could be appreciated in biscuits with 17% EBSG SEM

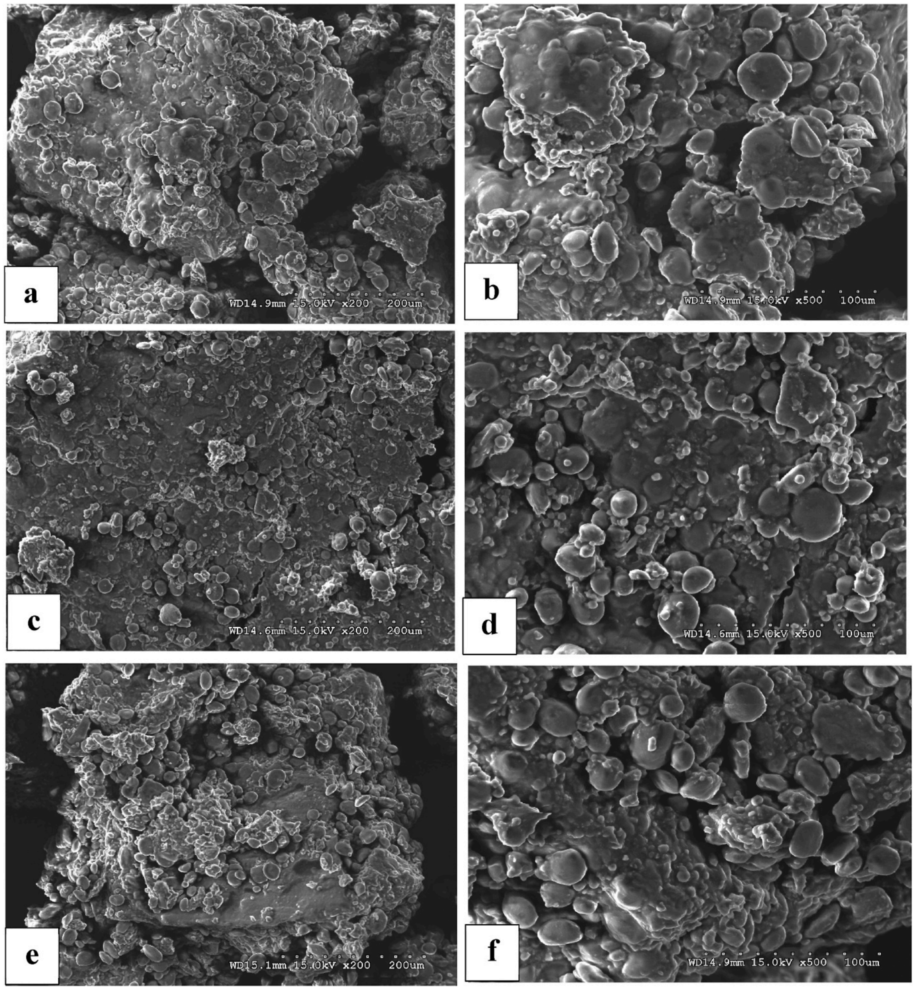


**Fig. 1.** Biscuits' scan electron microscopic images. FOS-EBSG0: Fig. 1a and b. FOS-EBSG8: Fig. 1c and d. FOS-EBSG17: Fig. 1e and f. FOS-EBSG0: Biscuit with 15.2% FOS and no EBSG. FOS-EBSG8: Biscuit with15.2% of FOS and 8% of EBSG. FOS-EBSG17: Biscuit with15.2% of FOS and 17% EBSG. FOS: Fructooligosaccharides. EBSG: Extruded Brewers' Spent Grain.

images at ×200 (Fig. 1e) interrupting the starch-protein matrix. The structure consisted presumably of small densely packed starch granules or free starch granules seem to occur interrupted in the matrix next to larger particles (Herken et al., 2016).

### 3.2. Sensory analysis

Results for biscuits sensory acceptance and purchase intention are shown in Table 3. Consumers analysis showed a significant lower acceptability and purchase intention for biscuits with 17% EBSG than for the rest ($p < 0.05$). In the 9-point hedonic scale, an overall liking level or acceptability equal to 6 or more is considered adequate according to Muñoz, Civille, and Carr (1992). Therefore, FOS-EBSG17 was the only biscuit which did not achieve the expected overall liking scores. Previous studies have also found that as the content of BSG in biscuits' increased, their acceptability decreased (Ajanaku, Dawodu, Ajanaku, & Nwinyi, 2011; Heredia-Sandoval et al., 2019; Petrović et al., 2015). Besides, it has been reported that food products' sensory attributes deteriorate following the addition of more than 15% BSG in flour blend basis, owing to an increasing unacceptable flavor (Kissell & Prentice, 1979). However, 20%–25% BSG wheat flour substitution has also been well accepted (Heredia-Sandoval et al., 2019; Petrović et al., 2015).

