Influence of extraction solvents on the recovery of antioxidant phenolic compounds from brewer's spent grains

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ABSTRACT

This study evaluated the efficacy of different solvents (methanol, ethanol, acetone, hexane, ethyl acetate, water, methanol:water mixtures, ethanol:water mixtures, and acetone:water mixtures) for extracting antioxidant phenolic compounds from brewer's spent grains (BSGs). The extracts were characterized regarding the contents of total phenols, flavonoids, proteins and reducing sugars. Antioxidant activity was determined by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical, and the ferric reducing antioxidant power (FRAP) assay. The solvents had different efficiencies for extraction of antioxidant phenolic compounds. All the produced extracts showed antioxidant activity, but the extract produced with 60% v/v acetone had the most elevated content of total phenols and antioxidant potential by the two methods. BSG was demonstrated to be a valuable source of antioxidant phenolic compounds, and solid-to-liquid extraction using 60% v/v acetone was a low cost and quite efficient method to recover these value-added compounds.

1. Introduction

Brewers' spent grains (BSGs) are the most abundant solid by-products generated during the brewing process, resulting from the wort elaboration step. This material is basically composed of the barley malt residual constituents and includes the barley grain husk in the greatest proportion, and also minor fractions of pericarp and fragments of endosperm [1]. BSG is generated in large amounts every year and, although some recent studies suggest the possibility of reusing this material for industrial applications such as the production of alpha-amylase [2], activated carbon [3], ethanol [4], lactic acid [5] and xylitol [6], BSG is still traditionally supplied to local farmers for elimination, and the development of economically viable technologies for valorization of this agro-industrial by-product has been encouraged.

In the last few years, great attention has been paid to the bioactive compounds since these compounds have ability to promote a number of benefits for human health. The most common bioactive compounds include secondary metabolites such as antibiotics, mycotoxins, alkaloids, food grade pigments, plant growth factors, and phenolic compounds [7], which present antioxidant, anti-mutagenic, anti-allergenic, anti-inflammatory, and anti-microbial properties. Due to these beneficial characteristics for human health, researches have been intensified in order to find natural resources (fruits, vegetables, plants, agricultural and agro-industrial residues) as potential sources of bioactive compounds. Additionally, there is still a growing interest in finding natural resources with antioxidant activity to effectively replace the synthetic antioxidants, which have been related to toxic and carcinogenic effects [8].

Phenolic compounds are bioactive substances widely distributed in plants. However, the amount of these compounds as well as their structure (the number of phenolic hydroxyl groups and their position) vary to each material source, leading to variations in their antioxidant capacity [9]. Barley grain is reported to be an excellent source of phenolic compounds including phenolic acids (benzoic and cinnamic acid derivatives), flavonoids, tannins, proanthocyanidins, and amino phenolic compounds [10], which are widely recognized as having important antioxidant and antiradical properties [11]. Some studies report that BSG contains antioxidant phenolic compounds including the hydroxycinnamic acids ferulic and p-coumaric, among others [12–14].

Solid-to-liquid extraction is the most common method used to recover natural antioxidants from plant materials. However, the efficiency of extraction is affected by several factors, among of which, the type of solvent have been considered one of the most important [15]. A large amount of information can be found on the effect of the type of solvent on the extraction of antioxidant phenolic compounds from different raw materials. Nevertheless, studies on the extraction of these compounds from BSG are scarce. To the best of our knowledge, there are no studies on the solvents extraction of antioxidant phenolic compounds from BSG. Only

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some few studies can be found on the particular extraction of ferulic and/or p-coumaric acids from this raw material by using methods based on saponification (with NaOH solution) [12], enzymatic hydrolysis [13,14], or microwave-assisted extraction [16].

The aim of this study was to extract antioxidant phenolic compounds from BSG. Initially, the chemical composition of this material was determined, and subsequently, the extraction of antioxidant phenolic compounds was studied. Different solvents including methanol, ethanol, acetone, hexane, ethyl acetate, water, and mixtures of methanol, ethanol or acetone with water were evaluated. The antioxidant potential as well as the contents of phenols, flavonoids, reducing sugars and proteins in the produced extracts were determined.

