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Bioactive compounds from brewer's spent grain: phenolic compounds, fatty acids and *in vitro* antioxidant capacity

Aline da Rosa Almeida[1], Márcia Regina Ferreira Geraldo[2], Leomara Floriano Ribeiro[1], Marcos Vieira Silva[2], Matheus Vinicius de Oliveira Brisola Maciel[4] and Charles Windson Isidoro Haminiuk[3*]

*[1]Programa de Pós-Graduação em Engenharia de Alimentos, Universidade Federal do Paraná, Curitiba, Paraná, Brasil. [2]Departamento Acadêmico de Alimentos, Universidade Tecnológica Federal do Paraná, Campo Mourão, Paraná, Brasil. [3]Programa de Pós-Graduação em Tecnologia de Alimentos, Universidade Tecnológica Federal do Paraná, Rua Rosalina Maria Ferreira, 1233, 87301-899, Campo Mourão, Paraná, Brazil. [4]Programa de Pós-Graduação em Ciência de Alimentos, Universidade Federal de Santa Catarina, Florianópolis, Santa Catarina, Brasil. *Author for correspondence. E-mail: haminiuk@utfpr.edu.br*

ABSTRACT. Brewer's spent grain (BSG) was characterized by physicochemical, total phenolic compound and flavonoids contents. Antioxidant activity was evaluated by four different assays. The chromatographic analyses were used to quantify the phenolic compounds and the fatty acids in BSG. Ethanolic extracts were tested to evaluate antibacterial activity. The higher concentration of total phenolic compounds for BSG was obtained in the extraction with ethanol 20%. BSG showed an antioxidant potential for all tests evaluated. In the case of chromatographic analysis, phenolic acids and flavonoids, such as syringic acid and catechin, respectively, were detected in high quantities. Regarding to the fatty acids profile, polyunsaturated fatty acids, such as linoleic and oleic acids, were found in significant amounts. No antibacterial activity was reported for bacterial cultures and concentrations tested. BSG may be considered a protein source, rich in fiber, polyunsaturated fatty acids and bioactive compounds with antioxidant potential.

Keywords: by-products, brewer's spent grain, flavonoids, antioxidant.

Compostos bioativos do bagaço de malte: compostos fenólicos, ácidos graxos e capacidade antioxidante *in vitro*

RESUMO. Bagaço de malte (BM) foi caracterizado pela avaliação do conteúdo total de compostos fenólicos e flavonóides e propriedades físico-químicas. A atividade antioxidante foi avaliada por quatro diferentes ensaios. Análises cromatográficas foram utilizadas para quantificar os compostos fenólicos e os ácidos graxos do BM. O extrato etanólico foi testado para avaliar a atividade antibacteriana. A concentração mais elevada de compostos fenólicos totais para o BM foi obtida na extração com etanol a 20%. O BM mostrou um potencial antioxidante para todos os testes avaliados. Na análise cromatografia líquida, ácidos fenólicos e flavonóides, tais como o ácido siringico e catequina, respectivamente, foram detectados em quantidades elevadas. Em relação ao perfil de ácidos graxos, ácidos graxos poli-insaturados, como ácidos oleico e linoleico, foram encontradas em quantidades significativas. Nenhuma atividade antibacteriana foi observada para culturas de bactérias e concentrações testadas. BM pode ser considerado como uma fonte de proteína, rico em fibra, ácidos graxos poli-insaturados e compostos bioativos com potencial antioxidante.

Palavras-chave: sub-produto, bagaço de malte, flavonoides, antioxidante.

Introduction

Brazil is the third largest producer of beer worldwide, with a production approximately of 13.5 billion liters per year. The beer is produced from barley, hop, water and yeast, resulting in a beverage rich in carbohydrates, amino acids and minerals. During the brewing process, relatively large amounts of by-products are generated, the most common ones being spent grains, spent hops and surplus yeast (Granato, Branco, Faria, & Cruz, 2011; Mussatto, 2009).

The brewers` spent grain (BSG) is the most abundant solid by-product produced during the brewing process, resulting from the wort elaboration stage. In 2012, Brazil manufactured around 2.70 million tons of spent grain, approximately 20 kg of BSG per 100 L of beer produced. Although it is produced in large quantities during the whole year, BSG is used mainly as animal feed. BSG is composed of barley malt residues mostly barley grain husk, but small amounts of pericarp and endosperm fragments may also be found. Further, they may also include additions (non-malt sources) such as wheat, rice or maize, added during mashing, even though this depends on the type of beer produced (Reinold, 1997; Townsley, 1979). BSG´s

chemical composition may vary according to barley variety, harvest time, malting and mashing conditions, and to the quality and type of extras added or not in the brewing process (Huige, 1994).

