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Characterization of Lipids and Lignans in Brewer's Spent Grain and Its Enzymatically Extracted Fraction

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ABSTRACT: Brewer's spent grain (BSG), the major side stream of brewing, consists of the husks and the residual parts of malts after the mashing process. BSG was enzymatically fractionated by a two-step treatment with carbohydrate- and protein-degrading enzymes, which solubilized 66% of BSG. BSG contained 11% lipids, which were mostly triglycerides, but also a notable amount of free fatty acids was present. Lipids were mostly solubilized due to the alkaline pH applied in the protease treatment. The main fatty acids were linoleic, palmitic, and oleic acids. Several lignans were identified in BSG, syringaresinol and secoisolariciresinol being the most abundant, many associated with the cell wall matrix and released by the alkaline-protease treatment.

KEYWORDS: *brewer's spent grain, enzymatic solubilization, lipid, lignan*

INTRODUCTION

Brewer's spent grain (BSG) is the most abundant side stream from brewing with an annual production of 30 million tons worldwide. Traditionally, this material rich in carbohydrates, protein, and lignin goes mainly to cattle feed, with little or no profit to the breweries. BSG contains several potentially valuable components suitable for utilization as food ingredients or raw materials for microbial or chemical conversions. Thus, advanced technologies for deconstruction and fractionation of BSG in the development of new value chains for breweries are still required. Both chemical and enzymatic fractionations of BSG have been previously studied in order to separate the carbohydrate, protein, and lignin moieties for further uses.[1−3] For instance, the carbohydrates have been studied as fermentable sugars,[4] arabinoxylan for functional oligosaccharides,[5] and xylitol,[6] and the solubilized peptides from BSG could have potential as foaming agents and emulsifiers.[7] Nevertheless, large-scale studies, not to mention practical applications, have been rarely reported.

In mashing, almost all of the starch and β-glucan and some proteins within the malts are enzymatically solubilized. BSG thus contains the husk and the outermost layers of the barley kernel and is a heterogeneous material rich in arabinoxylan (22−28%), cellulose (17−25%), lignin (12−28%), and protein (15−24%).[8] As BSG originates from the brewing process, it is a food-grade material and could be an interesting ingredient in food production, provided that cost-efficient processing methods to separate the different components can be developed.

In food processing, enzymatic treatments are preferable compared to chemical treatments, and enzymatic fractionation methods for protein and carbohydrates from BSG have been especially studied over the last 10 years.[1,2,7,9,10] However, BSG contains bacterial and fungal spores that are not destroyed by the high temperature in mashing;[11] to avoid their extensive growth and hence spoilage of the material, the hydrolysis time has to be limited. Incubations of 4−5 h have been often reported,[1,10] but with current enzymatic methods, this time period can only release 50% of the total carbohydrates in a solubilized form.[12] However, the proteins are more easily removed and can be almost completely released by protease activity during the short time under optimal conditions.[10] Due to the different pH and temperature optima of carbohydrate- and protein-degrading enzymes, the hydrolysis treatments are more efficient when carried out in a sequential manner rather than combined in one step. A sequential carbohydrase−protease treatment can solubilize up to 40−60% of the total material, depending on the enzymes and conditions applied.[10,13,14]

In the previous studies, the focus has been mostly on the carbohydrates and proteins, whereas the lipids and phenolic compounds other than hydroxycinnamic acids have not been well characterized. If BSG is aimed to be utilized as a food component, the compositions of these aforementioned compounds are also of interest. Lignans are low molecular weight, polyphenolic compounds composed of similar structural units as lignin. They are known to have several health-promoting effects, such as antioxidant and anticarcinogenic activity,[15−19] but their content in BSG has not been previously determined. Furthermore, lipids are also an important constituent in BSG, but they have not been previously characterized. The aim of this study therefore was to characterize the lipids and lignans in BSG and their extractability in a hydrolysis process.

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MATERIALS AND METHODS

Materials. BSG was obtained from Sinebrychoff brewery (Kerava, Finland) and stored as is at −20 °C. The composition of BSG is shown in Table 1. The main components were arabinoxylan, glucan (mainly cellulose), protein, lignin, lipids, and ash. Small amounts of starch (1.3%) and mixed link β-glucan (0.36%) were also present. The amounts of ferulic and *p*-coumaric acids, which are mostly associated with lignin, were 3.0 and 1.1 mg/g, respectively.