**Table 3**
Acceptability and purchase intention for tested biscuits.

| | Acceptability | Purchase intention |
|---|---|---|
| FOS-EBSG0 | $6.941 \pm 1.318^{b}$ | $5.176 \pm 1.195^{b}$ |
| FOS-EBSG8 | $6.740 \pm 1.306^{b}$ | $5.020 \pm 1.222^{b}$ |
| FOS-EBSG17 | $5.863 \pm 1.613^{a}$ | $4.333 \pm 1.380^{a}$ |

Results shown as mean ± standard deviation. Different letters within the same column show significant differences ($p < 0.05$). FOS-EBSG0: Biscuit with 15.2% FOS and no EBSG. FOS-EBSG8: Biscuit with15.2% of FOS and 8% of EBSG. FOS-EBSG17: Biscuit with15.2% of FOS and 17% EBSG. FOS: Fructooligosaccharides. EBSG: Extruded Brewers' Spent Grain.

As expected, a lower acceptability of the product coincided with a lower purchase intention (Table 3). Besides a strong and significant Pearson correlation was found between the overall liking and purchase intention ($r = 0.75$, $p < 0.0001$). Further studies (with $n > 100$) are needed to determine the reasons why consumers' would choose to buy the product or not, and how can information about each product, such as nutritional and health claims, influence their purchase intent (Kaya, 2016). Besides, products with BSG were better accepted than regular ones when information was provided regarding the fiber source claim and sustainability claim (Curutchet, Serantes, Pontet, Prisco, & Arcia, 2022). Nevertheless, it has been found that the most important factor for conditioning consumers food choice are taste beyond health factors (Kaya, 2016). Although, these trends are changing after the COVID-19 pandemic as consumers are more concerned on health aspects (Kerry, 2021).

Fig. 2 shows the results of the JAR analysis. Regarding texture, most

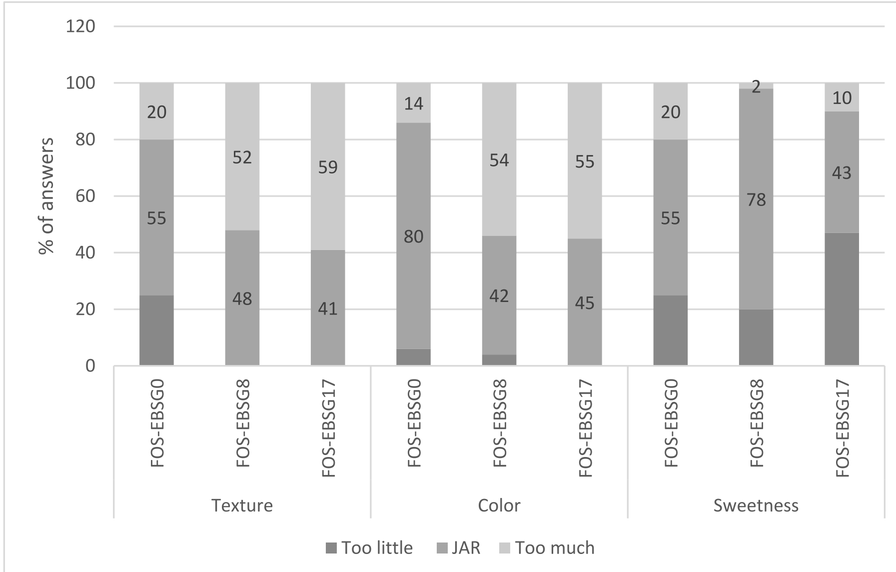


**Fig. 2.** Just about right frequencies as percentage of answers (n = 51). For texture attribute: to little corresponds to "too soft" and too much to "too hard". For color attribute: too little corresponds to "too light" and too much to "too dark". For sweetness: too little is "very little sweet" and too much means "very sweet". FOS-EBSG0: Biscuit with 15.2% FOS and no EBSG. FOS-EBSG8: Biscuit with15.2% of FOS and 8% of EBSG. FOS-EBSG17: Biscuit with15.2% of FOS and 17% EBSG. FOS: Fructooligosaccharides. EBSG: Extruded Brewers' Spent Grain.

of the participants identified biscuits with EBSG (FOS-EBSG8 and FOS-EBSG17) as too hard, while they found the texture of biscuits without EBSG (FOS-EBSG0) adequate. Most consumers found FOS-EBSG8 and FOS-EBSG17 color was too dark, while FOS-EBSG0 color was acceptable. These trends are in line with results presented in Table 2, where it was shown that FOS-EBSG8 and FOS-EBSG17 were harder and had lower luminosity than FOS-EBSG0 ($p < 0.05$). Finally, FOS-EBSG0 and FOS-EBSG8 sweetness was found adequate, while FOS-EBSG17 sweetness was mostly identified as too little. Moreover, FOS-EBSG8 biscuit was the one with the highest number of consumers who identified its sweetness as just-about-right. Thus, it seems free glucose content in EBSG (Gutiérrez-Barrutia et al., 2022) may enhanced sweet flavor, up to a point where it may be masked by BSG's bitter taste or aftertaste (Naibaho, 2021).

