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Effect of Enzymatic Hydrolysis of Brewer's Spent Grain on Bioactivity, Techno-Functional Properties and Nutritional Value When Added to a Bread Formulation †

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Abstract: The interesting nutritional value and abundance of brewer's spent grain (BSG) may be adequate for its use as a sustainable functional ingredient. The aim of the present work was to enhance BSG bioactive properties, along with studying the BSG bread technological feasibility by rheological properties evaluation. To optimize the release of BSG bioactive compounds, an enzymatic hydrolysis was carried out using a composite central design, varying alcalase and cellulase percentage. Multiple regression (MR) and response surface methodology (RSM) were performed evaluating total polyphenol content (TPC), ABTS and ORAC as response variables, showing a positive effect for alcalase % and non-significant for cellulase %. The optimal conditions (0.1% alcalase) were used as BSG flour (FBSG) for the development of the functional bread (FBSG bread), substituting 20% *w*/*w* of wheat flour. The nutritional and bioactive characterization of the breads showed FBSG bread presented higher fiber content (>6%), TPC and antioxidant activity than control bread (CB). Breads' physicochemical characteristics were analyzed by measuring the parameters of volume, color, and texture. Regarding volume, FBSG bread presented a significant decrease ($p < 0.05$) (1890.4 ± 6.9 cm^3) with respect to CB (2359.5 ± 106.5 cm3), and also presented a significant increase ($p < 0.05$) in the development of brown/reddish tones in the crumb, which reflected in L and a parameters (53.62 and 6.10 respectively) compared to CB (75.70 and −0.16 respectively). Texture analysis showed FBSG bread chewiness (6.85 ± 0.13 Kg) and cohesiveness (0.608 ± 0.027) did not present significant differences ($p < 0.05$) with CB. On the other hand, FBSG bread parameters of resilience (27.5 ± 2.3), and rubberiness (7.63±0.16 $Kg{\cdot}m{\cdot}s^{-2}$) were increased while elasticity (89.81 ± 0.067) decreased. In conclusion, a sustainable "high fiber content" and antioxidant bread was obtained presenting suitable rheological properties as wheat flour bread. Further studies on sensory profile and acceptability of the novel food should be addressed to evaluate the consumers' perception on rheological parameters.

Keywords: antioxidant activity; brewer´s spent grain; enzymatic hydrolysis; functional bread; high fiber content; rheological properties

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1. Introduction

Beer is one of the most consumed beverages worldwide with an approximate production of 1.94 billion hectoliters in 2019. It is precisely the fifth most popular drink after tea, carbonated drinks, milk, and coffee [1]. Despite environmental awareness, technological advances and industry efforts, beer production inevitably generates large amounts of waste and by-products in the form of the so-called brewer's spent grain (BSG), spent hops, and surplus yeast [2]. The most abundant by-product is the BSG, which represents approximately 85% of the total by-products obtained [3,4]. It is mainly made up of the seed, pericarp and husk layers that cover the barley grain [5]. The current main destination of the BSG is animal feed, obtaining a low market value of approximately € 35 per ton [6,7]., The main components of BSG include fiber (30–50% *w/w*) and protein (19–30% *w/w*) making it an attractive material to improve the nutritional value of human food products [3]. In addition, the importance of this by-product as an ingredient and potential source of bioactive components that promote health has begun to be recognized, since BSG contains components such as arabinoxylans, phenolic compounds, bioactive peptides derived from protein hydrolysis, among others, that have recently gained attention for their health benefits [4,8].

Several studies have evaluated the incorporation of BSG into cereal-based foods [9], these being the most common staple foods in the world [10]. Among cereal-based foods, bread is one of the most consumed worldwide. The wide consumption, low cost and versatility of bread represents great potential as a vehicle for bioactive compounds for the development of a functional food [7,11,12].

The aim of the present work was to release bioactive compounds from BSG by enzymatic hydrolysis to enhance its antioxidant properties and to study the techno-functional feasibility and nutritional value of a bread formulation with the addition of the hydrolysate as a functional ingredient.

2. Materials and Methods

2.1. Preparation of Dry BSG

BSG was provided by the craft beer maker Birra Bizarra (Montevideo, Uruguay). It was dried in a conventional oven at 60 ° C for 24 h to obtain a moisture lower than 10%. The dry BSG was milled with a coffee grinder (Bosch, TSM6A013B) and subsequently sieved (mesh size < 250 μm).

2.2. Enzymatic Hydrolysis of BSG: Conditions and Antioxidant Capacity Assays

Enzyme-assisted extraction technology was used to optimize the release of bioactive compounds from BSG for obtaining BSG flour (FBSG). A central composite design was applied using two variables [two commercial enzymes, a protease [Alcalase® (Novozymes, Denmark)] and a carbohydrase [Celluclast 1.5 L (Novozymes, Denmark)] at two levels (0 and 0.1% *w/w*)]. Multiple regression (MR) and response surface methodology (RSM) were performed evaluating total polyphenol content (TPC), ABTS and ORAC as response variables. TPC of the hydrolysates was determined by using the Folin-Ciocalteau method [13]. ABTS and ORAC-FL assays were performed as described by Fernández-Fernández et al. [13].