Table 1. Composition of BSG and the Residual Fractions Obtained after the Hydrolysis Steps[a]

		hydrolysis residue			
component	initial BSG	1st	2nd	P-AEF	extr P-AEF
carbohydrate	42.2	22.2	49.4	4.0	6.7
arabinoxylan	22.2	15.9	31.2	2.3	2.7
glucan	17.1	4.5	15.9	0.7	1.1
lignin	19.3	29.1	33.5	24.4	40.7
protein	22.8	28.6	4.8	27.2	45.2
lipid	11.0	15.5	4.1	39.9	–
ash	4.7	4.6	8.1	4.5	7.4

[a]The values are given in mass %. Extr. P-AEF: P-AEF from which lipids were removed with heptane extraction. The amount of lignin is determined by subtracting other components from 100%.

BSG Composition Analysis. Total lipids were analyzed by extracting a dry sample with heptane for 5 h in a Soxhlet apparatus. After extraction, the solvent was evaporated at room temperature, and the lipids were quantitated gravimetrically. The Klason lignin content was determined gravimetrically after removing extractives by heptane extraction and carbohydrates by acidic hydrolysis.[20] The acid-soluble lignin was measured from the hydrolysate by UV absorbance.[21] The carbohydrate composition was determined by analyzing the monosaccharides from the hydrolysate with HPAEC-PAD using a Dionex CarboPac PA-1 column (guard column 4 × 50 mm^2 and analytical column 4 × 250 mm^2) in a Dionex ICS-3000 system (Dionex Corp., Sunnyvale, CA) with electrochemical detection. The analysis was performed as described previously[22] with minor modifications, that is, equilibration with 15 mM NaOH and isocratic elution with water. The starch and β-glucan contents were determined with Megazyme kits total starch (amyloglucosidase/α-amylase method) and mixed-linked β-glucan, respectively, according to the manufacturer's (Megazyme, Bray, Ireland) instructions. The nitrogen content was analyzed by total nitrogen analysis at Analytische Laboratorien Prof. Dr. H. Malissa and G. Reuter GmbH (Lindlar, Germany) with a standard method ASTM D-5291. The nitrogen content was converted to protein content by multiplying by a factor of 6.25. The ash content was determined gravimetrically after burning the samples in a muffle furnace at 550 °C overnight. Phenolic acids were extracted from BSG by adding 2 M NaOH to the dry sample and incubating for 16 h at room temperature. The samples were then acidified with HCl and extracted with ethyl acetate three times. The organic phase was collected, and the solvent was evaporated. The dried residue was dissolved in a 50:50 mixture of methanol and water. High-performance liquid chromatography (HPLC) analysis of the phenolic acids was performed with a Waters 600S system controller equipped with a Waters 616 pump (Waters, Milford, MA) using a 4.6 × 150 mm^2, 5 μm, Hypersil BDS-C18 column (Agilent) with UV detection at 324 nm. A gradient solvent system using 5% formic acid in water (solvent A) and 100% acetonitrile (solvent B) was used. The volumetric proportions of solvents were 95%/5% A/B (5 min), 90%/10% A/B (10 min), 60%/40% A/B (5 min), and 10%/90% A/B (5 min). The flow rate was 0.7 mL/min. All analyses were carried out in duplicate.

Enzymatic Hydrolyses. BSG was defrosted at 4 °C overnight and suspended in tap water; a 6% BSG suspension was milled with a Masuko supermasscolloider MKZA10-15J, (Masuko Sangyo Co. Ltd., Kawaguchi-city, Japan) with a 7 times passing through MKGA10−80 grinding stones with a grinding speed of 1500 rpm. After grinding, a portion of the water was removed by centrifugation, and the dewatered suspension was stored overnight at 4 °C. Enzymatic hydrolysis consisted of two steps (Figure 1) and was carried out in a 10 L reactor using a 10% (w/w) solids content. The quantity of BSG in the first hydrolysis step was 1000 g, and the enzymes used were Depol740L (Biocatalysts Ltd., Cefn Coed, Wales, U.K.) (5000 nkat of xylanase activity per g of BSG) and Celluclast 1.5 L (Novozymes, Bagsvaerd, Denmark) (50 FPU per g of BSG). The BSG suspension was preheated to 50 °C before adding the enzymes. The pH of the reaction mixture after adding the enzymes was 5.4 and was not further adjusted. The mixture was continuously stirred at 48 rpm. After 5 h, the suspension was cooled down by centrifuging in a precooled SorvallRC12BP centrifuge with a H12000 rotor (Sorvall Products L.P., Newton, CT, USA) at 4 °C and 4000 rpm for 30 min. The supernatant was collected, and the solid residue was washed twice with tap water to remove solubilized compounds and enzymes. The residue was weighed, and small aliquots were withdrawn to determine the dry matter content of the centrifuged material and for composition analysis of the insoluble material. The rest of the insoluble material was used in the second step of the hydrolysis. The second proteolytic step was carried out with Alcalase 2.4 L (Novozymes, Bagsvaerd, Denmark) (20 μL of enzyme preparation per g of BSG). The hydrolysis was carried out as before but this time at 60 °C for 4 h and in 100 mM, pH 10 sodium carbonate buffer. The decrease in pH during the hydrolysis was adjusted back to pH 10 with 10 M NaOH. The hydrolysis was terminated as done previously by cooling the suspension while separating the solids from liquids and by washing the solids.