Penalty analysis showed the mean drop in liking scores in attributes which had a significant negative effect ($p < 0.05$) and an occurrence higher than 20%. Penalties were calculated for each product and for all biscuits together to identify the trends that affected biscuits acceptability in general.

Results for the penalty analysis made for the three biscuits together are shown in Fig. 3. Sweetness ($p < 0.0001$) and color ($p < 0.001$) presented a significant effect on overall liking scores. Texture penalties could not be calculated. Results are in accordance with previous studies which have established that biscuits' sweetness is a key sensory attribute that determines liking (Biguzzi et al., 2015), and in the present study it seems too much sweet was not found to affect so negatively the overall liking of biscuits as very little sweet. Furthermore, Biguzzi et al. (2015) found that fat- or sugar-reduced biscuits perceived as less sweet, are less liked by consumers. This goes in line with our findings as FOS-EBSG0 and FOS-EBSG8 did not have significant differences in overall liking (Table 3) and both were perceived by the majority of consumers to have the appropriate sweetness (Fig. 2). Apart from that, too dark color of biscuits was found to have a higher liking mean drop than lighter biscuits' color. FOS-EBSG8 and FOS-EBSG17 were described by most of the

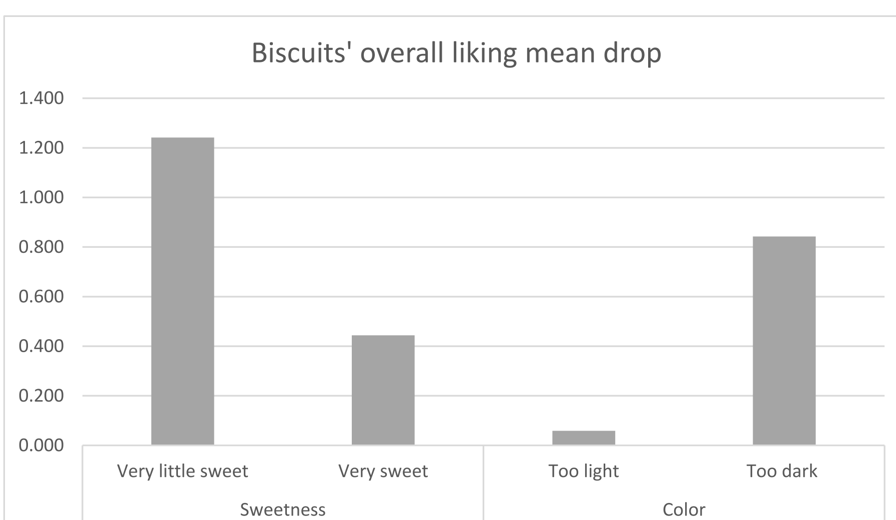


**Fig. 3.** Penalty analysis for tested biscuits.

consumers as too dark (Fig. 2). To a point, the unique dark brown color of BSG has a beneficial impact on the color of the product, which is assumed to be healthy (Naibaho, 2021). However, FOS-EBSG8 and FOS-EBSG17 were not perceived as healthier than FOS-EBSG0 by consumers based on the CATA results (Fig. 4).

Focusing on individual product's penalty analysis, sweetness was the only analyzed attribute to have a significant effect ($p < 0.05$) on overall liking of FOS-EBSG0 and FOS-EBSG8, while no significant ($p > 0.05$) effect was found for FOS-EBSG17. In the case of FOS-EBSG0, the mean drop registered was 1.690 if sweetness was too little and 0.836 if sweetness was too much. However, FOS-EBSG8 presented a mean drop equal to 1.149 if sweetness was too little and −1.051 if sweetness was too much, i.e. the liking mean was higher when the biscuit was identified as too sweet compared to the general liking mean. The color had a non-significant ($p > 0.05$) effect on the overall liking of the biscuits, and in the case of FOS-EBSG17 color penalties could not be calculated. Penalties could not be calculated for texture because most of the responses were at the extremes.

