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Enzymatic and Chemical Treatment Limits on the Controlled Solubilization of Brewers' Spent Grain

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ABSTRACT: The enzymatic hydrolysis of brewers' spent grain (BSG) has been investigated through treatment with commercial carbohydrases and proteases. Resultant residues were then chemically fractionated and delignified. Enzymatic treatments released 25−30% of the BSG mass and yielded precursors suitable for subsequent conversion to potentially value-added products. Controlled chemical fractionation selectively solubilized arabinoxylan but with no differences apparent due to prior enzyme treatment. The loss of non-polysaccharide components during alkali treatment suggests the presence of a high proportion of alkali-soluble lignin. Further delignification of the alkali-insoluble residues and further chemical fractionation released the remaining hemicellulose, to yield a residue which was >90% cellulose. Further knowledge of the properties and interaction between BSG polymers will facilitate an improved enzyme-assisted total deconstruction of BSG and hence the exploitation of its biomass.

KEYWORDS: brewers' spent grain, polysaccharide, lignin, phenolic acid, enzyme, digestion, fractionation

INTRODUCTION

Brewers' spent grain (BSG), the residual malted barley grains resulting from wort preparation, is a high volume byproduct arising during the brewing process. It is rich in polysaccharides, proteins and lignin, and except for differences in protein content in specific source malted grain, there is little variation in the composition of BSG between breweries.[1] This makes BSG a readily available global commodity suitable for exploitation. However, stabilization of such a high moisture commodity postproduction is required to avoid microbial spoilage.[2] Enzymatic approaches to fractionate BSG are favored as a means to recover selective high-value components, following its stabilization against microbial spoilage.[2−7]

Whereas chemical treatments, like acid hydrolysis, can release a spectrum of component monosaccharides, their severity also destroys potentially valuable components and generates environmentally harming effluents. Ideally a selective mechanical or chemical fractionation of BSG coupled with tailored enzyme treatments is required, but little knowledge is currently available about matrix interactions, especially between the guaiacyl-rich lignin, with a S/G ratio of 0.55,[8] and the hemicellulose. Commercial multienzyme cocktails have been used for polysaccharide solubilization from BSG,[6,7] and these can release up to 30% of the original carbohydrate content within 5 h and hence a microbiologically safe treatment time.[2] Enzyme activity is maintained during treatment, so the apparent limit of solubilization is due to other factors, such as steric hindrance. Traditionally, the presence of lignin is considered a barrier to enzymatic degradation,[9,10] though in cereal grains the presence of lignin and lignin-associated material can lead to an overestimation of the true lignin content.[11−13] Hence it is important to distinguish lignin from non-lignin-like material and to avoid nonspecific measures of lignin, like Klason lignin and its association with proteins, when dealing with the effects of lignin on cell wall degradability.

Protein content in BSG can be reduced by up to 77% by different commercial proteases, and possible protein encapsulation of polysaccharides is considered a minor restriction on digestibility.[14] Combinations of carbohydrase and protease treatments have also shown that, within 8 h, 40% of BSG can be digested.[5,7,14] This includes over 80% of the proteinaceous material being lost, rather than only an enhanced deconstruction of polysaccharides. Thus protein residues will persist and be a potential contributor to nonspecific measurements of lignin.

It has been proposed that a reduction in lignin concentration, hydrophobicity, and cross-linking will improve the enzymatic release and deconstruction of structural polysaccharides.[15] In general, lignification can decrease the rate and extent of hemicellulose and cellulose solubilization.[15,16] However, this does not necessarily lead to a significant increase in biomass solubilization,[7] which suggests that qualitative or structural features, rather than extent of lignification, remain important for the release of polysaccharides. Indeed, ferulate-induced cross-linking may account for nearly half of the inhibitory effects ascribed to lignin on cell wall fermentation.[17]

In the current investigation, BSG, treated to test its potential to release polysaccharide-related value-added products, has shown that a high proportion of lignin-like material may be associated with BSG. Treatments to improve release of polysaccharides have considered the effects of pre- and postdigestion with commercial enzyme cocktails on the profile and composition of polysaccharides and bound phenolics present. This has been complemented by a chemical fractionation to determine the loss of non-polysaccharide material present and through delignification treatments to estimate the relative importance of lignin in the deconstruction of BSG.