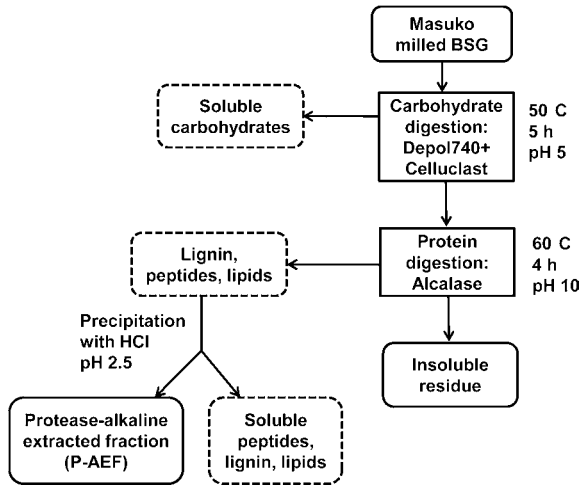


Figure 1. Scheme of BSG hydrolysis steps.

The soluble fraction of the second hydrolytic step was precipitated by lowering the pH to 2.5 with 5 M hydrochloric acid. The solids were separated by centrifugation and washed twice with acidic water (pH 2.5). The precipitate is referred as the protease-alkaline extracted fraction (P-AEF). For lignan analysis, P-AEF was extracted with heptane as described above. All the insoluble fractions and samples taken between different hydrolytic steps were freeze-dried for further analyses.

Microscopy Analyses. Light microscopy was carried out as described previously.[23] In brief, samples were embedded in hydroxyethyl methylacrylate matrix from which 2 μm thick sections were cut. Prior to imaging with the microscope, the sections were treated with chemical dyes Calcofluor and Acid Fuchsin to enable visualization of different components of BSG. Acid Fuchsin stains protein red, and Calcofluor stains β-glucan bright blue. The autofluorescence of lignin and other phenolics are seen as yellow and green. Cutin appears as orange due to its autofluorescence. For the autofluorescence images, the samples were not stained, and the