Results for CATA questionnaire are presented in Fig. 4. The fact that a lower amount of consumer identified FOS-EBSG17 as delicious compared to FOS-EBSG0 and FOS-EBSG8, and more consumers identified an aftertaste and strange taste in it compared to FOS-EBSG0 and FOS-EBSG8, may elucidate another reason for FOS-EBSG17 's lower acceptability ($p < 0.05$) (Table 3). Besides, these attributes were also identified in chocolate milk enriched with BSG and were linked to the lower acceptability of this product (Curutchet, Serantes, Pontet, Prisco, & Arcia, 2022). The strange taste and aftertaste found in FOS-EBSG17 may corresponded to the bitter taste registered in previous formulated biscuits with the same level of wheat flour substitution (Heredia-Sandoval et al., 2019). In fact, BSG high polyphenol content has been related to a decreased sweetness and increased bitterness when incorporated into baked cereal goods (Waters, Jacob, Titze, Arendt, & Zannini, 2012).

### 3.3. Appetite ratings

Appetite is a term applied to several dimensions of eating behavior including preference, selection and motivation to eat. It can be considered as being the "desire for food" and can be present even in the absence of a physiological need (Miquel-kergoat, Azais-braesco, Burton-freeman, & Hetherington, 2015). Satiety is defined as the inhibition of hunger as a result of having eaten, while satiation is the satisfaction of appetite during feeding that marks the end of eating (Slavin & Green, 2007). To understand the factors that control perceived satiety multiple domains: physiology, psychology and food, need to be considered (Ni, Smyth, Cozzolino, & Gidley, 2022). The present study aimed only to understand whether biscuits' different composition and structure may impact on subjectively perceived satiety.

Fig. 5 shows the VAS ratings for all the appetite ratings analyzed as specified in section 2.4.3. Significant differences ($p < 0.05$) were found when comparing biscuits with 17% of EBSG (FOS-EBSG17) with the biscuits without EBSG (FOS-EBSG0). After FOS-EBSG17 ingestion, a significant reduction of hunger and willingness to eat, while an increased in satiety and fullness was observed. Although, no significant differences ($p > 0.05$) were found when comparing biscuits with 8% EBSG (FOS-EBSG8) and biscuits without EBSG (FOS-EBSG0), a non-statistical trend to increase satiety and fullness was also observed for FOS-EBSG8 compared to FOS-EBSG0. No significant differences ($p > 0.05$) were found for thirst among all tested biscuits. Therefore, 17% wheat flour substitution by EBSG in tested biscuits, resulted in a better appetite control.

Results may be due to FOS-EBSG17 's higher ($p < 0.05$) content of insoluble and high molecular weight soluble dietary fiber compared to FOS-EBSG0 and FOS-EBSG8, as the FOS amount was the same in every biscuit (Supplementary Material Table S1). Although not all dietary fibers have the same impact on satiety, the majority of studies with controlled energy intake reviewed by Slavin and Green (2007) reported an increase in post-meal satiety and a decrease in subsequent hunger with increased in dietary fiber. Regarding whole foods overweight college-age men who received during 1 month high dietary fiber bread supplemented with 24 g of cellulose indicated they were not hungry at any time, while four of the six men eating the control bread were hungry (Mickelsen et al., 1979). Additionally, a significant inverse correlation was found between fiber content of cereals eaten at breakfast and energy intake during lunch and participants reported feeling less hungry after having very-high-fiber cereal than very-low-fiber cereal (Grace, Parker, Levine, Tallman, & Biiington, 1989).

Different mechanisms were suggested for dietary fiber role in controlling appetite or hunger. As exposed by Warrilow, Mellor, Mckune, and Pumpa (2019) it is generally considered that an increase in dietary fiber will result in additional delay in gastric emptying. However, it is also thought to be due to slower nutrient absorption. Nutrients' slower absorption may be due to physical obstruction of nutrients in the digestive tract by insoluble dietary fiber and by an increase in the viscosity of the small intestine digesta caused by the presence of soluble dietary fiber. Nevertheless, a significant ($p < 0.01$) and strong correlation ($r = -0.89$) was found in the digesta viscosity and the free water content of digesta in rats which depended on the water holding capacity