2.3. BSG Flour (FBSG) and Bread Preparation and Characterization

For the preparation of FBSG the optimal conditions obtained in Section 2.2 were used. The hydrolysate obtained was lyophilized, then milled and sieved as indicated in Section 2.1. The FBSG was stored at −20 °C for further analyses.

Two bread formulations were prepared: a control bread (CB) with wheat flour and a functional bread (FBSG bread) with a wheat flour substitution of 20% *w/w* by FBSG. The level of addition of FBSG was selected to achieve the labeling of "high fiber content" according to MERCOSUR regulation N° 01/12 [14]. The ingredients composing both breads are shown in Table 1. All the ingredients were placed in a Kassel pan (KS-PM16) and program 1 was followed for a basic bread preparation (total time 175 min, cooking temperature 180 °C). The breads were unmold when they were still hot and were allowed to cool down. Both breads were made in duplicate. Then, breads were weighed and volume was measured (measuring length, width and height).

Afterwards, color and texture were measured in the samples. For crumb texture analysis, four 25 mm thick slices were taken from the central part of the crumb of each bread. A “Texture Profile Analysis” (TPA) was performed using a TA-XT2i texture analyzer (Stable Micro Systems Ltd., Surrey, UK) with the “Texture Expert” software where the parameters of hardness, elasticity and cohesion were measured at 24 °C.

The crumb and crust color of the breads were measured using a CM-2300d Konica Minolta portable spectrophotometer. The results were expressed using the CIE system L * (luminosity) a * (redness/greenness) and b * (yellowness/bluish).

Table 1. Bread ingredients.

Ingredients	Quantity (g)	
	Control Bread	**FBSG Bread**
Wheat flour	500	400
FBSG	-	100
Water	245	370
Sugar	10	10
Olive oil	10	10
Yeast	10	10
Salt	3.5	3.5

2.4. Proximal Composition and Antioxidants of Dry BSG, CB and FBSG Bread

The proximal composition of BSG, and the breads was determined following the methods of AOAC [15]: moisture, ash, chloride, total dietary fiber (method 985.29) (including soluble and insoluble fractions), lipids, proteins with a conversion factor of 6.25 and carbohydrates by difference once the other components of the sample were determined. TPC and antioxidant capacity of BSG, FBSG, CB and FBSG bread was determined as indicated in Section 2.2.

2.5. Statistical Analysis

Data were analyzed by means of the analysis of variance (ANOVA) and Tukey test was applied to determine significant differences between values ($p < 0.05$) using the Infostat v. 2015 program.

3. Results and Discussion

3.1. Enzymatic Hydrolysis of BSG

The Multiple Regression (MR) and Response Surface Methodology (RSM) for the three response variables (TPC, ABTS and ORAC) showed a good linear fitting of the model obtaining $R^2 > 95$ %, with $p < 0.01$ for the coefficients. The study showed a positive effect for % alcalase ($p < 0.05$), and not significant for % cellulase. The optimal condition for the extraction was 0.1% alcalase and 0% cellulase.

3.2. Bread Techno-Functional Properties

Both breads (control bread and FBSG bread) were obtained as Figure 1 shows. Bread parameters (Table 2) showed, FBSG bread presented a significant decrease ($p < 0.05$) in volume with respect to CB, which is in agreement with the breads photograph (Figure 1). This decrease in volume could be due to the presence of arabinoxylans, the main components of the BSG fiber. Arabinoxylans affect the formation of the gluten network influencing bread quality parameters such as volume and texture [16]. The formation of gluten network is negatively influenced by arabinoxylans (pentosans) direct interaction with gluten proteins. On the other hand, arabinoxylans compete against gluten proteins for water molecules, changing the conditions for the network development [17,18]. As to color parameters, FBSG bread showed a significant increase ($p < 0.05$) in the development of brown/reddish tones in the crumb, typical of BSG color. Texture analysis showed FBSG bread chewiness and cohesiveness did not present significant differences ($p > 0.05$) with CB, while resilience and rubberiness were increased and elasticity decreased. These changes may be due to the presence of the high fiber content of BSG, particularly arabinoxylans.

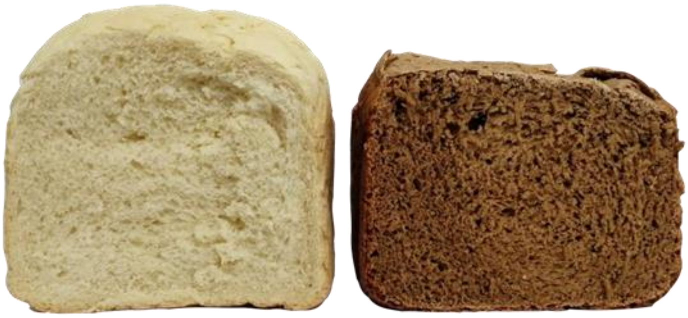

Figure 1. Control bread (left) and FBSG bread (right) photographs.