The main constituents in BSG´s chemical composition are protein (26-30%) and high fiber contents (approximately 70%) (Mussatto, Dragone, & Roberto, 2006; Öztürk, Özboy, Cavidoglu, & Köksel, 2002). Furthermore, some studies report that BSG contains bioactive compounds including hydroxycinnamic acids, such as ferulic and p-coumaric acids, flavonoids, tannins, and proanthocyanidins, widely acknowledged as featuring important antioxidant, anti-mutagenic, anti-allergenic, anti-inflammatory and anti-microbial qualities (Bravo, 1998; Hernanz et al., 2002). Several research studies have recently associated consumption of foods rich in bioactive compounds with the promotion of human health, such as the consumption of tea (catechins), coffee (chlorogenic acid), wine (resveratrol) and fruits (Bravo, 1998; Moreira et al., 2013). Fatty acids (FAs) in foods have also been studied for promoting health and preventing disease. This is due to their high polyunsaturated fatty acid contents and their effects in reducing cardiovascular diseases (Leaf & Weber, 1988). However, information on bioactive compounds, antioxidant potential and profile fatty acids of by-products generated by the beer industry, such as BSG, are still scarce.

Driven by intense global pressure for a green environmental technology, academic and industrial researchers are trying to reduce the amount of such wastes by discovering alternatives besides the current general usage in animal feed. Since most are agricultural products, they may be readily recycled and reused. In fact, they are real economic opportunities for brewing improvements (Ishiwaki, Murayama, Awayama, Kanauchi, & Sato, 2000). Furthermore, the fact that beer is a natural product makes brewers pay attention to their marketing image and take seriously the treatment of their industrial by-products (Fillaudeau, Blanpain-Avet, & Daufin, 2006).

Therefore, current assay aims at extracting, characterizing and quantifying the bioactive compounds in brewers' spent grain, determining the antioxidant capacity for different assays, evaluating fatty acids and assaying the antibacterial potential. The above parameters were investigated to investigate the reuse potential of the brewing industry waste.

Material and methods

Chemicals

From Sigma-Aldrich (St. Louis, MO USA) we purchased 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonate) (ABTS), ferrozine (0.5 mmol L^{-1}), β-carotene,2,2-diphenyl-1-picrylhydrazyl (DPPH), chloroform, linoleic acid, Tween 40, Folin-Ciocalteau reagent, standards of fatty acid methyl esters (FAME 189-19), chemical HPLC-grade standards (purity ≥ 95%) of gallic acid, syringic acid, catechin, and kaempferol. Methanol and phosphoric acid were of HPLC grade, while the other reagents used in the experiments were of analytical grade. The aqueous solutions were prepared by ultra-pure water (Milli-Q, Millipore, São Paulo, São Paulo State, Brazil).

Sample

In the current assay, Brewer's spent grain (BSG), obtained from a single lot after the filtration stage during the production of Pilsen malt pure beer, was kindly supplied by Micro Brewery Bier Hoff (Curitiba, Paraná State, Brazil). BSG sample was dried in an air circulation oven (Marconi MA 035) at 50°C for 24 hours in the laboratory; ground, sealed in polyethylene bags, vacuum packed and stored at – 20°C until further use.

Physical and chemical analysis

Moisture content (constant weight at 105°C), protein content (Kjeldahl method), lipid content (Soxhlet extraction), ash (by incineration), crude fiber and dietary fiber (enzymatic method), minerals, such as calcium, iron, phosphorus, manganese, potassium and zinc (by inductively coupled plasma atomic emission spectrometry - ICP-AES), pH and titratable acidity were analyzed, in triplicate, following method by Adolfo Lutz Institute, to determine the physical and chemical composition of the BSG sample (Instituto Adolfo Lutz, 2005).