MATERIALS AND METHODS

Materials. BSG was obtained (65−70 °C) directly from the mash tun as one bulk sample from a local brewery and was divided into 1 kg

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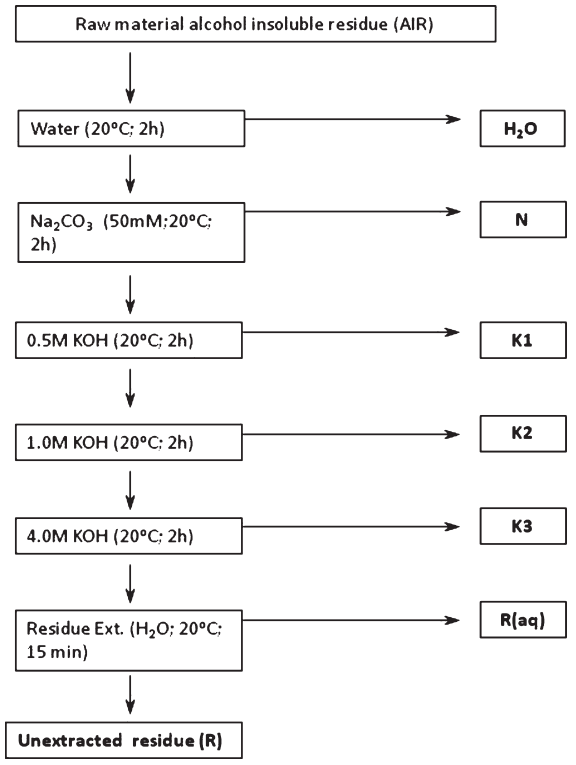

Figure 1. Scheme for the fractionation of BSG pre- and postenzyme digestion treatments.

aliquots in sealed polythene bags to minimize microbial spoilage.[1] After cooling to room temperature, samples were frozen at −20 °C. A sample of the fresh material was retained for moisture determination. Frozen samples were either lyophilized and milled prior to use or thawed and used as the fresh sample material. Food grade enzymes were obtained from commercial suppliers: Econase CE [Rohament CL] (AB Enzymes, Rajamäki, Finland), Depol 740L, Depol 686 and the feruloyl esterase TsFaeC from *Talaromyces stipitatus* (Biocatalysts Ltd., Cefn Coed, Wales, U.K.), and Alcalase 2.5 L (Novozymes, Bagsvaerd, Denmark). The recombinant feruloyl esterase AnFaeA from *A. niger* was heterologously expressed in *Pichia pastoris*, as described previously.[18] All chemicals used were at least AnalaR quality.

Methods. *Digestion Protocols.* BSG samples were digested using sequential treatments of Econase CE, Alcalase, Depol 740L, Depol 686 and Econase supplemented with feruloyl esterases as indicated in the text, (e.g., E1/A2 = Econase treatment followed by Alcalase treatment). Samples were digested at 66 mg·mL^{-1} sample dry weight and at the temperature optimum for each enzyme. For Econase (120 μL·g^{-1} sample), digestion was at 60 °C in deionized water (pH 5.5−6.0). For Alcalase (20 μL·g^{-1} sample), digestion was at 60 °C in 30 mM ammonium carbonate (pH 8.5−8.7). Boiling to inactivate enzymes between treatments removed ammonium carbonate. Depol 740L and Depol 686 digestions (25 and 22 μL·g^{-1} BSG respectively) were performed at 50 °C in deionized water and included a digestion with the Depol, followed by a digestion with Alcalase. Enzyme profiles and activity levels were as reported previously.[6] The feruloyl esterases, AnFaeA and TsFaeC, were used at 0.0015 U/g BSG and at the pH of the accompanying commercial enzyme preparation. Sample insoluble residues were recovered by filtration and freeze-dried prior to the preparation of alcohol-insoluble residues (AIR). Supernatants from selected digests were retained after centrifugation, boiled to inactivate enzymes and freeze-dried prior to analysis for constituent sugars and phenolic acids.

Alcohol-Insoluble Residue (AIR). AIR was prepared from untreated material and enzyme-digested residues.[1] BSG (100 g; ∼22% dry weight; stored up to 24 h at 4 °C) was homogenized for 30 s and then plunged into boiling absolute ethanol (300 mL: ∼80% ethanol final concentration) for 1 h. The insoluble residue was recovered by filtration (GF/C paper) and re-extracted (×2) in boiling 70% ethanol (each 300 mL; 2 h), then in boiling absolute ethanol (×2; 300 mL; 5 min), and finally with cold absolute ethanol (150 mL). The insoluble residue (AIR) was washed (×2) in 2 volumes of acetone prior to drying to constant weight at 40 °C.

Residue Fractionation. BSG AIR was treated as outlined in Figure 1, to provide a range of extracts released from BSG at increasing strengths of alkali. The extraction procedure was similar to that used previously[19] but without the preliminary hot water wash and Pronase treatment, since these were integral to the digestion procedures tested, and with the addition of an aqueous wash of the final residue. The AIR (5 g) was suspended in degassed deionized water (200 mL) and extracted for 2 h at room temperature, with stirring. Extraction at each subsequent stage was in 200 mL of the appropriate solvent for 2 h. Supernatant extracts were recovered by centrifugation (20000*g*, 20 min) and filtered through GF-C. The filtrates provided sample extracts N, K1, K2, K3 and R_{aq} and an unextracted residue (R), as shown in Figure 1.