2. Experimental

2.1. Raw material and chemicals

Brewer's spent grains (BSGs) were supplied by UNICER Bebidas de Portugal, S.A. (S. Mamede de Infesta, Portugal). As soon as obtained, the material (approx. 80% moisture content) was dried at 60 °C to 90% dry matter to be stored safely.

Sodium carbonate, gallic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH), sodium acetate, quercetin, aluminum chloride, 2,4,6-tris (1-pyridyl)-5-triazine (TPTZ), ferrous sulfate, iron (III) chloride, hydrochloride acid and Folin–Ciocalteu phenol reagent were purchased from Sigma–Aldrich GmbH (Sternheim, Germany). Reagent-grade hexane, ethyl acetate, methanol, ethanol and acetone were from Panreac (Barcelona, Spain). Potassium acetate was purchased from AppliChem (Darmstadt, Germany). HPLC-grade acetonitrile and reagent-grade glucose were obtained from Fisher Scientific (Leicestershire, UK). Reagent-grade 3,5-dinitrosalicylic acid was purchased from Fluka Chemika (Buchs, Switzerland). All other chemicals used were of analytical grade and obtained from either Sigma–Aldrich or Merck (Darmstadt, Germany). Ultrapure water was obtained from a Milli-Q System (Millipore Inc., USA).

2.2. Chemical characterization of BSG

In order to determine the chemical composition, a BSG sample of 2 g was mixed with 10 ml of sulfuric acid 72% w/w and maintained under discontinuous agitation at 50 °C over 7 min. Distilled water was then added to the reaction medium to dilute the acid to a concentration of 2.5% w/w, and the reaction medium was autoclaved at 121 °C for 45 min. At the end of this process, the material was cooled and filtered through filter paper. The solid residue retained in the filter paper was washed exhaustively with distilled water until neutralization and subsequently dried at 105 °C to constant weight. The liquid solution samples were filtered and analyzed to determine the concentrations of glucose, xylose, and arabinose, by HPLC. The percentage of cellulose, hemicellulose and lignin present in the raw material was calculated as described by Mussatto and Roberto [17]. Ashes in BSG were determined after incineration in a muffle furnace at 550 °C for 4 h. The content of nitrogen was determined by combustion using a Thermo Scientific Flash 2000 Elemental Analyzer, and the protein content was estimated by using the $N \times 6.25$ conversion factor. Finally, the content of extractives was determined by difference.

The mineral content was determined by inductively coupled plasma atomic emission spectrometry (ICP-AES). BSG samples (200 mg) were digested with HNO_3 (5 ml) and H_2O_2 (3 ml) in closed vessels (XF100, Anton Paar) using a Multiwave 3000 microwave (Anton Paar). For the digestion, the microwave power was increased from 0 to 1150 W during 9 min, and was then maintained at 1150 W during 10 min. After cooled to room temperature,

the final volume of the samples was adjusted to 100 ml, and they were analyzed by ICP-AES in a Thermo Scientific iCAP 6300 equipment.

2.3. Solvent extraction

Different solvents were used for extraction of antioxidant phenolic compounds from BSG, which included hexane, ethyl acetate, methanol, ethanol, acetone and water. Methanol, ethanol and acetone were used in the pure form (absolute) and also in mixtures with water to obtain different concentrations (80%, 60%, 40%, and 20% v/v), while hexane and ethyl acetate were used only in the pure form (95% v/v).

For the extractions, 1 g of dried BSG was mixed with 20 ml of solvent in 100-ml Erlenmeyer flasks, which were duly covered to avoid solvent loss, and maintained during 30 min in a water-bath with magnetic agitation at 80 °C when using water, and at 60 °C when using the organic solvents, due to their lower boiling point. After this time, the produced extracts were filtered through filter paper and 0.22 μm membranes, and stored at −20 °C until analyses. The volume of extract recovered after each extraction was quantified and used for calculation. Triplicate extractions were made for each solvent.