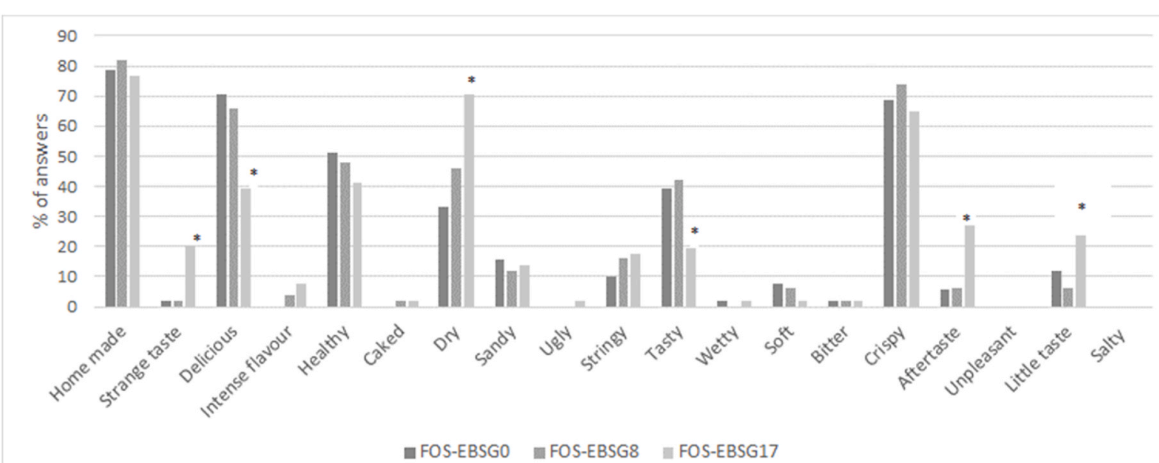


**Fig. 4.** Frequency of answers for sensory attributes evaluated by check-all-that-apply (CATA) questions. * shows significant differences ($p < 0.05$). FOS-EBSG0: Biscuit with 15.2% FOS and no EBSG. FOS-EBSG8: Biscuit with15.2% of FOS and 8% of EBSG. FOS-EBSG17: Biscuit with15.2% of FOS and 17% EBSG. FOS: Fructooligosaccharides. EBSG: Extruded Brewers' Spent Grain.