Preparation of brewer's spent grain extracts

Extractions were performed at the ratio 1:20 (w/v - solute/solvent) and mixtures were shaken for 24 hours on a shaker (Tecnal/TEC-420), at 100 rpm and at 25ºC. Five different concentrations were used with different percentages ranging between 0 and 100 % (100:0; 80:20; 60:40; 40:60; 20:80) (v/v), with ethanol and distilled water, respectively. Solutions were centrifuged at 3493 x *g* for 25 minutes and the supernatants were separated, filtered and stored under refrigeration.

Total phenolic compounds analysis (TPC)

TPC of BSG extracts was estimated by a colorimetric assay, following methodology by Singleton and Rossi (1965) for extracts at different

concentrations. Diluted BSG extracts 100:0, 80:20, 60:40, 40:60, 20:80, ethanol and water, respectively, or blank (150 μL) were mixed with 7500 μL distilled water and 750 μL Folin–Ciocalteau reagent. After 3 min., 2250 μL of 15% sodium carbonate and 4350 μL of distilled water were added. The mixture was incubated in the dark, at room temperature (25°C), for 2 hours. The absorbance was then measured at 765 nm using an UV-Vis 1600 spectrophotometer (Pró-Análise, Brazil). Measurement was compared to a calibration curve of gallic acid (GA) ($p \leq 0.01$ and $R^2 = 0.99$) and results were given in mg gallic acid equivalents (GAE) per kg of BSG. All measures were performed in triplicates.

Total flavonoids content (TFC)

Total flavonoids were determined by aluminum chloride colorimetric method, in triplicate (Chang, Yang, Wen, & Chern, 2002). A 500 μL aliquot of BSG extract (20% ethanol) was mixed with 1500 μL of 95% ethanol, 100 μL of 10% aluminum chloride, 100 μL of 1M potassium acetate and 2800 μL of distilled water. After incubation at room temperature (25 °C) for 30 min, the absorbance of the reaction mixture was measured at 415 nm using an UV-Vis 1600 spectrophotometer (Pró-Análise, Brazil). The 10% aluminum chloride was replaced by the same amount of distilled water in blank. Measurement was compared to a calibration curve of quercetin ($p \leq 0.01$ and $R^2 = 0.99$) and results were given as mg quercetin equivalents (QE) per kg of BSG.

Antioxidant assays

1.1-Diphenyl-2-picrylhydrazyl radical (DPPH) test

Free radical scavenging activity was assessed by the DPPH• method, as previously described by Mensor et al., (2001) with minor changes. Based on the total phenolic compound values, five different concentrations (2–10 mg mL^{-1} in methanol) of BSG extracts (20% ethanol) were used to perform the DPPH• assay. Thus, 1 mL of DPPH• methanol solution (0.3 mmol L^{-1}) was added to 2.5 mL of sample solutions of different concentrations and the mixtures were allowed to react at room temperature (25°C) in the dark. After 30 min., the absorbance was measured at 518 nm with a UV-Vis 1600 spectrophotometer (Pró-Análise, Brazil), expressed in antioxidant activity percentage (AA %), according to Equation 1:

$$AA(\%) = 100 - \{[(Abs_{sample} - Abs_{blank}) x\ 100]/Abs_{control}\} \quad (1)$$

where:

Abs_{sample} is the absorbance of sample;
Abs_{blank} is the absorbance of blank;
$Abs_{control}$ is the absorbance of control.

Methanol (1.0 mL) mixed with BSG extract solution (2.5 mL) was used as a blank. The 0.3 mmol L^{-1} DPPH• solution (1.0 mL) mixed with methanol (2.5 mL) was used as a negative control. Positive controls included the samples with the standard solutions. EC_{50} values were calculated by non-linear regression ($p \leq 0.01$ and $R^2 = 0.98$) of plots where the abscissa represented the concentration of tested BSG extracts; the ordinate was the average percentage of antioxidant activity from three separate tests.

2.2'-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) assay

Evaluation of antioxidant activity by ABTS assay was performed according to methodology by Thaipong, Boonprakob, Crosby, Cisneros-Zevallos and Byrne, (2006) with minor changes. The stock solutions included 7.4 m MABTS solution and 2.6 mM potassium persulfate solution. The working solution was then prepared by mixing the two stock solutions at equal quantities and by allowing them to react for 12 hours at room temperature (25°C) in the dark. The solution was then diluted by mixing 1 mL ABTS solution with 60 mL methanol to obtain an absorbance of 1.1 ± 0.02 at 734 nm using an UV-Vis 1600 spectrophotometer (Pró-Análise, Brazil). A fresh ABTS solution was prepared for each assay. Five different concentrations (10-50 mg mL^{-1}) were prepared from the BSG extract (ethanol 20%). BSG extracts (150 μL) were then allowed to react with 2850 μL of ABTS solution for 2 hours in the dark and the absorbance was taken at 734 nm. Distilled water was used for negative control. Absorbance values were converted into percentage of antioxidant activity using Equation 4. Results were expressed in EC_{50} values calculated by non-linear regression ($p \leq 0.01$ and $R^2 = 0.99$). All measurements were performed in triplicate.