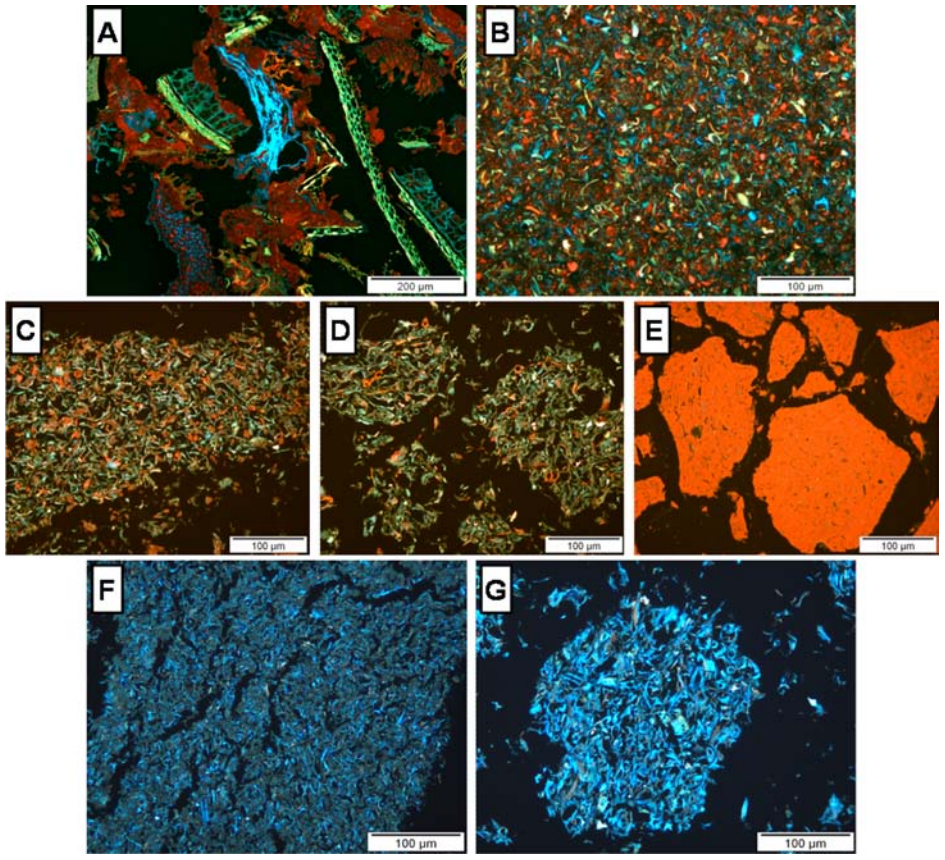


Figure 2. Microscopy images of BSG and the enzyme-treated fractions: light microscopy images of (A) unmilled BSG, (B) Masuko-milled BSG, (C) residue after the first carbohydrase treatment, (D) residue after the protease treatment, and (E) protease-alkaline extracted fraction; autofluorescence images of (F) the residue after the first carbohydrase treatment and (G) the residue after the protease treatment.

excitation and emission wavelengths used were 330−385 nm and >420 nm, respectively.

Lipid Analysis with Pyrolysis−GC/MS. Methylation pyrolysis in the presence of tetramethylammonium hydroxide (TMAH) was performed using a platinum foil pulse pyrolyzer Pyrolab2000 (Pyrolab, Lund, Sweden) connected to a gas chromatograph−mass spectrometry (GC/MS) Varian 3800 GC-Varian 2000 MS. The column used was a 30 m × 0.25 mm, i.d., 1 μm, fused silica capillary column DB-1701 (Agilent). About 40 μg of the sample was weighed accurately and transferred to the foil. A 10% TMAH aqueous solution (4 μL) was added and mixed with the sample. The sample mixture was inserted in the pyrolyzer maintained at 175 °C and heated at 400 °C for 4 s. The degradation products were led into the capillary column for separation using helium as the carrier gas, with a flow rate of 1.0 mL/min. The column temperature was programmed from 80 °C (2 min) to 160 °C at 8 °C/min and extended from 160 to 280 °C at 5 °C/min. The final temperature was held for 15 min. The mass spectrometer was operated in EI mode (70 eV). Fatty acid composition was calculated after normalization of peak areas to 100%.

Lipid Classification with TLC−GC. The separation of major lipid classes (phospolipids, free fatty acids, di- and triglycerides) was carried out with thin layer chromatography (TLC).[24] The heptane-extracted lipids were redissolved in a small amount of chloroform/methanol (100:1) and supplemented with the internal standards. The mixture was applied on silica plates, and the plates were developed with petroleum ether/diethyl ether/acetic acid (80:30:1). Lipid classes were visualized under UV light after spraying 0.01% Rhodamine 6B GO, scraped off, and used for quantitation.

Fatty acids were quantitated as their methyl esters.[25] The samples were first suspended in an excess (1 mL) of saponification reagent (3.7 M NaOH in 49% methanol) at 100 °C for 30 min. Samples were cooled down to room temperature, and 4 mL of methylation reagent (3.3 M HCl in 48% methanol) was added. After mixing, samples were held at 80 °C for 10 min. Fatty acid methyl esters were extracted in 1.5 mL hexane/methyl-*tert*-butyl ether solution (1:1). The methyl esters were analyzed by GC with a flame ionization detector (GC-FID) with an HP-88 column (30 m × 0.25 mm, phase 0.20 μm; Agilent). The amount of fatty acid methyl esters were calculated with corresponding standards. Three replicate samples were analyzed.

Analysis of Lignan Contents. BSG and the heptane-extracted P-AEF sample were extracted using accelerated solvent extraction (ASE). Approximately 0.5−0.7 g of the samples was ASE extracted as described by Smeds et al.[26] with some modifications, that is, 3 × 5 min static cycles were applied, and the heptane-extracted P-AEF was extracted with acetone and acetone/water (70:30, v/v), only. The acetone and acetone/water fractions were combined and diluted to 100 mL with acetone. A portion (10 mL) of this extract was withdrawn, and the solvent was evaporated to dryness in a water bath at 40 °C using a stream of nitrogen gas. The extracts were then enzymatically hydrolyzed by adding 490 units of purified β-glucuronidase (from *Helix pomatia*) (Sigma−Aldrich) dissolved in 1 mL of 10 mM sodium acetate buffer (pH 5.0) and kept at 37 °C for 19 h. The internal standards matairesinol-d_6, enterolactone-d_6, and dimethylated pinoresinol-d_6 (dissolved in methanol) were added in amounts of 484, 285, and 570 ng, respectively. The solutions were liquid−liquid extracted with 2 × 0.75 mL of ethyl acetate, and the ethyl acetate phase was evaporated to dryness using a stream of nitrogen gas. Methanol/0.1% acetic acid (500 μL, 20:80, v/v) was added, the samples were sonicated for approximately 30 s, and the solutions were filtered using PTFE 0.45 μm syringe filters. A portion (10 μL) of the solutions were injected into a HPLC-MS/MS system, and the lignans were determined as described previously.[26,27]