Table 2. Texture and color results of control bread and FBSG bread.

Bread parameters		Control Bread	FBSG Bread
	Volume (cm^3)	2359.5 ± 106.5 ^{b}	1890.4 ± 6.9 ^{a}
Texture	Chewiness (Kg)	6.53 ± 0.91 ^{a}	6.85 ± 0.13 ^{a}
	Cohesiveness	0.588 ± 0.086 ^{a}	0.608 ± 0.027 ^{a}
	Resilience	24.6 ± 5.5 ^{a}	27.5 ± 2.3 ^{b}
	Elasticity	96.1 ± 2.8 ^{a}	89.8 ± 0.07 ^{b}
	Rubberiness ($Kg \cdot m \cdot s^{-2}$)	6.8 ± 1.1 ^{a}	7.6 ± 0.2 ^{b}
Color	L crumb	75.70	53.62
	L shell	74.36	53.86
	a crumb	−0.16	6.10
	a shell	6.08	9.77
	b crumb	16.35	16.90
	b shell	25.10	16.83
	Chroma crumb	16.35 ± 1.26	18.16 ± 1.69
	Chroma shell	26.46 ± 1.94	19.71 ± 2.41

ANOVA analysis was performed per row using Tukey's test. The means in the row with different letters indicate significant differences ($p < 0.05$).

3.3. Proximal and Antioxidant Composition of BSG, Control Bread and FBSG Bread

Table 3 shows the proximal composition of the BSG, CB and FBSG bread. BSG results are in agreement with the reported by Lynch et al. [3]. Regarding the composition of the breads, FBSG bread significantly increased ($p < 0.05$) lipids and fiber content compared to CB. The fiber content of FBSG bread (6.9 g/100 g) was 2.5 times higher than CB, obtaining the nutritional claim "high fiber content" according to MERCOSUR regulation No. 01/12 [14].

Table 3. Proximal composition of BSG, control bread and FBSG bread.

Sample Composition	BSG	Control Bread	FBSG Bread
Lipids	6.66 ± 0.26	0.642 ± 0.037 [a]	1.73 ± 0.62 [b]
Proteins	13.88 ± 0.16	7.70 ± 0.57 [a]	7.49 ± 0.35 [a]
Moisture	2.34 ± 0.16	36.45 ± 0.77 [a]	42.2 ± 2.9 [a]
Ash	n.d.	0.566 ± 0.070 [a]	2.51 ± 0.22 [a]
Chloride	n.d.	0.023 ± 0.003 [a]	0.043 ± 0.014 [a]
Total Dietary Fiber	50.8 ± 2.0	2.82 ± 0.53 [a]	6.9 ± 1.3 [b]
Carbohydrates	n.d.	51.9 ± 1.3 [a]	39.6 ± 0.3 [b]

n.d.: not determined. ANOVA analysis was performed per row using Tukey's test. The means in the row with different letters indicate significant differences ($p < 0.05$).

Regarding the antioxidant activity of the samples (Table 4), the enzymatic hydrolysis of the BSG produced a release of bioactive compounds and an increase in the antioxidant activity, which are reflected on FBSG results. As for breads, FBSG bread presented a significant increase ($p < 0.05$) in TPC and antioxidant activity by ABTS and ORAC-FL compared to CB. The increase in the antioxidant capacity of BSG and bread compared to FBSG and FBSG bread, respectively, could be due to the release of phenolic acids (ferulic and p-coumaric acids) and bioactive peptides during enzymatic hydrolysis of BSG and bread fermentation [19,20].

Table 4. Antioxidant potential of BSG, FBSG, control bread and FBSG bread.

Antioxidant Composition	BSG	FBSG	Control Bread	FBSG Bread
TPC (mg GAE/g)	1.61 ± 0.02 [c]	1.97 ± 0.03 [d]	0.27 ± 0.01 [a]	0.47 ± 0.06 [b]
ABTS (μmol Trolox/g)	10.63 ± 0.03 [c]	12.3 ± 0.3 [d]	1.7 ± 0.1 [a]	2.0 ± 0.2 [b]
ORAC-FL (μmol Trolox/g)	6.7 ± 1.3 [c]	9.4 ± 1.5 [d]	0.11 ± 0.01 [a]	0.40 ± 0.04 [b]

ANOVA analysis was performed per row using Tukey's test. The means in the row with different letters indicate significant differences ($p < 0.05$).

4. Conclusions

BSG enzymatic hydrolysis showed the release of antioxidant compounds obtaining a BSG flour with improved antioxidant capacity. The incorporation of BSG flour in a bread formulation resulted in a "high fiber content" bread with suitable rheological properties and increased antioxidant capacity when compared to a wheat flour control bread. In conclusion, a novel sustainable bread with high nutritional quality was obtained.

Although positive results were obtained regarding increased dietary fiber and antioxidant capacity when adding BSG flour to bread formulation, sensory analysis of the novel food should be addressed in order to evaluate the consumers' perception on rheological parameters and consumer's acceptance. Moreover, bioaccessibility studies should be carried out in order to determine the remaining bioactivity after digestion.

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