Metal chelating ability

The ferrous ion chelating ability of BSG extracts (20% ethanol) was measured at five different concentrations (10-50 mg mL^{-1}) (Senevirathne & Siriwardhana, 2006). A 700 µL aliquot of BSG extract at different concentrations was mixed with 700 µL distilled water, 175 µL $FeCl_2$ (0.5 mmol L^{-1}) and shaken; absorbance (Abs 1) was measured at 550 nm with a spectrophotometer (PG Instruments, T 80, China). Absorbance (Abs 2) at 550 nm was determined after reaction for 10 minutes with added 175 µL ferrozine (0.5 mmol L^{-1}). Distilled water was used for blank and negative controls, whereas EDTA was the positive control. Ferrous ion chelating ability was expressed as an antioxidant activity percentage (AA %) according to Equation 2 :

$$\text{Ferrous ion chelating ability (\%)} = \{1\ [(Abs2_s - Abs1_s)(Abs2_c - Abs1_c)x\ 100\} \quad (2)$$

Where Abs_s is the absorbance of the sample and Abs_c is the absorbance of control. Results were expressed in EC_{50} and calculated by non-linear regression ($p \leq 0.01$ and $R^2 = 0.97$).

β-carotene/Linoleic acid assay

Antioxidant activity of BSG extract was determined by measuring the coupled autoxidation of β-carotene and linoleic acid, following Emmons, Peterson and Paul (1999) with minor changes. Samples were diluted with ethanol to the equivalent of 2 grams of starting material per 40 mL, whereas β-carotene (5 mg) was dissolved in 100 mL of chloroform and 6 mL were added to 80 mg of linoleic acid and 800 mg of Tween 40. Chloroform was removed using a rotary vacuum-evaporator (Tecnal, TEC-211, Brazil). Oxygenated de-ionized water (200 mL) was added and suitably mixed. Aliquots (3 mL) of the β-carotene/linoleic acid emulsion were mixed with 50 μL of sample at five different concentrations (5-50 mg mL^{-1}) and incubated in a water bath at 50°C. Emulsion oxidation was monitored spectrophotometrically (PG Instruments, T 80, China) by measuring absorbance at 470 nm every 20 minutes. Control samples contained 50 μL of ethanol 20% replacing BSG extract. Degradation over time was non-linear and antioxidant activity was expressed as % inhibition relative to control after incubation for 120 minutes, by Equation 3.

$$AOA = 100\ (DR_C - DR_S)/DR_C \quad (3)$$

where:

AOA is the antioxidant activity;

DR_C is the degradation rate of the control = ln (*a*/*b*)/120;

DR_S is the degradation rate of the sample = ln (*a*/*b*)/120;

a is the initial absorbance at time 0;

b is the absorbance at 120 minutes. Results were given in EC_{50} and calculated by non-linear regression ($p \leq 0.01$ and $R^2 = 0.99$).

Gas chromatography (GC) analysis

Total lipids were determined according to the gravimetric method described by Bligh and Dyer (1959), and fatty acid methyl esters (FAME) were prepared by the method proposed by Hartman and Lago (1973). The fatty acids´ methyl esters were identified and quantified on gas chromatograph (GC) Finnigan, Model 9001 (Finnigan GC Group, Texas, U.S.A.) equipped with a flame ionization detector (FID) and a capillary column (60 m x 0.25 mm x 0.25 μm) (SGE BPX70, Australia). The injector and detector temperatures were kept at 255 and 250°C, respectively. Nitrogen was used as a carrier gas at a flow rate of 30.0 mL $min.^{-1}$ and the split ratio was 1:80. Oven temperature program comprised the following: the column was held initially at 185°C for 10 min.; then temperature was increased to 240 at 4°C $min.^{-1}$ and held for 1 minute. The total analysis time was 25 min. and 15 s. The injection volume was 1 μL. Retention times of the FAME were compared to those of standard methyl esters for identification. Retention times and peaks area percentages were automatically computed by integration with Clarity Lite software (Data Apex, version 2.7.03.498, Czech Republic). Quantification of FAME (in g kg^{-1} of total lipids) was performed against internal standard methyl tricosanoate (C23:0Me) (Visentainer & Franco, 2006).

