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Assessment of antioxidant capacity of brewer's spent grain and its supercritical carbon dioxide extract as sources of valuable dietary ingredients

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ABSTRACT

The antioxidant activity of brewer's spent grain (BSG) and malt during different wort elaboration steps, were assessed by employing QUENCHER approach for the DPPH·, ABTS·+, ORAC, FRAP and Folin–Ciocalteu's assays. The antioxidant potential of malt and BSG lipophilic fractions, obtained by means of supercritical carbon dioxide extraction (SCE-CO_2), and remaining solid residues were evaluated too. The obtained results indicate that in most cases BSG possessed significantly higher radical scavenging capacity, reducing power and total phenolic content as compared to malt prior to wort preparation. SCE-CO_2 yielded 5.49 g of lipophilic fraction per 100 g of BSG, with the activity of 212.36 mg TE/g extract as measured in L-ORAC assay. The SCE-CO_2 reduced the radical scavenging activity and reducing power of BSG by 1–23% in different assays, indicating that considerable part of BSG compounds with particular antioxidant capacity still remains in the solid residue.

1. Introduction

Brewer's spent grain (BSG) is the barley malt residue, obtained during the first step of brewing process and amounting approximately 20 kg per 100 L of beer produced. Therefore, it is the main solid by-product of the brewing industry, produced worldwide by both large and small scale breweries in large quantities throughout the year. Nowadays BSG is mainly utilized for the production of animal feeds, fertilizers, as an alternative source of energy and, more recently, in biotechnological processes as substrate for microorganism cultivation and enzyme production (Mussatto et al., 2006). However, a number of malt constituents (e.g. cellulose, non-cellulosic carbohydrates, proteins and essential amino acids, lignin, lipids and phenolic compounds) remains in BSG after the mashing and lautering of wort. Also, the qualitative and quantitative composition of macro- and micronutrients of BSG can vary according to barley variety, malt type, malting and mashing conditions, and the type of beer produced (Santos et al., 2003; Mussatto et al., 2006; Robertson et al., 2010; Moreira et al., 2013). Therefore, the application of BSG as a low-cost source of nutrients and phytochemicals for the dietary purposes is of particular interest nowadays and was successfully demonstrated for a number of bakery products, including breads, muffins, cookies, mixed grain cereals, cakes, waffles, tortillas, brownies, doughnuts, etc. (Mussatto et al., 2006). Additionally, it was shown that BSG phenolic compound extracts (Mussatto et al., 2007; McCarthy et al., 2012, 2013a; Moreira et al., 2013; Meneses et al., 2013; Reis and Abu-Ghannam, 2014), arabinoxylans (Reis and Abu-Ghannam, 2014), protein isolates (McCarthy et al., 2013b), fermentation products (Gupta et al., 2013), also phytosterol and alkylrezorcinol-containing lipophilic fractions (del Río et al., 2013) could be further utilized towards the search of natural food constituents with potential antioxidant capacity and other functional properties for nutraceutical and pharmaceutical applications.

A number of the so-called direct and indirect methods are available to evaluate the antioxidant capacity of bioactive compounds; however, the data available are mainly related with the antioxidant activity assessment of water-soluble or extractable products (Prior et al., 2005). The variety of extraction procedures applied and organic solvents used significantly affect the recovery of bioactive constituents and, in consequence, the antioxidant capacity of the

Abbreviations: ABTS·+, 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) radical; BSG, brewer's spent grain; DPPH·, 2,2-diphenyl-1-picrylhydrazyl radical; DW, dry weight; FRAP, ferric reducing antioxidant power; GAE, gallic acid equivalents; H-ORAC, hydrophilic-oxygen radical absorbance capacity; L-ORAC, lipophilic-oxygen radical absorbance capacity; TEAC, Trolox equivalent antioxidant capacity; TPC, total phenolic content; SCE-CO_2, supercritical carbon dioxide extraction.