2.4. Chemical characterization of BSG extracts

2.4.1. Total phenols

Total phenols were determined by using the Folin–Ciocalteu reagent according to the colorimetric method described by Singleton and Rossi [18] adapted for use in a 96-well microplate. Briefly, 5 μl of the filtered extracts duly diluted in the same solvent used for extraction were mixed with 60 μl of sodium carbonate solution (7.5% w/v) and 15 μl of Folin–Ciocalteu reagent in a 96-well microplate. Then, 200 μl of distilled water were added and the solutions were mixed. After standing for 5 min at 60 °C, the samples were allowed to cool at room temperature, and the absorbance was measured at 700 nm in a spectrophotometric microplate reader (Sunrise Tecan, Grödig, Austria), using each respective solvent used for extraction as blank. A calibration curve was prepared using standard solution of gallic acid (200, 400, 600, 800, 1000, 2000, and 3000 mg/l). The total phenols content was expressed as milligram gallic acid equivalent per dry weight of material (mg GAE/g BSG).

2.4.2. Flavonoids

Flavonoids were quantified by colorimetric assay [19]. Briefly, 30 μl of the filtered extracts duly diluted in the same solvent used for extraction were added to 90 μl methanol in a 96-well microplate. Subsequently, 6 μl aluminum chloride (10% w/v), 6 μl potassium acetate (1 mol/l) and 170 μl distilled water were sequentially added to the mixture. After 30 min of reaction in the dark at room temperature, the absorbance of the mixture was measured at 415 nm in a spectrophotometric microplate reader (Sunrise Tecan, Grödig, Austria), using each respective solvent used for extraction as blank. A calibration curve was prepared using standard solution of quercetin (25, 50, 100, 150, and 200 mg/l). The total content of flavonoids was expressed as milligram quercetin equivalent per dry weight of material (mg QE)/g BSG).

2.4.3. Protein

Protein was estimated by the Bradford assay [20] adapted for use in a 96-well microplate. Briefly, 10 μl of the filtered extracts duly diluted in the same solvent used for extraction were mixed with 300 μl of Coomassie Blue reagent in a 96-well microplate. The microplate was then stirred for 30 s and, after 10 min at room temperature, the absorbance was measured at 595 nm in a spectrophotometric microplate reader (Sunrise Tecan, Grödig, Austria), using a

blank prepared with distilled water. The reagent was prepared by dissolving 100 mg of Commassie Brilliant Blue G25 in 50 ml of ethanol 95%. Then, 100 ml of phosphoric acid 85% were slowly added and the solution was diluted to 1 l with distilled water, filtered through 0.22 μm membranes, and stored at 4 °C in a dark room. A calibration curve was prepared using aqueous solutions of BSA (25, 100, 250, 500 and 1000 mg/l). The protein content was expressed as milligram per dry weight of material (mg BSA/g BSG).

2.4.4. Free radical scavenging activity (DPPH)

The free radical activity was determined by measuring the ability of the extracts to scavenge the free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) [21]. For the reactions, 10 ml of each extract, duly diluted in methanol, were added to 290 μl of DPPH solution (6×10^{-5} M in methanol and diluted to an absorbance of 0.700 at 517 nm) in a 96-well microplate. The resulting solutions were vortexed, and allowed to stand for 30 min in darkness at room temperature. Then the absorbance was measured at 517 nm in a spectrophotometric microplate reader (Sunrise Tecan, Grödig, Austria), using methanol as blank. The control solution consisted in using methanol instead of the sample. The radical scavenging activity was expressed as the inhibition percentage using the equation (1), where A_C and A_S are the absorbance of the control solution and the absorbance of the sample solution, respectively.

$$\% \text{ Inhibition of DPPH} = (1 - A_S/A_C) \times 100 \qquad (1)$$

2.4.5. Ferric reducing/antioxidant power assay (FRAP assay)

The antioxidant activity by the ferric reducing antioxidant power (FRAP) assay was determined by mixing 10 μl of the filtered and duly diluted extract with 290 μl of FRAP reagent in a 96-well microplate. Then, the reaction mixture was incubated at 37 °C for 15 min. After that, the absorbance was determined at 593 nm against a blank prepared with distilled water [22]. FRAP reagent was freshly prepared by mixing a 10 mM 2,4,6-tris (1-pyridyl)-5-triazine (TPTZ) solution in 40 mM HCl with a 20 mM $FeCl_3$ solution and 0.3 M acetate buffer (pH 3.6) in a proportion 1:1:10 (v/v/v). A calibration curve was prepared with aqueous solution of $FeSO_4$.7-H_2O (200, 400, 600, 800, and 1000 μM). FRAP values were expressed as millimoles of ferrous equivalent per dry weight of material (mM Fe (II))/g BSG).