Phenolic compounds determined by HPLC

Extracts were filtered with a syringe filter 0.22 μm of nylon (Millipore, São Paulo, São Paulo State, Brazil) prior to analysis, and 10 μL of sample was injected. Dionex UltiMate 3000 HPLC system (Dionex, Idstein, Germany), equipped with UltiMate 3000 Pump, Ulti-Mate 3000 Autosampler Column Compartment, Ulti-Mate 3000 Photodiode Array Detector and Chromeleon software, was used for analysis. Separation was performed on a reversed phase Acclaim® 120 column, C18 5 μm 120 Å (4.6 mm × 250 mm). It was used in the experiments at 40°C. The mobile phases consisted of acidified water with phosphoric acid 1% (A) and methanol (B). The solvent gradient comprised 0–15% B in 2 min.; 15–25% B in 5 min.; 25–30% B in 10 min.; 30–35% B in 15 min.; 35–50% B in 25 min.; 50–60% B in 30 min.; 60–80% B in 35 min.; 80–100% B in 45 min., followed by washing and reconditioning of the column. Flow rate was 1 mL $min.^{-1}$ and runs were monitored at 280, 300 and 320 nm. Phenolic compounds were identified by comparing their retention times using calibration curves with standards dissolved in methanol (Kelebek & Selli, 2014). Analyses were performed in triplicates.

Antibacterial activity assay

The agar disc diffusion method was employed to determine the antimicrobial activities of the BSG extract. Sterile filter paper discs were placed on agar Miller Hinton inoculated with test bacteria (*Staphylococcus aureus, Escherichia coli and Salmonella typhimurium*), filled with 15 μL BSG extract at four different concentrations (0.01, 0.1, 1.0 and 50 mg mL^{-1}), incubated at 37°C for 48h. All tests were

performed in triplicate. After incubation, inhibition zone diameters were measured. The tetracycline hydrochloride antibiotic (50mg mL^{-1}) was used as positive control material whilst solvent was the negative control (Bauer, Kirby, Sherris, & Turck, 1966).

Statistical analysis

Data were expressed as mean ± standard deviation (SD). Statistical analysis was carried out with STATISTICA 7.0 (StatSoft, 2004.).

Results

BSG sample showed high protein and fiber contents as well as high mineral elements, such as phosphorus and potassium, as Table 1 shows.

Table 1. Physicochemical composition of BSG.

Constituent	Content
Moisture(g kg^{-1})	63.2 ± 0.50
Protein (g kg^{-1})	185 ± 1.20
Lipids (g kg^{-1})	64.1 ± 0.20
Ash (g kg^{-1})	32.3 ± 0.50
Dietary fiber(g kg^{-1})	436.9 ± 0.00
Crude fiber(g kg^{-1})	125 ± 1.00
Titratable acidity(g kg^{-1})	80.3 ± 0.00
pH	5.93 ± 0.05
Calcium (mg kg^{-1})	81.60 ± 0.1
Iron (mg kg^{-1})	210 ± 0.8
Phosphorus (mg kg^{-1})	4882.7 ± 3.2
Manganese (mg kg^{-1})	34.3 ± 0.1
Potassium (mg kg^{-1})	1570.9 ± 2.6
Zinc (mg kg^{-1})	67.2 ± 1.2

Results are given as the mean value ± SD; n = 3.

Figure 1 shows fatty acid contents in BSG determined by gas chromatography, showing linoleic (C18:2), palmitic (C16:0) and oleic (C18:1) acids as the most abundant. Small amounts of other fatty acids, such as linolenic (C18:3) and stearic (C18:0) acids, were also found (Table 2).

According to results shown in Figure 2, the total phenolic compounds in BSG extracts ranged between 1.30 and 3.80 mg GAE kg^{-1} of BSG. The extraction with 20% v/v (3.80 mg GAE kg^{-1} of BSG) was, therefore, the most efficient for the extraction of phenolic compounds from BSG (Table 3).