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products obtained, as was recently illustrated for various BSG extracts by Meneses and co-workers (2013). Also, a considerable part of bioactive compounds in cereals are bound to cell wall polysaccharides (Acosta-Estrada et al., 2014), therefore the impact of insoluble fractions on the total antioxidant capacity may be highly underestimated. To avoid these disadvantages, a novel procedure to measure the antioxidant capacity of insoluble food components was recently proposed by Serpen and co-workers (2007). This so-called QUENCHER approach does not require extraction step prior to measurements, since the insoluble part of the sample exerts the antioxidant activity due to the surface reactions with, for example, radical solution at the solid–liquid interface, while the soluble moiety participates in the typical liquid–liquid interactions simultaneously (Gökmen et al., 2009). Also it does not depend on the mechanism behind the assay and can be combined with all antioxidant capacity assessment methods that are recommended for representative evaluation of antioxidant properties (Huang et al., 2005).

Therefore, this research was aimed to assess radical scavenging properties, reducing power and the total phenolic content of BSG and, in comparison, malt samples during wort elaboration applying the QUENCHER approach for the DPPH$^{\cdot}$, ABTS$^{\cdot+}$, ORAC, FRAP, and Folin–Ciocalteu's assays. Additionally, BSG and malt samples were fractionated by means of supercritical carbon dioxide extraction (SCE-CO_2), employed as an alternative and environmentally friendly technique to conventional extraction with organic solvents. As reviewed by Herrero et al. (2010), CO_2 is nontoxic and non-flammable solvent, and yields high purity products, therefore SCE-CO_2 extracts could be successfully explored in food and pharmaceutical industries. To the best of our knowledge, the SCE-CO_2 of BSG was previously performed only by Fernández et al. (2008), while the antioxidant potential of isolated fractions has not been studied yet. Therefore, the antioxidant capacity of SCE-CO_2 extracts and remaining solid residues was additionally evaluated by means of L-ORAC assay and QUENCHER procedure. Such systematic approach is expected to provide more comprehensive data on the antioxidant capacity of BSG and to facilitate the development of processing schemes for the isolation and fractionation of BSG into valuable ingredients.

2. Materials and methods

2.1. Chemicals and reagents

2,2-Diphenyl-1-picrylhydrazyl hydrate (DPPH, free radical, 95%), 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (TROLOX, 97%), 2,2′-azobis(2-amidinopropane) dihydrochloride (AAPH), microcrystalline cellulose (20 μm), Folin & Ciocalteu's phenol reagent (2 M) and gallic acid (99%) were obtained from Sigma–Aldrich (Bornem, Belgium); $FeCl_3{\cdot}6H_2O$ (>99%), sodium acetate (>99%) from Acros Organics (Geel, Belgium); 2,4,6-tris(2-pyridyl)-*s*-triazine (TPTZ) and fluorescein (FL) from Fluka Analytical (Bornem, Belgium); 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) diamonium salt (ABTS), NaCl, KCl, Na_2HPO_4 and $K_2S_2O_8$ were from Merck (Darmstadt, Germany); KH_2PO_4 was from Jansen Chimica (Beerse, Belgium); Na_2CO_3 (98%, anhydrous) from RPL (Grauwmeen, Belgium). Randomly methylated β-cyclodextrin (RMCD) (Trappsol, pharmacy grade) was purchased from CTD Holdings, Inc. (High Springs, FL). Carbon dioxide (99.9%) was obtained from Gaschema (Jonava r., Lithuania). *Pilsen*, *Munich* and *Carahell Dark* malts were obtained from JSC "Viking Malt" (Panevėžys, Lithuania). Solvents were of analytical grade.