RESULTS AND DISCUSSION

Enzyme-Aided Solubilization of BSG. A two-step hydrolysis of BSG was carried out with carbohydrate- and protein-degrading enzymes. Prior to the hydrolyses, BSG was pretreated with a Masuko grinder. Reducing the particle size of BSG by grinding has been shown to improve the efficiency of enzymatic hydrolysis of BSG carbohydrates releasing some soluble material by the mechanical impact alone.[12] The changes in the microstructure of BSG were followed with light microscopy starting from unmilled BSG to Masuko-ground BSG and through the enzymatic treatments (Figure 2). In Figure 2A–E, the bright blue stain represents cell walls of residual endosperm cells, the red stain indicates protein, and lignin or phenolic acids contained in the aleurone, pericarp, and husk cells are seen because of their autofluorescence (green and yellowish colors).

The initial BSG contained several millimeter long particles with intact cellular structures (Figure 2A). Due to grinding of BSG with the Masuko mill, the particle size was significantly decreased, and the cellular structures were disrupted (Figure 2B). The hydrolysis was initiated with a mixture of two carbohydrase preparations, Depol740 and Celluclast 1.5, previously found to be effective in the solubilization of BSG carbohydrates.[12] Celluclast is high in cellulolytic and xylanolytic activities whereas Depol740 contains feruloyl esterase activity in addition to several hemicellulolytic and cellulolytic activities.[12] In the first hydrolysis step, the enzymes solubilized 26% of the milled BSG. After the first treatment, the carbohydrate content had decreased from 42% to 22%, whereas the contents of protein, lignin, and lipids had increased accordingly (Table 1). It should be noted that the analysis of lignin content with nonwood materials is challenging and due to several factors the Klason lignin method often exaggerates the results.[28] The same phenomenon was observed also with the BSG fractions, and therefore, the lignin contents (Table 1) were calculated by subtracting the amounts of other components from 100%.

When observed by microscopy, the blue color had disappeared almost completely after the carbohydrase treatment (Figure 2C). Presumably, the nonlignified endosperm and aleurone cell walls were more accessible for enzymes than the more resistant, protective outer layers. It has been shown with an endoxylanase on wheat bran[29] that the enzyme preferentially acted on the aleurone cells and progressed into the bran only after degradation of the aleurone layer. This was due to lower arabinose substitution of the aleurone layer and because the xylanase was too large to diffuse into the cells without further arabinoxylan degradation. A small amount of protein was also solubilized in the first hydrolysis as Depol740 contains some protease activity.[2] However, protein content of the residue increased due to the carbohydrate removal, which was confirmed by an increase in red-colored components (Figure 2C).

The aim of the second hydrolysis treatment was to digest and hence release the proteins from the residual matrix. Alcalase has previously been shown to work effectively on BSG proteins, especially at alkaline pH (9.5–10).[1,30] While the first hydrolysis step with Depol740/Celluclast removed 6% of the protein, the Alcalase treatment resulted in 93% of all BSG protein being solubilized by the two steps. The removal of protein was confirmed by microscopy as the amount of red stain decreased significantly (Figure 2D). Most of the water-soluble barley protein is removed already in mashing, and the proteins left in BSG are mainly the water-insoluble storage proteins, hordeins, and structural proteins, glutelins.[31] In addition, BSG often contains some precipitated protein from wort boiling. The insoluble residue after the protease treatment contained thin and long orange particles, which should not be confused with protein (Figure 2D). Most likely, they were parts of the cutin, which is a polyester in the hydrophobic cuticle layer covering the outer surface of the husk. Cutin has also aromatic components and can therefore be seen due to its autofluorescence. In samples rich in protein, the red color was so dominant that it was difficult to distinguish the cutin, but as the protein was removed, the cutin fragments became more visible.