2.4.6. Reducing sugars

Total reducing sugars were quantified by the DNS assay [23] adapted for use in a 96-well microplate. Briefly, 100 μl of the filtered and duly diluted extract were added to 100 μl of DNS reagent in an eppendorf, which was placed in a hot water bath at 100 °C for 5 min. Afterwards, the samples were allowed to cool in a cold water bath and 1 ml of distilled water was added to each sample. Two hundred microliters of each sample were then placed in a 96-well microplate and the absorbance was measured at 540 nm in a spectrophotometric microplate reader (Sunrise Tecan, Grödig, Austria) using a blank prepared with distilled water. DNS reagent was prepared by dissolving 2.5 g of 3,5-dinitrosalicylic acid in 25 ml of distilled water at 80 °C. The mixture was cooled in an ice bath and 50 ml of 2 N NaOH and 75 g of potassium sodium tartrate were added. Finally, distilled water was added to complete the volume to 250 ml. A calibration curve was prepared using standard solution of glucose (100, 200, 400, 600, 800, and 1000 mg/l). The content of reducing sugars was expressed as milligrams per dry weight of material (mg/g BSG).

2.5. Statistical analysis

The extraction experiments were carried out in triplicate for each solvent, and analytical determinations were made as five repetitions. Results were presented as means values with standard deviations. The results were statistically analyzed by using the Tukey's range test, where a p value of less than 0.05 was regarded as significantly different. Origin Pro 8 (Origin Lab Corporation, USA) was the software used for data analysis.

3. Results and discussion

3.1. Chemical composition of BSG

Chemical composition of BSG is presented in Table 1. As can be seen in this table, BSG is mainly constituted by sugars (glucose, xylose and arabinose) polymerized in the structures of cellulose and hemicellulose, which represent 41% w/w of its composition on a dry weight basis. Besides sugars, BSG also contains noticeable fractions of proteins (24.69% w/w) and lignin (19.40% w/w), while extractives and ashes are present in minor proportions. Extractives may include components such as waxes, alkaloids, pectins, mucilages, gums, resins, terpenes, saponins and tall oil [24]. Ashes are formed by a large variety of minerals, including phosphorus, potassium, calcium, magnesium, sulfur, iron, manganese, boron, zinc, copper, molybdenum, sodium, aluminum, barium, strontium, chromium, tin, lead, cobalt, iodine, cadmium, nickel, selenium and gallium, at levels up to 6000 mg/kg dry weight (Table 2). Phosphorus and calcium are the mineral elements present in the highest amounts in BSG.

The lignin content in BSG (19.4% w/w) is particularly high when compared to other lignocellulosic materials such as barley straw (15.5% w/w) [25], wheat straw (14.1% w/w) [26], rice straw (13.0% w/w), sugarcane bagasse (18.8% w/w), silvergrass (18.6% w/w) [27], and oil palm empty fruit bunch (18.8% w/w) [28]. Lignin is a macromolecule constituted by different functional groups, which include aliphatic hydroxyl groups, phenolic groups, and carboxylic groups [29], but its structure and composition vary to each raw material. Lignin in BSG has been reported to contain significant amount of phenolic acids, particularly the hydroxycinnamic acids ferulic and p-coumaric [13,14], which have important physiological functions including antioxidant activity. Due to the importance of the phenolic compounds and taking into account the significant amount of lignin present in BSG composition, the extraction of antioxidant phenolic compounds could be considered as an alternative of great interest for the valorization of this agro-industrial by-product.

3.2. Chemical composition of BSG extracts

Different solvents, in mixtures or not with water, were used to extract antioxidant phenolic compounds from BSG. Since the temperature increase has been reported to improve the efficiency of extraction due to enhanced diffusion rate and solubility of the

Table 1
Chemical composition of brewers' spent grains (dry matter).