2.2. Sample preparation

For the purposes of this research, BSG was obtained after mashing and lautering of wort (Fig. 1) in the MiniBrau beer production line (NERB, Germany) in Food Science and Technology Competence Centre at Kaunas University of Technology. In a mash tun, 4.6 kg of the milled malt mixture (97.7% *Pilsen*, 2.2% *Munich* and 0.1% *Carahell Dark*) was mixed with 15 L of water (40–45 °C) for 20 min, followed by the stepwise increse of the mash temperature from 45 to 73 °C to promote an enzymatic hydrolysis of starch and proteins (protein rest at 50–55 °C for 30 min; maltose rest at 60–65 °C for 30 min; dextrin rest at 70–73 °C for 20 min), mashout (75–77 °C for 20 min) and lautering to separate the mash into a clear liquid wort, further transferred to a brew kettle for boiling with hops, and residual solid fraction (BSG), which was additionally rinsed with 78 °C water. BSG and mash solid fraction samples collected after protein, maltose and dextrin rests (Fig. 1) were freeze-dried (−50 °C, 0.5 mbar), ground by ultra centrifugal mill ZM 200 (Retsch, Haan, Germany) using 0.2 mm hole size sieve and kept in a dry, well-ventilated and dark place prior to the analysis.

2.3. Supercritical CO_2 extraction (SCE-CO_2)

The SCE-CO_2 of BSG and, in comparison, initial malt mixture (Fig. 1), was carried out using supercritical fluid extractor Helix (Applied Separation, Allentown, PA) as described by Kraujalis and Venskutonis (2013), with slight modifications. Each extraction was performed using 10 g of ground malt or BSG, which were loaded into a 50 mL stainless steel extraction vessel (inner diameter – 14 mm, length – 320 mm) between two layers of cotton wool in both ends to avoid particle clogging in the system. The temperature of the extraction vessel was controlled by the surrounding heating jacket. The extracts were collected in glass bottles. The volume of CO_2 consumed was measured by a ball float rotameter and a digital mass flow meter in standard liters per minute (SL/min) at standard state (P_{CO2} = 100 kPa, T_{CO2} = 20 °C, ρ_{CO2} = 0.0018 g/mL). The flow rate of CO_2 in the system was controlled manually by the micro-metering valve (back-pressure regulator) and kept constant during all experiments at 2–3 SL/min. The conditions for extraction were set following the procedure of Fernández et al. (2008): extraction temperature 40 °C, pressure 35 MPa, time 70 min (including 10 min of a static extraction time). The amount of extract was determined gravimetrically (±0.001 g). The solid residue after the SCE-CO_2 was collected and kept in a dry, well-ventilated and dark place prior to the analysis. All the experiments were performed in duplicates.

2.4. In vitro antioxidant activity assessment

The preparation of BSG and malt samples for the antioxidant activity assessment (Fig. 1) employing the QUENCHER approach was carried out as described by Serpen et al. (2007), with some modifications. Separate stock mixtures were prepared in microcrystalline cellulose at a concentration of 500 μg/mg. Prior to the analysis, a series of "solid dilutions" of stock mixture with microcrystalline cellulose were performed to a final concentration of 62.5 μg/mg for the DPPH$^{\cdot}$, H-ORAC and Folin–Ciocalteu's assays, and 15.625 μg/mg for the ABTS$^{\cdot+}$ and FRAP assays. Additionally for the QUENCHER procedure, 20 mg of BSG or malt SCE-CO_2 extracts were re-dissolved in 20 mL of hexane, well-mixed with 180 mg of microcrystalline cellulose at a concentration of 100 μg of extract/mg, followed by the solvent evaporation in a rotary evaporator and the removal of solvent residue under the gentle nitrogen-flow. Absorbances were measured with Spectronic Genesys 8 spectrophotometer (Thermo Spectronic, Rochester, NY). All the experiments were performed in triplicates. Antioxidant capacity was expressed as Trolox equivalent antioxidant capacity (TEAC, mg TE/g DW of malt or BSG and mg TE/g SCE-CO_2 extract), unless indicated differently.