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Chemical composition of lipids in brewer's spent grain: A promising source of valuable phytochemicals

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ABSTRACT

Brewer's spent grain (BSG) is an important by-product from the brewing process produced in high amounts worldwide. BSG is rich in carbohydrates, lignin, proteins and lipids. In this work, the chemical composition of the lipids in BSG was studied in detail by gas chromatography and mass spectrometry. The predominant lipids were triglycerides (67% of total extract), followed by a series of free fatty acids (18%). Lower amounts of monoglycerides (1.6%) and diglycerides (7.7%) were also identified among the lipids in BSG, together with minor amounts of other aliphatic series such as *n*-alkanes and alkylresorcinols. Steroid compounds (steroid hydrocarbons, steroid ketones, free sterols, sterol esters and sterol glycosides) were also found in important amounts in BSG (ca. 5%), with free and conjugated sterols being the most abundant steroids. BSG can thus be regarded as a valuable source of phytochemicals of interest for the pharmaceutical, cosmetic, food or other industries.

1. Introduction

Brewer's spent grain (BSG) is the solid residue obtained from barley (*Hordeum vulgare* L.) after mashing and filtration from the brewing process. BSG basically consists of the husk–pericarp–seed coat layers that covered the original barley grain (Mussatto et al., 2006). BSG represents up to 30% (w/w) of the starting malted grain, which makes this a readily available, high volume and low cost by-product within the brewing industry, and a potentially valuable resource for industrial exploitation. BSG is produced in high amounts from brewing companies worldwide, with an annual production of 30 million tons, among which about 3.4 million tons are produced in Europe each year (Niemi et al., 2012; Stojceska et al., 2008).

BSG is a lignocellulosic material containing cellulose (17–25%), non-cellulosic carbohydrates (25–35%), protein (15–24%) and lignin (8–28%), with lower amounts of lipids (10%) and ash (5%) (Mussatto et al., 2006; Robertson et al., 2010; Santos et al., 2003). The main application of BSG has been basically limited to animal feeding due to its high content of protein and fiber or simply as a landfill. For this reason, the development of new techniques for a more appropriate use of this agro-industrial by-product is of great interest since BSG is produced in large quantities throughout the year. One of the main areas of exploitation of this type of residue would be the recovery of valuable constituents. BSG contains several potentially valuable components suitable for utilization as raw materials for production of added-value products. Lipids in particular, which are a major part of BSG composition, are of considerable interest since they have a wide range of industrial applications in pharmaceutical, food, cosmetics, personal care products, polishes and coatings as well as in other industrial sectors, including the production of liquid biofuels. Lipids were once the primary sources of aliphatic compounds used by the industry, but with the arrival of petroleum, their consumption declined in most industrial applications. Today, market forces, regulations, and concerns about declining of energy resources and the need to mitigate green-house gas emissions and decrease our dependency on fossil fuel reserves bring lipid materials once again to the front, as an alternative to petroleum-derived chemicals and fuels (Octave and Thomas, 2009; Singh Nigam and Singh, 2011). Greater utilization of natural, renewable resources of lipids is vital for an economically viable and environmentally sound society. Therefore, new and alternative sources of biobased lipids need to be investigated. The high amounts of lipids in BSG make this material an interesting feedstock for the production of high value-added lipids in the context of the so-called lignocellulose biorefinery.

For an appropriate evaluation of BSG as a source for added-value products, the complete characterization of the different components present is of high interest. Previous studies have mostly dealt with the composition of carbohydrates, proteins and *p*-hydroxycinnamic acids (Faulds et al., 2002; Mussatto et al., 2007; Robertson et al., 2010). In comparison, studies concerning the composition of lipids in BSG have been relatively scarce and limited

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(Niemi et al., 2012). According to a previous work, BSG contained 11% lipids, which consisted mostly of triglycerides, with important amounts of free fatty acids. In that work, a gross lipid class profiling was made by TLC, while a more detailed lipid analysis was performed by pyrolysis-gas chromatography-mass spectrometry (Py-GC/MS) in the presence of tetramethylammonium hydroxide (TMAH) as a methylating agent, and indicated the predominance of palmitic, oleic and linoleic acids (Niemi et al., 2012). However, this technique is not adequate for detailed lipid analysis since it produces transesterification (and subsequent methylation of the carboxyl and hydroxyl groups) of both free and bound fatty acids in the material, including non-solvent extractable lipids that may be bound in the matrix to structures such as cutin (del Río et al., 1996; del Río and Hatcher, 1998), which makes it impossible to distinguish the origin of the released fatty acids. In addition, this analytical technique prevented the analysis of intact high molecular weight lipids such as sterol esters, sterol glycosides or triglycerides that cannot be discerned and characterized.

In the present work, we have performed a detailed and comprehensive characterization of the lipids present in BSG. The lipid composition was carried out by gas chromatography (GC) and gas chromatography–mass spectrometry (GC–MS) using short- and medium-length high temperature capillary columns, respectively, with thin films, which enables the elution and analysis of a wide range of compounds from fatty acids to intact high molecular weight lipids such as sterol esters, sterol glycosides or triglycerides (Gutiérrez et al., 1998). The knowledge of the precise composition of the lipids in BSG will help to maximize the exploitation of this important agro-industrial lignocellulosic waste product.