Component	Concentration (g/100 g)[a]
Cellulose (glucan)	21.73 ± 1.36
Hemicellulose	19.27 ± 1.18
Xylan	13.63 ± 0.82
Arabinan	5.64 ± 0.35
Lignin	19.40 ± 0.34
Insoluble in acid	17.54 ± 0.31
Soluble in acid	1.86 ± 0.03
Proteins ($N \times 6.25$)	24.69 ± 1.04
Ash	4.18 ± 0.03
Extractives	10.73 ± 0.32

[a] Values correspond to mean ± standard deviation.

Table 2
Mineral elements in brewers' spent grains composition (dry matter).

Minerals	Concentration (mg/kg)[a]
Phosphorus	6000 ± 0.00
Calcium	3600 ± 0.00
Sulfur	2900 ± 0.00
Magnesium	1900 ± 0.00
Potassium	600 ± 0.00
Iron	154.90 ± 0.60
Sodium	137.10 ± 6.10
Manganese	40.90 ± 0.90
Zinc	82.10 ± 0.60
Aluminum	81.20 ± 2.20
Cobalt	17.77 ± 0.22
Copper	11.40 ± 0.50
Iodine	11.00 ± 0.72
Strontium	10.36 ± 0.00
Barium	8.62 ± 0.00
Boron	3.20 ± 0.80
Molybdenum	1.35 ± 0.34
Lead	<1.60
Selenium	<1.60
Gallium	<1.47
Tin	<1.30
Chromium	<0.54
Nickel	<0.54
Cadmium	<0.15

[a] Values correspond to mean ± standard deviation.

compounds in solvents [15], the extraction reactions were not performed at room temperature, but in temperatures near the boiling point of the solvents. Therefore, extractions were performed at 80 °C when using water, or at 60 °C when using the organic solvents. The use of higher temperatures is not encouraged since elevated temperatures could affect the antioxidant activity of the extracts as well as to decrease the stability of the phenolic compounds [15].

All the BSG extracts contained phenolic compounds (including flavonoids) in their composition, and the content of these compounds varied to each solvent used for extraction. Besides phenolic compounds, most of the extracts contained also some proteins and reducing sugars (Table 3), but in low amounts taking into account that proteins and sugars correspond to 24.69% and 41.00% w/w of BSG composition, respectively (Table 1). These low amounts of proteins and reducing sugars are justifiable since the extraction conditions used in the present study were not suitable to release these fractions from the material structure. Sugars, for example are efficiently extracted from BSG at temperatures higher than 100 °C, especially 120 °C when using dilute acid [17] or between 150 °C and 190 °C when pure water is used as extraction solvent [30]. Proteins can be extracted from BSG by ultrasonic-assisted extraction [31] or ultrafiltratioin [32].

The content of total phenols in BSG extracts, which was expressed as gallic acid equivalents (GAEs), varied from 2.14 mg GAE/g BSG when ethyl acetate was used as extraction solvent to 9.90 mg GAE/g BSG when 60% v/v acetone was used, which represent a 4.6 fold increase in the extraction yield. Among the tested solvents, acetone was the most efficient for extraction of phenolic compounds from BSG, mainly when used at 60% v/v. Under this condition, the extract produced from BSG contained a phenolic concentration significantly different ($p < 0.05$) of all the other extracts produced (Table 3). Additionally, flavonoids, which constitute the largest group of plant phenolics accounting for over half of the eight thousand naturally occurring phenolic compounds [33] were present in all the BSG extracts produced with acetone, in concentrations varying between 0.51 and 2.12 mg QE/g BSG. Except this solvent, only ethanol (⩾40% v/v), methanol (⩾60% v/v) and hexane were able to extract flavonoids from BSG (Table 3).

Acetone has also been demonstrated to be more efficient than other organic solvents for the extraction of phenolic substances from different raw materials such as star fruit [34], elderberry and grapes [35], mango seeds [15], banana peel [36], and plants including *Orthosiphon stamineus* [37], *Larrea tridentata* [38], and *Syzygium cumini* [39]. Similar to the present study, acetone:water mixtures (50–95% v/v) have been reported to be one of the most effective solvents for extraction of phenols from different natural sources, especially from protein matrices, since this mixture is able to degrade polyphenol–protein complexes [40]. This fact would explain the high efficiency of this solvent to extract phenolic compounds from BSG, as this material has high protein content (24.69 g/100 g DW, Table 1).