The Alcalase treatment solubilized 40% of BSG, and therefore, after the two-step treatment, a total of 66% of BSG had been solubilized. A portion (58%) of the calculated BSG lignin was present in the insoluble residue after the protease treatment, whereas practically no solubilization of lignin occurred during the carbohydrate digestion. Previously, it has been speculated that, when BSG is treated with carbohydrate- and protein-degrading enzymes, lignin is partly cosolubilized during an alkaline protease treatment[13] due to its linkages to other components or because of the high pH in the proteolytic treatment. Therefore, in the current work, the supernatant collected after the Alcalase treatment was acidified to precipitate the solubilized lignin (protease-alkaline extracted fraction, P-AEF). The amount of the obtained precipitate was 113 g (after removal of lipids 68 g). Nearly half of the lignin was released by the action of Alcalase (Table 1), but only a small part of it, approximately a third, precipitated when the pH was lowered to 2.5. It is known that lignin is covalently linked to carbohydrates,[32] and possibly, some carbohydrates were associated with the lignin present in the soluble phase making it more soluble in water. The protease treatment also released 87% of the lipids present in BSG (Table 1), with almost half of them associated with the precipitate after the subsequent acidification. The main components of P-AEF were lipids, protein (or peptides), and lignin (Table 1). In Figure 2E, precipitated proteinaceous material presumably covered lignin and thus hid some of the autofluorescence as well. The degree of solubilization in the second hydrolytic step was 40% of the initial BSG, and as the amount of the subsequent precipitate was 11%, it demonstrated that most of the material solubilized in the proteolytic treatment remained in the soluble form despite the lowering of the pH.

Figure 2F, G represents the autofluorescence of the hydrolysis residues when excited with UV light. The major autofluorescent component in BSG is lignin, but several other aromatic compounds such as phenolic acids and lignans contribute as well.[33] Although some of the aromatic compounds are released by the enzymatic treatments, the autofluorescence of the residues increased in the processing. This is consistent with the composition data (Table 1) showing also that the lignin content of the solid residue was enriched by the hydrolytic treatments. In addition to the enrichment of lignin, its autofluorescence may become more visible as the protein and carbohydrates covering it are removed.

Lignin is one of the major constituents of BSG, but so far, nothing is really known about the characteristics of BSG lignin. Recently, there have been studies on the delignification of BSG lignin[34] and the further use of the obtained lignin fraction,[35] but in these studies, no characteristics of the processed lignin were determined. In the literature, the lignin content of BSG is generally expressed simply as "Klason lignin", without further

considerations of what this acid-insoluble residue actually consists.[28] The autofluorescence of the insoluble residue combined with the lignin content data strongly supports the assumption that this material truly is lignin for the most part.

BSG Lipids. Figure 3A presents the results of fatty acid analyses with pyrolysis−GC/MS. The fatty acid content of BSG

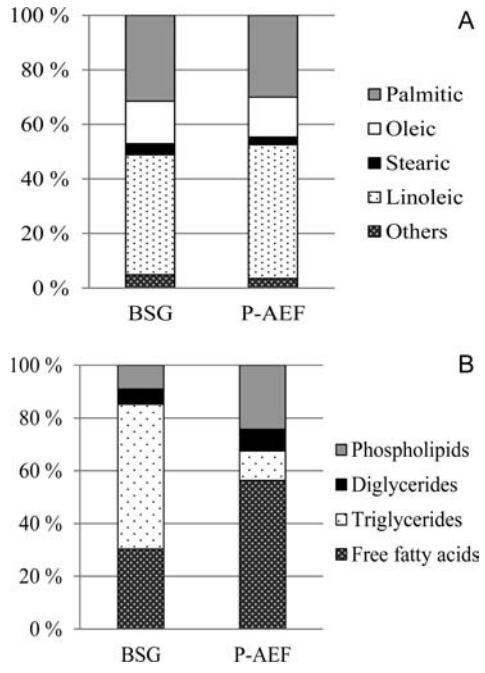


Figure 3. (A) Proportions of different fatty acids in BSG and protease-alkaline extracted fraction (P-AEF). (B) Proportions of different lipid classes in BSG and P-AEF.

was 11%, and the most abundant lipids were linoleic (18:2), palmitic (16:0), and oleic acids (18:1). Small amounts of other fatty acids, such as stearic (18:0) and linolenic (18:3) acids, were also present. The benefit of pyrolysis−GC/MS is that it is able to detect all fatty acids in the material. More often solvent-extractable lipids are analyzed, but all lipids are not necessarily extractable as they may be bound in the matrix to structures such as cutin. The lipid content of unmalted barley varies between 1.0−2.6%,[36,37] but in mashing, the lipids are enriched, and their concentration in BSG is from 5.8% up to 11%.[6,8,12] Only minor changes in fatty acid composition occur during malting and mashing,[38] and therefore, the fatty acid composition of BSG is similar to that of barley, although in barley, the linoleic acid content has been reported to be slightly higher and palmitic acid content consequently somewhat lower than in BSG. However, this is most likely due to differences in environmental conditions in which the barley plants were cultivated.[39]