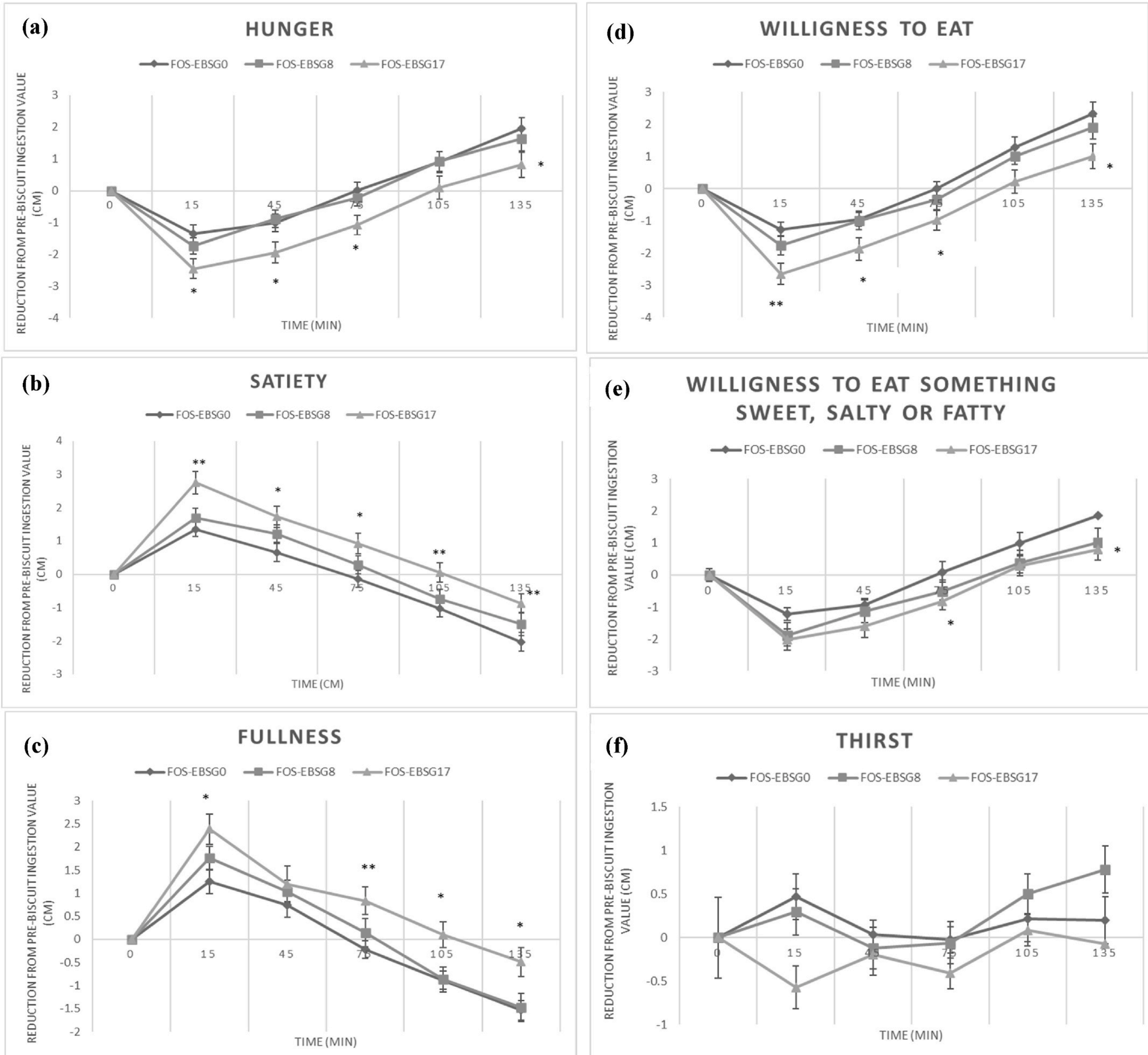


**Fig. 5.** VAS scores of hunger (a), satiety (b), fullness (c), willingness to eat (d), willingness to eat something sweet, salty and fatty (e) and thirst (f) rated from the morning meeting in fasting conditions before the standard breakfast to prior to lunch time. Results presented as mean ± SEM. * indicate significant difference $p < 0.05$ and ** indicate significant differences with $p < 0.01$ compared to FOS-EBSG0. FOS-EBSG0: Biscuit with 15.2% FOS and no EBSG. FOS-EBSG8: Biscuit with15.2% of FOS and 8% of EBSG. FOS-EBSG17: Biscuit with15.2% of FOS and 17% EBSG. FOS: Fructooligosaccharides. EBSG: Extruded Brewers' Spent Grain.

of insoluble dietary fiber (Takahashi et al., 2009). Thus, FOS-EBSG17 's higher ($p < 0.05$) dietary fiber content resulted in a higher WHC compared to FOS-EBSG0 and FOS-EBSG8, what may have had a positive effect on hunger suppression and prolong satiety sensation. Furthermore, fiber-rich foods usually are accompanied by increased efforts and/or time of mastication. Besides, FOS-EBSG17 's higher hardness (Table 2), more compact structure (Fig. 1) and dryness (Fig. 4) compared to FOS-EBSG0 may have resulted in more mastication cycles before swallowing. Foods that are harder to break down require a higher number of masticatory cycles and are eaten at a slower rate (Hollis, 2018). Additionally, a longer oral processing time before swallowing influences post-prandial subjective appetite as it may influence the post-prandial metabolic or endocrine response in a manner that reduces appetite: increased gut hormones (CCK, GLP-1, PPY) and suppressed ghrelin (Hollis, 2018; Miquel-kergoat et al., 2015).

### 3.4. Gastrointestinal wellbeing

Increased dietary fiber intake is often associated with adverse gastrointestinal symptoms (Stewart et al., 2010). Participants received 30g of each biscuit formulation, which based on data presented in Table 1 resulted in 4g of FOS in each experiment, while insoluble dietary fiber and high molecular weight soluble dietary fiber varied. In 30g of biscuits, insoluble dietary fiber content was 0.463, 1.251 and 2.166 g for FOS-EBSG0, FOS-EBSG8 and FOS-EBSG17 treatment, respectively. High molecular weight soluble dietary fiber content was 0.205, 0.299 and 0.330g for FOS-EBSG0, FOS-EBSG8 and FOS-EBSG17, respectively.

After 2 h of biscuit ingestion, the only gastrointestinal symptom identified was constipation in a “1-slightly more than usual” level for 3.9% of the participants after eating FOS-EBSG0. The occurrence of gastrointestinal symptoms between 2- and 24 h after biscuits ingestion is shown in Table 4. No significant differences ($p > 0.05$) were observed between FOS-EBSG8 and FOS-EBSG17 ingestion compared to FOS-EBSG0. In fact, all the biscuits presented low gastrointestinal symptomatology as their total scores were between 0.6 and 0.92 out of 3. For the control biscuit (FOS-EBSG0) the two most reported gastrointestinal symptoms were bloating and flatulence and 15% of the participants reported more than usual occurrence of these symptoms after eating FOS-EBSG0. Same trends were found for FOS-EBSG8 and FOS-EBSG17. In the case of FOS-EBSG8, bloating was the most reported symptom: 21.6% of the participants identified this symptom as more than usual and 5.8% much more than usual. FOS-EBSG17 presented lower levels of bloating occurrence but higher flatulence occurrence, compared to FOS-EBSG8. In the case of FOS-EBSG17, nine (17.6%) and eleven (21.5%) participants identified more than usual bloating and flatulence, respectively, while only 3.9% presented much more than usual bloating and flatulence.