It is worth noting that acetone:water mixtures were more efficient than pure acetone to extract phenols from BSG. According to some authors, the addition of water to organic solvents such as acetone, methanol, and ethanol, creates a more polar medium that facilitates the extraction of phenolic compounds [41]. Other studies report also more efficient extraction of phenolic compounds when using the organic solvent in mixture with water [38,42]. However, the use of pure water as solvent is not efficient to extract phenols because these compounds are often more soluble in organic solvents less polar than water [43]. This is in agreement with the results obtained in the present study (Table 3).

Finally, according to the maximum value obtained in the present study, BSG presents phenols content of 9.9 mg GAE/g dry matter (Table 3). This result is lower than the contents present in berries (28–51 mg GAE/g) for example, but is similar or higher than the phenols content reported to several fruits, vegetables, and plants, such as onion (2.5 ± 0.1), Swede peel (1.6 ± 0.0), cucumber leaf (3.8 ± 0.1), carrot leaf and peel (7.4 ± 0.1 and 6.6 ± 0.1), tomato (2.0 ± 0.1), potato peel (4.3 ± 0.2), chamomile flower (9.1 ± 0.8), pineapple weed (4.2 ± 0.9), lake reed leaf (5.7 ± 0.3), goldenrod (8.2 ± 0.1), red clover (7.8 ± 0.2), and bulrush leaf (8.2 ± 0.1) [44]. Such result suggests that BSG is a raw material with significant content of phenolic compounds.

3.3. Antioxidant potential of BSG extracts

The antioxidant potential of the BSG phenolic extracts was determined by two methods based on different approaches, namely the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method, and the ferric reducing antioxidant power (FRAP) assay, which have been extensively used to determine the antioxidant potential of various plant extracts and natural products. Irrespective of the solvent used for extraction, all the extracts produced from BSG showed antioxidant potential at least by one of these methods (Table 3). Among them, the extracts obtained with 80% v/v methanol, 80% and 60% v/v acetone or ethanol had the highest antioxidant activity by both DPPH and FRAP methods. Similar results were observed during the extraction of antioxidant compounds from mango peel, where mixtures between methanol, ethanol or acetone with water produced extracts with higher antioxidant potential than extracts produced with these solvents in the pure form [15].

The highest values of DPPH inhibition were obtained for the BSG extracts produced with acetone at 60% and 40% v/v, whose values were not different at 95% confidence level. The DPPH method is based on the ability of DPPH radical to react with hydrogen donor species such as phenols and flavonoids present in the extract material [45]. Analyses of the correlation between the DPPH results obtained for acetone extracts and the total phenols and flavonoids contents in these extracts revealed a positive correlation for both cases, which means that the antioxidant activity increased as the concentration of total phenols and flavonoids increased. However, a strong correlation was observed with the flavonoids results,

Table 3

Characterization of the extracts produced by solid-to-liquid extraction of brewer's spent grains (BSGs) using different solvents.