Lipid classification (Figure 3B) showed that most lipids in BSG are triglycerides (55%), although the amount of free fatty acids was also notable (30%). Phospholipids and diglycerides accounted for 9.1% and 5.7%, respectively. Compared to unmalted barley, BSG has less triglycerides and phospholipids and more free fatty acids, whose proportions in barley are 69%, 27%, and 2%, respectively.[38] The increased amount of free fatty acids in BSG is probably a result of endogenous lipase activity that released free fatty acids from triglycerides and phospholipids during malting and mashing. Triglycerides are the main form of storing lipids for energy in barley, whereas phospholipids constitute biological membranes. The lipids in barley are located in the endosperm and embryo, as their role is to provide nutrients and energy for the new, germinating barley plant. Although the endosperm is almost completely solubilized in mashing, most of the lipids remain with spent grains and are not transferred to wort.[38,40] During the protease treatment, the high pH caused hydrolysis of alkali-labile ester bonds of the di- and triglycerides and saponifying of the fatty acids, as in P-AEF, the proportion of triglycerides was only 11%.

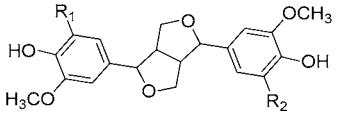
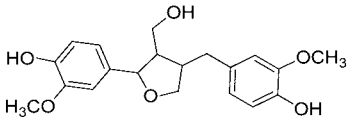
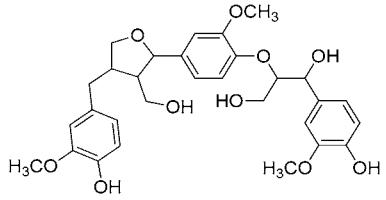
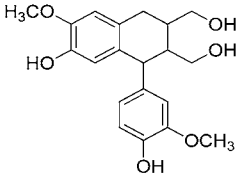
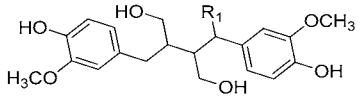
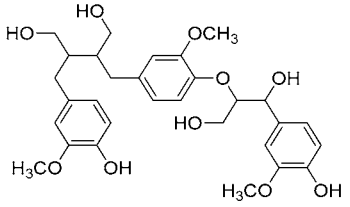
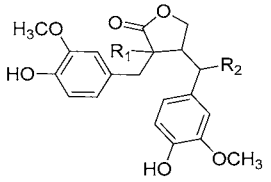
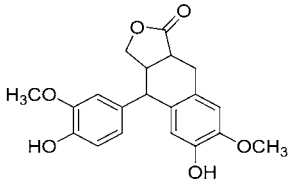
Lignans in BSG and the Protease-Alkaline Extracted Fraction. Several lignans were detected in BSG (Table 2). The total content of the analyzed lignans amounted up to 1300 μg/100 g, which is similar to the lignan content of unmalted barley.[26] Compared to other cereals, such as wheat and rye, barley has much lower lignan content.[26] The most abundant lignans in BSG were syringaresinol **3** and secoisolariciresinol **7**, which had significantly higher concentrations than any other lignan. Lignan concentrations in P-AEF were notably higher than in BSG indicating that most lignans were released from the matrix in the conditions of the second hydrolysis step and were then concentrated in the precipitate. Syringaresinol **3** was the most abundant lignan in both BSG and P-AEF, but the amounts of medioresinol **2**, nortrachelogenin **13**, and α-conidendrin **14** were considerably increased as well in P-AEF. For lariciresinol **4**, nortrachelogenin **13**, α-conidendrin **14**, and secoisolariciresinol-sesquilignan **9**, the concentrations in P-AEF were higher than what seemed theoretically possible to obtain from BSG. In addition, medioresinol **2** and 7-hydroxymatairesinol **11** were not detected at all in BSG but were found in P-AEF in significant amounts.