**Table 4**
Frequency of occurrence and total and individual scores of gastrointestinal symptoms between 2- and 24-h following consumption of the biscuits in healthy adults (n = 51).

| | Biscuit formulation | | |
|---|---|---|---|
| | FOS-EBSG0 | FOS-EBSG8 | FOS-EBSG17 |
| **Bloating** | | | |
| No symptoms | 43 | 37 | 40 |
| More than usual | 8 | 11 | 9 |
| Much more than usual | 0 | 3 | 2 |
| Extremely more than usual | 0 | 0 | 0 |
| Significance | – | ns | ns |
| Individual score | 0.156 ± 0.367 | 0.333 ± 0.588 | 0.255 ± 0.523 |
| **Nausea** | | | |
| No symptoms | 51 | 51 | 48 |
| More than usual | 0 | 0 | 3 |
| Much more than usual | 0 | 0 | 0 |
| Extremely more than usual | 0 | 0 | 0 |
| Significance | – | ns | ns |
| Individual score | 0.000 ± 0.00 | 0.000 ± 0.00 | 0.059 ± 0.237 |
| **Flatulence** | | | |
| No symptoms | 43 | 39 | 38 |
| More than usual | 8 | 10 | 11 |
| Much more than usual | 0 | 2 | 2 |
| Extremely more than usual | 0 | 0 | 0 |
| Significance | – | ns | ns |
| Individual score | 0.157 ± 0.367 | 0.275 ± 0.532 | 0.294 ± 0.582 |
| **Abdominal pain** | | | |
| No symptoms | 47 | 48 | 47 |
| More than usual | 4 | 2 | 2 |
| Much more than usual | 0 | 1 | 2 |
| Extremely more than usual | 0 | 0 | 0 |
| Significance | – | ns | ns |
| Individual score | 0.078 ± 0.271 | 0.078 ± 0.337 | 0.117 ± 0.431 |
| **Diarrhea** | | | |
| No symptoms | 48 | 49 | 48 |
| More than usual | 3 | 2 | 3 |
| Much more than usual | 0 | 0 | 0 |
| Extremely more than usual | 0 | 0 | 0 |
| Significance | – | ns | ns |
| Individual score | 0.059 ± 0.237 | 0.039 ± 0.392 | 0.118 ± 0.431 |
| **Constipation** | | | |
| No symptoms | 50 | 48 | 49 |
| More than usual | 1 | 1 | 2 |
| Much more than usual | 0 | 1 | 0 |
| Extremely more than usual | 0 | 0 | 0 |
| Significance | – | ns | ns |
| Individual score | 0.020 ± 0.140 | 0.059 ± 0.310 | 0.039 ± 0.196 |
| **GI Rumbling** | | | |
| No symptoms | 45 | 47 | 45 |
| More than usual | 5 | 1 | 5 |
| Much more than usual | 1 | 3 | 1 |
| Extremely more than usual | 0 | 0 | 0 |
| Significance | – | ns | ns |
| Individual score | 0.137 ± 0.400 | 0.137 ± 0.490 | 0.137 ± 0.400 |
| **Total Score** | 0.607 | 0.921 | 0.917 |

The results are expressed as frequency of occurrence of individual gastrointestinal symptoms. Differences between the occurrence of individual gastrointestinal symptoms for the different biscuit's samples were analyzed using a Chi-square test comparing the frequencies reported for biscuit without EBSG (FOS-EBSG0) with the ones for biscuit with 8% EBSG (FOS-EBSG8) and 17% EBSG (FOS-EBSG17). ns = not significant. FOS: Fructooligosaccharides. EBSG: Extruded Brewers' Spent Grain.

Therefore, the main results of this study were that EBSG addition did not contribute significantly to an increase in gastrointestinal symptoms at the given doses, probably because EBSG insoluble type of dietary fiber (cellulose and hemicellulose) are partially fermentable in human gut (Adams, Sello, Qin, Che, & Han, 2018). Secondly, biscuits formulation was found to be well tolerated at tested conditions. Besides, no volunteer decided to quit the experiment due to gastrointestinal symptoms occurrence.