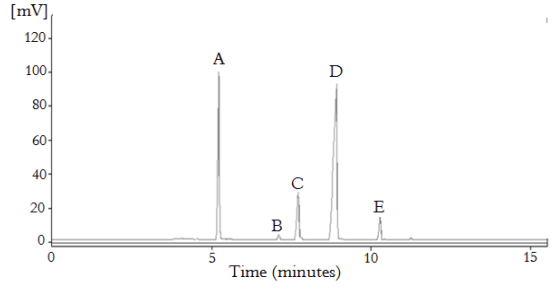

Figure 1. GC-FID chromatogram of main methyl esters of the fatty acids. Palmitic acid (A), stearic acid (B), oleic acid (C), linoleic acid (D) and linolenic acid (E) of BSG.

Table 2. Composition of fatty acids in BSG.

Fatty acids	Content (g kg^{-1})
Palmitic acid (C16:0)	252.78 ± 0.57
Stearic acid (C18:0)	9.81 ± 0.15
Oleic acid (C18:1 n9)	103.71 ± 0.85
Linoleic acid (C18:2 n6)	566.74 ± 0.36
Linolenic acid (C18:3 n3)	43.47 ± 0.54
SFA[a]	262.99 ± 0.43
MUFA[b]	103.71 ± 0.85
PUFA[c]	610.21 ± 0.82

[a] Total saturated fatty acids, [b] Total monounsaturated fatty acids, [c] Total polyunsaturated fatty acids

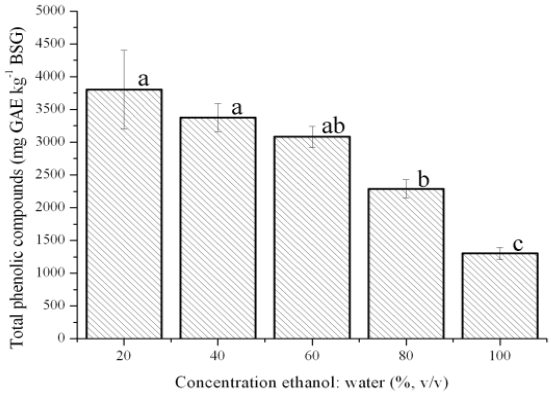

Figure 2. Extraction of total phenolic compounds from BSG extracts in five different concentrations ethanol-water (20:80; 40:60; 60:40; 80:20 and 100:0 v/v)

Total flavonoids contents reached 1493.75 ± 91.65mg QE kg^{-1} and the results of antioxidant assays are shown in Table 3.

Table 3. Total phenolic compounds, flavonoids and *in* vitro antioxidant activity assays of BSG.

Bioactive compounds				
Sample	Flavonoids[1]		TPC[2,3]	
BSG	1493.75 ± 91.65		3802.03 ± 599	
Antioxidant activity assays (EC_{50} = mg mL^{-1})				
Sample	DPPH	ABTS	BCLA[4]	MCA[5]
BSG	6.63 ± 0.09	26.09 ± 0.81	21.11 ± 1.20	16.33 ± 0.11

[1]Expressed as mg quercetin kg^{-1}, [2]Total phenolic compounds analysis, [3]Expressed as mg kg^{-1}gallic acid equivalents (GAE), [4]BCLA: β-carotene/ linoleic acid assay, [5]MCA: metal chelating ability assay.

Table 4 shows individual phenolic compounds identified and quantified by HPLC in BSG extract. The phenolic compound with the highest concentration in BSG extract was syringic acid (hydroxybenzoic acid), followed by catechin (flavan-3-ol), gallic acid (hydroxybenzoic acid) and kaempferol (flavonol).

Table 4. Individual phenolic compounds analyzed by high-performance liquid chromatography in BSG.

Phenolic Compounds	RT (min.)	λ (nm)	RSD (%)	R^2	mg kg^{-1}
Gallic Acid (I)	6.65	280	8.26	0.998	32.2
Catechin (II)	9.95	280	9.09	0.997	84.4
Syringic acid(III)	14.09	280	8.34	0.998	122.2
Kaempferol (IV)	36.10	370	8.36	0.998	31.2

λ: wavelength; RT: Retention time. Results are given as the mean value, n = 2, RSD – Relative Standard Deviation, R^2 – Coefficient of determination.

Antibacterial activity was not found for *S. aureus*, *E. coli* and *S. typhimurium* in the different tested concentrations (0.01, 0.1, 1, 50 mg mL^{-1}).