Lignans are mostly polyphenolic compounds, excluding a few lignans that lack the phenolic hydroxyl group such as sesamin and hinokinin. Lignans are commonly found in plants, in which their function is to act as defensive substances, and they have been reported to have several disease-preventing and health-promoting properties.[15−19] The amounts of lignans in several cereal grains, including barley, have been quantified[26] and show that a number of these lignans are a part of our everyday diet. In addition, the conversion of plant lignans to the mammalian lignans enterodiol and enterolactone by gut microbiota has been demonstrated.[41,42]

When comparing barley[26] and BSG, it can be noticed that lignans behave differently in the mashing process. The concentrations of certain lignans, such as 7-hydroxymatairesinol **11** and lariciresinol **4**, which are among the most abundant lignans in barley, were much lower in BSG indicating that they are solubilized in mashing and appear in the wort. Many lignans in cereals exist as glycosides,[26] which may improve their solubility. On the other hand, some lignans, such as syringaresinol **3** and secoisolariciresinol **7**, appeared more enriched in BSG than barley indicating that they are not soluble in aqueous environment. However, as lignan concentrations vary between barley varieties and the harvesting year,[43] comparison between barley and BSG may not be completely accurate but should be considered only as indicative.

During the enzymatic processing, most lignans seemed to be solubilized by the alkaline-protease treatment and subsequently precipitated by the acidification, as the concentrations of lignans in P-AEF were systematically higher than in BSG. The increase in the concentration could be due to some lignans being bound in the cell walls with ester linkages and released by the alkaline pH effect.[26] It has been shown for certain lignans, including medioresinol **2** and lariciresinol **4**, that alkaline and

Table 2. Molecular Structures and Concentrations of Lignans in BSG and the Delipidated Protease-Alkaline Extracted Fraction (P-AEF)[a]

Molecular structure	Name	μg/100g in BSG	μg/100g in P-AEF
	R_1=R_2=H: Pinoresinol **1**	29	203
	R_1=OCH_3, R_2=H: Medioresinol **2**	nd	1249
	R_1=R_2=OCH_3: Syringaresinol **3**	982	2357
	Lariciresinol **4**	4.8	229
	Lariciresinol-sesquilignan **5**	13	82
	Cyclolariciresinol **6**	22	26
	R_1=H: Secoisolariciresinol **7**	233	595
	R_1=OH: 7-Hydroxy-secoisolariciresinol **8**	nd	nd
	Secoisolariciresinol-sesquilignan **9**	6.2	112
	R_1=R_2=H: Matairesinol **10**	18	142
	R_1=H, R_2=OH: 7-Hydroxymatairesinol **11**	nd	477
	R_1=H, R_2=O: 7-Oxomatairesinol **12**	1.0	nd
	R_1=OH, R_2=H: Nortrachelogenin **13**	24	1791
	α-Conidendrin **14**	12	1239

[a]Nd: not detected.

acidic treatments are required to achieve the best possible extraction yield from barley.[26] This could well explain why these lignans were detected in higher concentrations in P-AEF than BSG. In addition, structures of some lignans, for example, pinoresinol **1** and lariciresinol **4**, are incorporated in lignin,[44] and it has been suggested that they are released by acidic conditions.[26] Interestingly, 7-hydroxymatairesinol **11** and medioresinol **2**, which were below the detection levels in the initial BSG, were identified in P-AEF. 7-Hydroxymatairesinol **11** is the most abundant lignan in barley[26] but seems to be released in mashing, as it was not detected in BSG. 7-Hydroxymatairesinol **11** is not stable in alkaline or acidic conditions,[26] and therefore, its reappearance in P-AEF was unexpected.

The quantitation of lignans is complicated by several factors. Acidic and alkaline pHs are known to cause structural changes in several lignans and conversion of certain lignans to other lignans.[26] Furthermore, finding the optimal extraction method

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for lignans is not always straightforward and strongly depends on the properties of the starting material. For example, for barley, the ASE extraction gives the highest overall lignan yield, but there are some lignans that may not be entirely extractable with this method.[26]

A two-step enzymatic treatment solubilized 66% of BSG. In the first step, carbohydrates were partially resistant to the action of the carbohydrate-degrading enzymes, whereas the proteins and lipids were almost completely solubilized by the alkaline-proteolytic treatment. The main form of lipids in BSG was triglyceride, but a notable amount of free fatty acids were also detected. The main fatty acids were linoleic, palmitic, and oleic acids. Most of the lipids were solubilized by the proteolytic treatment, most likely due to the high pH. Several lignans were detected in BSG, and many of them were concentrated in the precipitated fraction. Lignans seemed to be partially bound in the cell wall matrix and were probably released by the effects of alkaline and acidic pH. Also, some chemical conversion may have occurred due to the changes in the pH during the enzymatic processing. The results of this study showed that, in addition to dietary fiber and protein, which have been previously characterized, BSG is rich in unsaturated fats and lignans with beneficial properties for health. Therefore, BSG or its enzymatically extracted fractions have great potential to be used in applications such as added-value food components.

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Notes

The authors declare no competing financial interest.

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