Our results are in accordance with previously published data which indicated that flatulence and bloating were the most frequent reported symptoms after 20g of FOS ingestion in snack bars (Wright, Mathews, Christman, Radford, & Dahl, 2012) or in lemonade (Ten Bruggencate, Bovee-Oudenhoven, Lettink-Wissink, Katan, & Meer, 2006). However, flatulence and bloating reached high scores when FOS were taken with lemonade (Ten Bruggencate et al., 2006), while mild symptoms were reported for the same FOS intake in snacks (Wright et al., 2012). Thus, it seems that FOS presents better gastrointestinal tolerance when it is added to solid food matrixes than liquid ones (Bonnema, Kolberg, Thomas, & Slavin, 2010). These symptoms are due to fermentation process which produces gas that may also induce borborygmi and abdominal pain. If they are not fermented, they may exert an osmotic effect in the intestinal lumen what may eventually result in diarrhea (Marteau & Seksik, 2004). The FDA consider FOS as Generally Recognized As Safe (GRAS) the AESAN Scientific Committee recommend a maximum daily intake of 9 g with the warning that “excessive consumption may cause intestinal upset”.

Many factors affect the gastrointestinal acceptability of foods containing prebiotic fibers, including the physicochemical properties of fibers, notably the chain length (Le Bourgot et al., 2022). It is thought that rapid fermentation occurring in short-chain undigestible carbohydrates would lead to a larger production of gas and increase water uptake compared to the effects of a steady fermentation associated with higher carbohydrate polymerization degree (Bonnema et al., 2010). Studies involving short-chain fructooligosaccharides from sugar beet added to water or yogurt suggested 40g a day kept gastrointestinal symptoms at mild levels (Le Bourgot et al., 2022). Bruhwyler, Carreer, Demanet, and Jacobs (2009) showed more severe gastrointestinal symptoms appeared for inulin with higher polymerization degree compared to inulin with lower polymerization degree. However, Bonnema et al. (2010) suggested that 5g/serving in orange juice were well-tolerated for short and long chain inulin, while larger doses (10 g/serving) could be well-tolerated if longer chain inulin was consumed. Besides Iriondo-Dehond et al. (2020) found out that 3% inulin was the maximum inulin dose that could be added in yogurts so as to guarantee gastrointestinal tolerance and sensory acceptability.

## 4. Conclusion

In the present study, different formulations of reduced in sugar biscuits with sustainable food ingredient: extruded brewers' spent grain, were formulated in line with recent food market demands. Regarding the technological properties, the addition of EBSG resulted in a harder and darker product, with a more compact structure. These effects were detected by consumers when 17% of EBSG was added to biscuits and caused a detrimental effect on product sensory acceptability by masking sweetness and enhancing an aftertaste. Positively, no significant differences ($p > 0.05$) were observed in product acceptability for biscuits with 8% and 0% of EBSG. The 17% addition of EBSG resulted in a positive effect in appetite control compared to biscuits without EBSG, while this effect was not achieved with 8% of EBSG addition. Besides, FOS addition in biscuits resulting in low gastro-intestinal symptoms, and the resulting higher content of dietary fiber in EBSG biscuits did not worsen these symptoms. Thus, all tested biscuits showed good gastro-intestinal tolerance.

Therefore, biscuits with 8% EBSG could be commercialized without further reformulation as good consumer acceptability has been registered. If short-term appetite control effects want to be achieved, biscuits with 17% EBSG should be reformulated to achieve better overall liking trying to enhance sweetness and mask aftertaste. Additionally, new formulations testing EBSG addition between 8% and 17% could also be considered. In this sense, to validate the market potential that EBSG cookies would have, it would be necessary to conduct a study with a larger number of consumers.

Brewers' spent grain is not considered a novel food by the European Union: it has been widely consumed before May 1997 and its access to the market is not subject to pre-market authorization in accordance to Regulation (EU) 2015/2283. Therefore, formulation herein proposed can be improved and commercialized. The present study incorporates this sustainable food ingredient into one of the most consumed bakery snacks: biscuits, improving their nutritional quality while good consumer acceptability can be achieved, causing a positive effect on satiety with a correct gastrointestinal tolerance.

### CRediT authorship contribution statement

**Maria Belen Gutierrez-Barrutia:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Sonia Cozzano:** Writing – review & editing. **Patricia Arcia:** Writing – review & editing, Visualization, Data curation. **Maria Dolores del Castillo:** Writing – review & editing, Supervision, Methodology, Funding acquisition, Conceptualization.

### Informed consent statement

Informed consent was obtained from all subjects involved in the study and all the participants were aware that they could withdraw the study at any time if they wanted.

### Institutional Review Board statement

The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Institutional Review Board (or Ethics Committee) of Universidad Católica del Uruguay approved on the 25 of March 2022.


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### Declaration of competing interest

Authors declare no conflict of interest. The authors have no affiliation with any organization with a direct or indirect financial interest in the subject matter discussed in the manuscript.


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### Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fbio.2024.105583.

### Data availability

Data will be made available on request.

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