Discussion

Studies have reported the proximate composition of BSG, which contains high contents of protein, fiber and lipids. This is due to the fact that BSG predominantly comprises husk-pericarp-seed coat layers which are rich in cellulose, non-cellulose polysaccharides, lignin, protein and fats (Santos, Jimenez, Bartolome, Gomez-Cordove, & Del Nozal, 2003). Variations in its physicochemical composition may occur because of differences in barley variety, harvest time, malting and mashing conditions in the process. As previously pointed out (Table 1), protein content was similar to that reported by Kanauchi, Mitsuyama and Andaraki (2001) (24%) and Russ, Mortel and Meyer-Pittroff (2005) (19-23%). On the other hand, values reported by Meneses, Martins, Teixeira and Mussatto (2013) for phosphorous content (6000 mg kg-1) was slightly higher than the above; potassium was the exception since result was lower (600 mg kg^{-1}).

Many bioactive substances found in cereals and oils have their functional activities in providing health benefits (Siró, Kápolna, Kápolna, & Lugasi, 2008). Different fatty acids were found in the BSG sample. Since minor changes in fatty acid composition occurred during malting and mashing, the fatty acid composition of BSG was similar to that of barley which provided high linoleic and palmitic acid content, respectively (Fedak & De La Roche, 1977; Kaukovirta-Norja, Laakso, Reinikainen, & Olkku, 1993).

Linoleic acid belongs to the family of essential Omega-6 unsaturated fatty acids that humans and animals must ingest since their body cannot synthesize them. Linoleic acid, also used in pharmaceutical and cosmetic products, influences metabolic processes in the skin and promotes the activity of vitamins A and E and barrier properties after stratum corneum recovery (Huang, Ju, & Chiang, 1999).

BSG mainly consists of polyunsaturated fatty acids (67.46%), followed by saturated fatty acids (26.92%), and finally by monounsaturated fatty acids (10.62%), respectively (Arranz et al., 2008; Niemi et al., 2012). The results were similar to those by Niemi et al. (2012), with a predominance of polyunsaturated fatty acids. The consumption of fatty acids is related to health benefits. In fact, the vegetable oils in BSG contain essential fatty acids and significant levels of other bioactive compounds, such as tocopherols, phytosterols and carotenoids that help in the prevention of cardiovascular disease through their antioxidant effects that protect bio-molecules from the action of free radicals (Liu, Winter, Stevenson, Morris, & Leach, 2012).

BSG extract´s chemical composition evidences an interesting source of fatty acids which have a wide range of nutraceuticals, pharmaceuticals and cosmetics properties and are of great interest to other industrial sectors. For example, as functional food ingredients, they seem to be a safe and practical option to reduce cholesterol levels in the blood. They also exhibit cancer prevention activity as they exert cytotoxic effects on cancer cells (McCarthy et al., 2013; Quílez, García-Lorda, & Salas-Salvadó, 2003).

Due to their importance in food quality, phenolic compounds are highly relevant for consumers, since they have protection and prevention roles in certain types of diseases. Phenolics are mainly classified according to the number of phenol rings that contain, for example, phenolic acids, stilbenes, flavonoids, lignans and tannins. In current study, phenolic acids, such as hydroxycinnamic and hydroxybenzoic acids, were the predominant phenolic compounds in BSG (Tsao &Yang, 2003).

The phenolic compounds of barley grain are contained in the husk with hydroxycinnamic acids accumulated in the cell walls. Since the values of total phenolic compounds extracted with 20, 40 and 60% of ethanol (v/v) failed to show any significant difference ($p \leq 0.05$) (data not shown), the extraction with 20% v/v (3.80 mg GAE kg^{-1} of BSG) was, therefore, the most efficient for the extraction of phenolic compounds from BSG. This is due to the fact that a high amount of water increases the permeability of cell tissue, enables better mass transfer by molecular diffusion and improves recovery of soluble bioactive compounds in water.

Flavonoids are the main bioactive compounds found in plant food material. Flavonoids in BSG are important because they are antioxidants and act as reducing agents, hydrogen donors, with high redox and metal chelate potential (Zhao et al., 2006). Total flavonoids contents reached 1493.75 ± 91.65 mg QE kg^{-1} (Table 3), whereas other studies (for instance, Meneses et al. (2013)) failed to obtain any results for flavonoid contents in BSG extracted with ethanol 20% (Moreira et al., 2013). The presence of flavonoids in BSG is important because of their antioxidant activities (Zhao et al., 2006).