Solvent (% v/v)	Composition				Antioxidant potential	
	Total phenols (mg GAE/g BSG)	Flavonoids (mg QE/g BSG)	Protein (mg/g BSG)	Reducing sugars (mg/g BSG)	DPPH inhibition (%)	FRAP (mM FE(II)/g BSG)
H_2O	3.59 ± 0.46 ab	0.02 ± 0.01 a	0 a	27.47 ± 1.57 bc	3.33 ± 1.79 abc	0.88 ± 0.15 ab
Methanol						
100	3.90 ± 0.06 ab	0.75 ± 0.14 bc	0 a	25.07 ± 1.01 b	12.69 ± 1.72 e	1.07 ± 0.03 bc
80	6.46 ± 0.50 bc	1.02 ± 0.19 c	4.29 ± 0.38 c	33.45 ± 0.54 c	12.25 ± 1.80 de	2.31 ± 0.24 d
60	4.57 ± 0.62 ab	0.33 ± 0.09 ab	3.44 ± 0.61 bc	32.41 ± 0.79 bc	10.40 ± 2.19 cd	2.04 ± 0.18 cd
40	3.89 ± 0.38 ab	0 a	0 a	29.03 ± 0.80 bc	7.59 ± 0.81 bcd	0.79 ± 0.07 ab
20	2.90 ± 0.43 a	0 a	0 a	22.96 ± 2.71b	2.51 ± 0.74 abc	0.45 ± 0.08 a
Ethanol						
100	4.60 ± 0.35 ab	2.54 ± 0.04 d	1.87 ± 0.09 bc	4.75 ± 0.89 a	5.03 ± 0.56 abc	1.29 ± 0.12 bc
80	5.54 ± 0.31 b	4.61 ± 0.05 f	7.48 ± 0.90 d	23.05 ± 0.95 b	12.02 ± 0.07 de	2.88 ± 0.40 d
60	7.13 ± 0.24 bc	2.59 ± 0.18 d	9.29 ± 0.57 e	24.18 ± 2.43 b	16.91 ± 0.78 e	2.87 ± 0.16 d
40	6.18 ± 0.57 bc	0.98 ± 0.03 c	1.55 ± 0.19 ab	25.19 ± 2.88 b	1.64 ± 0.41 ab	1.30 ± 0.23 bc
20	4.26 ± 0.51 ab	0 a	0 a	22.57 ± 1.04 b	0 a	0.94 ± 0.22 ab
Acetone						
100	5.66 ± 1.00 b	1.03 ± 0.01 c	0 a	6.33 ± 0.83 a	13.45 ± 1.62 e	0 a
80	5.37 ± 0.11 b	2.12 ± 0.04 d	1.01 ± 0.10 ab	32.12 ± 2.93 bc	20.55 ± 1.53 f	2.75 ± 0.10 d
60	9.90 ± 0.41 d	2.02 ± 0.08 d	3.32 ± 0.02 c	37.60 ± 0.63 d	18.53 ± 0.95 f	4.15 ± 0.24 e
40	6.26 ± 0.61 bc	1.33 ± 0.05 c	2.40 ± 0.27 bc	36.74 ± 3.69 d	13.01 ± 2.06 e	2.53 ± 0.19 d
20	5.94 ± 0.22 b	0.51 ± 0.03 abc	2.27 ± 0.15 bc	33.52 ± 0.37 c	7.46 ± 1.29 bcd	1.66 ± 0.11 bc
Hexane	4.44 ± 0.44 ab	2.92 ± 0.23 e	0 a	3.35 ± 0.11 a	2.02 ± 0.48 abc	0.24 ± 0.02 a
Ethyl Acetate	2.14 ± 0.53 a	0 a	0 a	3.61 ± 0.62 a	5.36 ± 0.27 abcd	0 a

For each column, letters equals indicate that the means difference is not significant at $p < 0.05$.

while the correlation with total phenols was weak (Fig. 1). These results suggest that flavonoids contribute significantly to the overall antioxidant activity of the acetone BSG extracts. The remaining contribution is due to compounds that were not identified in the present study. These results are in agreement with the observations made by Kähkönen et al. [44] who reported that the antioxidant activity of a phenolic extract cannot be predicted only on the basis of its total phenolic content, but it can be influenced by specific phenolic compounds present in this mixture. Flavonoids, for example, as observed in the present study, and other related polyphenols have been reported to contribute significantly to the antioxidant activity of extracts from other natural sources such as medicinal plants [46].