2. Materials and methods

2.1. Samples

Brewers' spent grain was obtained from Adnams brewery (Southwold, UK) and was kindly provided by Prof. Craig B. Faulds (INRA, Marseille). The sample was the residue resulting from wort prepared from malted barley for ale production. Additional information regarding the bulk composition of this sample can be found in Faulds et al. (2008). BSG was lyophilized and milled using a knife mill (Janke and Kunkel, Analysenmühle). Around 500 mg of BSG were subsequently extracted with acetone in a Soxhlet apparatus for 8 h. The acetone extracts were evaporated to dryness, and resuspended in chloroform for chromatographic analysis of the lipids. The acetone-extracted sample was then extracted with hot water (100 mL, 3 h at 100 °C) to determine the water soluble material. Klason lignin content was estimated as the residue after sulfuric acid hydrolysis of the pre-extracted material, corrected for ash and protein content, according to the TAPPI method T222 om-88 (Tappi, 2004). The acid-soluble lignin was determined, after the insoluble lignin was filtered off (Duran filter crucible 4; nominal pore size max. 10–16 μm), by UV-spectroscopic determination at 205 nm wavelength using 110 L cm^{-1} g^{-1} as the extinction coefficient. Holocellulose was isolated from the pre-extracted fibers by delignification for 4 h using the acid chlorite method (Browning, 1967). The α-cellulose content was determined by removing the hemicelluloses from the holocellulose by alkali extraction (Browning, 1967). Ash content was estimated as the residue after 6 h of heating at 575 °C according to the TAPPI method T211 om-02 (Tappi, 2004). Three replicates were used for each sample.

2.2. GC and GC–MS analyses

An HP 5890 gas chromatograph (Hewlett Packard, Hoofddorp, Netherlands) equipped with a split–splitless injector and a flame ionization detector (FID) was used for GC analyses. The injector and the detector temperatures were set at 300 °C and 350 °C respectively. Samples were injected in the splitless mode. Helium was used as the carrier gas. The capillary column used was a high temperature, polyimide coated fused silica tubing DB5-HT (5 m × 0.25 mm I.D., 0.1 μm film thickness; J&W Scientific). The oven was temperature-programmed from 100 °C (1 min) to 350 °C (3 min) at 15 °C min^{-1}. Peaks were quantified by area, and a mixture of standards (octadecane, palmitic acid, sitosterol, cholesteryl oleate, sitosteryl 3β-D-glucopyranoside, and triheptadecanoin) with a concentration range between 0.1 and 1 mg/mL, was used to prepare calibration curves. The correlation coefficient was higher than 0.99 in all the cases. The data from the three replicates were averaged. In all cases, the standard deviations from replicates were below 10% of the mean values. The total amounts of the different lipid classes were determined by adding up the amounts of their constituent compounds.

The GC–MS analysis was performed on a Varian Star 3400 gas chromatograph (Varian, Walnut Creek, CA) coupled with an Ion-trap detector (Varian Saturn 4000; Electron Impact at 70 eV) equipped with a high-temperature capillary column (DB-5HT, 15 m × 0.25 mm i.d., 0.1 μm film thickness; J&W Scientific). The MS was run in scan mode (*m/z* 50–1000) with the Ion-trap temperature set at 200 °C. Helium was used as carrier gas at a rate of 2 mL min^{-1}. The oven was heated from 120 °C (1 min) to 380 °C (5 min) at 10 °C min^{-1}. The temperature of the injector during the injection was 120 °C, and 0.1 min after injection was programmed to 380 °C at a heating rate of 200 °C min^{-1} and held for 10 min. The temperature of the transfer line was set at 300 °C. Bis(-trimethylsilyl)trifluoroacetamide (BSTFA) silylation was used to prepare the trimethylsilyl ether derivatives before the analysis. Compounds were identified by comparing their retention times and mass spectra with authentic standards, except for alkylresorcinols, which were only tentatively identified by comparing their mass spectra with those reported in the literature.

3. Results and discussion

3.1. Chemical composition of lipids in BSG

The abundance of the main constituents of BSG (water-soluble material, lipids, Klason lignin, acid soluble lignin, polysaccharides, proteins, and ash) is shown in Table 1. The data from previous papers regarding this sample are shown for comparison (Faulds et al., 2008; Robertson et al., 2010). The lipids of BSG accounted for 9.2% of dry material, a value higher than that reported in Faulds et al. (2008) for the same sample but similar to the value previously reported in other papers (Mussatto et al., 2006; Niemi et al., 2012).

Table 1
Abundance of the Main Constituents (% Dry-Weight) of the Brewers' Spent Grain Sample Selected for this Study. Data from Other Papers (Faulds et al., 2008; Robertson et al., 2010) Regarding the Same Sample Are Shown for Comparison.

Constituent	Content[a]	Robertson et al., 2010	Faulds et al., 2008
Water-soluble material	8.3 ± 1.0	n.d.	n.d.
Lipids	9.2 ± 0.2	n.d.	5.4
Klason lignin	8.8 ± 0.9[b]	16.0	20.1
Acid-soluble lignin	4.9 ± 0.3	n.d.	n.d.
Polysaccharides	49.4 ± 2.0	43.3	51.0
Proteins	14.5 ± 1.0[c]	18.8	17.6
Ash	4.9 ± 0.1	n.d.	n.d.

[a] Average of three replicates.
[b] Corrected for proteins and ash.
[c] Determined indirectly by subtracting the other components to 100%.