Phenolic compounds in by-product extracts are complex and their antioxidant activities and

mechanisms will largely depend on the composition and conditions of the tested radical system. Many authors performed more than one type of radical system to evaluate the antioxidant activity of a selected sample (Madhujith & Shahidi, 2009; Re et al., 1999). Thus, DPPH, ABTS, β-carotene / linoleic acid and metal chelating ability assays have been employed to evaluate the antioxidant activity of the extracts obtained from the BSG sample. The use of different assays for evaluating BSG´s antioxidant activity is due to different reaction mechanisms.

DPPH assay is based on the reduction of the radical by hydrogen-donating antioxidant. This assay presented a low value (EC50 = 6.63 mg mL^{-1}) when compared to that in other assays. However, when the EC50 value is low, the consumption of DPPH by the sample is high and its antioxidant activity is high. Therefore, BSG sample presented high antioxidant activity for DPPH assay. The method that had the best antioxidant activity response is based on the transfer of electrons from an antioxidant compound to the DPPH•, converting it to its reduced form (Babbar, Oberoi, Sandhu, & Bhargav, 2012). Further, DPPH has the advantage of being unaffected by certain side reactions of polyphenols, such as metal ion chelation and enzyme inhibition (Holtekjolen, Kinitz, & Knutsen, 2006).

ABTS assay is based on a stable ABTS radical cation which has a blue-green chromophore absorption, produced by ABTS oxidation with potassium persulfate and is reduced in hydrogen-donating antioxidants (Costa, Moura, Marangoni, Mendes, & Teixeira, 2010). Although this method has a similar mechanism to the DPPH assay, the ABTS assay in current investigation presented a higher value when compared to that of the DPPH test (Table 3).

Although β-carotene/linoleic acid assay (BCB) has a different mechanism activity different from the ABTS assay, the result was similar (21.11 mg mL^{-1}). The β-carotene assay is based on the capacity of a sample containing antioxidants to retard oxidation of β-carotene in the presence of light due to the abstraction of a hydrogen atom from the methylene groups of linoleic acid.

One of the mechanisms of antioxidative action is the chelating of transition metals which may be measured by the decrease in the iron (II) and ferrozine complex absorbance. In the BSG sample, the metal chelating ability (MCA) provided results closer to those of the DPPH assay (Table 3). Madhujith and Shahidi (2009) reported that more than 50% of total phenolics present in rice and corn are in the insoluble-bound form. The high radical scavenging activity assessed by DPPH assays reported in insoluble-bound phenolic fraction of by-products extracts may be attributed to the ability of this process to release phenolic compounds ether- or ester-linked with cell wall constituents, especially to arabinoxylans and lignin (Naczk & Shahidi, 2004).

According to Dvořáková et al. (2008) phenolic acids are the major phenylpropanoid components in cereals and different levels of these phenolics are found in different cereal fractions. The starchy endosperm in cereals contains low levels, whereas the outer layers of the grain (pericarp, aleurone layer and germ) contain the highest levels. Madhujith and Shahidi (2009) also reported high concentrations of phenolic acids in the outer layers of the grains predominantly existing in insoluble bound form. Therefore, the high antioxidant activity of insoluble bound phenolic fraction may be attributed not only to the presence of different phenolics with different antioxidant potential, but also to the release of phenolics from the bound form.

Antibacterial activity was not found for microorganism tested in the different concentrations of the extract. (0.01, 0.1, 1, 50 mg mL^{-1}). The final result may be influenced because there are variables such as extraction method, bacterial culture, concentration of BSG extract and sample composition that may affect the final result. Studies with other organic residues, such as those by Baydar, Sagdic, Ozkan and Cetin (2006) in their investigation on defatted grape seeds, showed an antimicrobial potential.

Conclusion

The brewers' spent grain (BSG) revealed a source of minerals, proteins, fiber dietary, phenolic compounds and fatty acids. Antioxidant potential was verified by different methods for BSG extract and the residue showed antioxidant activity in all methods highlighting the DPPH assay. Therefore, BSG may be potentially considered a source of bioactive compounds for enrichment of foods with fatty oils, dietary fibers, phenolic compounds and antioxidant activity.

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