In the FRAP method, the antioxidant activity is determined based on the ability to reduce Fe^{3+} to Fe^{2+}, and the results are expressed as millimolar ferrous ion equivalents per gram of sample. Similar to the DPPH results, the most elevated antioxidant activity by the FRAP assay was also obtained for the extract produced with 60% v/v acetone (Table 3). According to Rodríguez-Rojo et al. [47], acetone is able to provide high antioxidant yield due to its hydrogen-bonding ability, which is crucial for the extraction of antioxidant phenolic compounds.
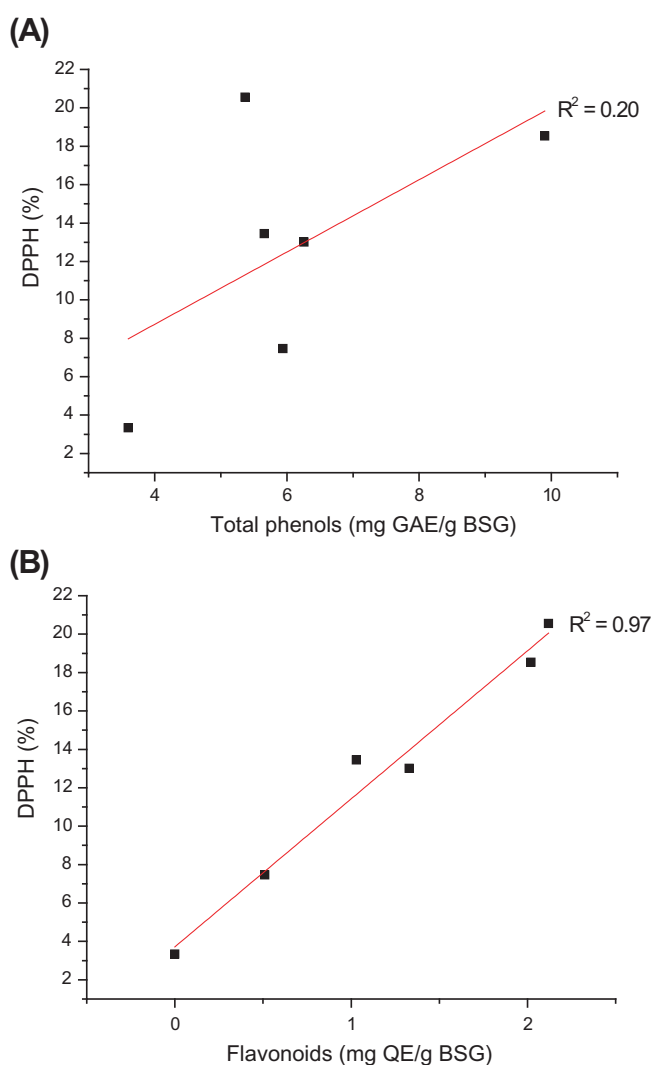
It is worth noting that the extracts produced with water presented low antioxidant potential and also low content of total phenols when compared with most of the extracts produced with organic solvents. Similar results have been reported during the extraction of antioxidant compounds from other raw materials such as mango peel and seed [15], grape by-products [48], murta leaves [49], *L. tridentata* leaves [38], and spent coffee grounds [42]. Such result suggests an unpolar character of the cell walls of these plant materials, including BSG. This characteristic would allow solvents that are less polar than water (which include all the solvents used in the present study) to extract the antioxidant compounds more easily [15].

The use of acetone as solvent for extraction is very interesting if it is desired the extract application in food products, for example. The use of organic solvents in the manufacturing process to obtain food ingredients is regulated, and from the perspective of food security it is preferable to use solvents such as acetone:water or ethanol:water mixtures since they are in compliance with good manufacturing practice. Methanol is usually a good extraction solvent but it has a toxic character. Therefore, extracts produced with methanol arises serious issues for application in food and pharmaceutical products [42].

4. Conclusions

BSG contains considerable amount of phenolic compounds with antioxidant activity, which can be recovered by solid-to-liquid extraction. However, the extraction solvent is a factor of great influence on the recovery of these compounds. Acetone:water mixtures, especially at 60% v/v, were highly efficient to extract antioxidant phenolic compounds from BSG, and the antioxidant capacity of the produced extract was strongly correlated with the content of flavonoids present.

This study is the first attempt for recovering antioxidant phenolic compounds by solvent extraction of BSG, and the present findings certainly contribute to ascertain the potential of BSG for applications in the food, cosmetic and pharmaceutical industries, since antioxidant phenolic compounds could be recovered from BSG and used as a natural and inexpensive alternative to synthetic antioxidants.

Fig. 1. Correlation (simple regression analysis) between antioxidant activity by the DPPH method and total phenols content (A) or flavonoids content (B) in the BSG extracts obtained by extraction with acetone.

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