Determination of Sugars. Sugars were released from AIR samples through Saeman hydrolysis (dispersion in 72% H_2SO_4: 3 h, room temperature followed by hydrolysis in 1 M H_2SO_4 for 1 h at 105 °C). Hydrolysates were derivatized as their alditol acetates and analyzed by GLC using flame ionization detection.[20] Analysis of supernatant fractions followed the same protocol, but starting from hydrolysis in 1 M H_2SO_4. The total uronic acid content in samples was determined in the sugar hydrolysates, spectrophotometrically, using glucuronic acid as a standard.[21] Starch or dextran contribution to the sugar composition in enzyme-solubilized fractions was determined as total starch (K-TSTA: Megazyme International Ireland Ltd.) as described previously.[1]

Determination of Esterified Phenolic Acids. Total phenolic acids esterified to residues and extracted fractions were determined after saponification with 4 M NaOH (∼5 mg·mL^{-1}; 18 h; room temperature in the dark). Supernatants were recovered by centrifugation, and an aliquot (0.8 mL) was acidified (pH ∼2) using concentrated HCl, prior to extraction with ethyl acetate (3 × 3 mL). Extracts, reduced to dryness under nitrogen, were resuspended in methanol:H_2O (50/50, v/v), with constituent alkali soluble phenolic acids separated and quantified, as total monomeric and diferulic acids and including *cis* and *trans* isomers,[22] using HPLC (LUNA C18 reverse phase HPLC column (Phenonomex, Macclesfield, U.K.)), with *trans*-cinnamic acid as internal standard.[22]

Chlorite Delignification of BSG and Enzyme-Resistant Residues. Residues from the sequential extraction (R) were delignified through an acid chlorite treatment.[23] Each sample (∼1 g) was extracted in sodium chlorite/glacial acetic acid (1.6 g; 1.2 mL/100 mL) at 70 °C for 1 h, with occasional stirring. This was followed by a further addition of sodium chlorite/glacial acetic acid and continued extraction for 1 h. After cooling (40 °C) the insoluble residue was recovered by filtration (Whatman, GFC) and washed with deionized water to pH 5−6. Residues were dried by ethanol/acetone solvent exchange. The dried insoluble residue was sequentially extracted to produce fractions K_1, K_2, K_3 and final residue R_2. The supernatants arising from the residue fractionation were adjusted to pH 6−7 prior to dialysis (MWCO 4,500) against deionized water and freeze-drying. This fraction constituted the aqueous extract from the delignified residue.

Moisture Determination. Samples of fresh BSG were dried to constant weight (16 h) at 104 °C in a forced draft oven to determine dry weight/g fresh weight.

Statistics. Composition analysis data for polysaccharides and phenolics are the mean and standard deviation of triplicate samples. Trend fitting data points used Origin 7 Scientific Graphing and Analysis Software (Sigma Plot).

Table 1. Component Polysaccharide Sugars Determined in BSG and Residues after Enzyme Digestion (mg·g^{-1}Residue Dry Weight)[a]

	res	Rha		Fuc		Ara		Xyl		Man		Gal		Glc		UA		total	
sample	(%)	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
untreated	88.0	0.8	0.1	0.3	0.1	77.4	4.9	162.9	11.2	5.9	0.8	12.3	0.8	224.8	15.0	43.4	2.3	528	21
E1/A2/R	60.0	1.0	0.1	0.3	0.1	101.5	2.1	199.2	16.3	5.3	0.3	14.2	0.4	207.8	26.3	62.4	3.5	592	24
A1/E2/R	67.1	1.0	0.1	0.3	0.1	99.2	9.3	207.3	13.4	6.1	1.2	14.5	2.0	223.7	12.5	39.9	2.2	582	22
D686/A/R	63.6	0.7	0.3	0.4	0.1	106.2	7.7	227.4	28.7	7.3	1.0	15.8	1.2	238.7	36.3	40.2	2.6	637	60
D740L/A/R	51.9	0.9	0.1	0.4	0.1	95.3	4.8	197.1	19.4	7.1	0.8	14.7	1.2	239.8	26.2	36.5	2.6	592	45

[a] Results are shown as mean and standard deviation; res (%) = proportion of source BSG recovered as an alcohol insoluble residue; UA = uronic acid determined as galacturonic acid; E = Econase; A = Alcalase; D = Depol; R = insoluble residue recovered.

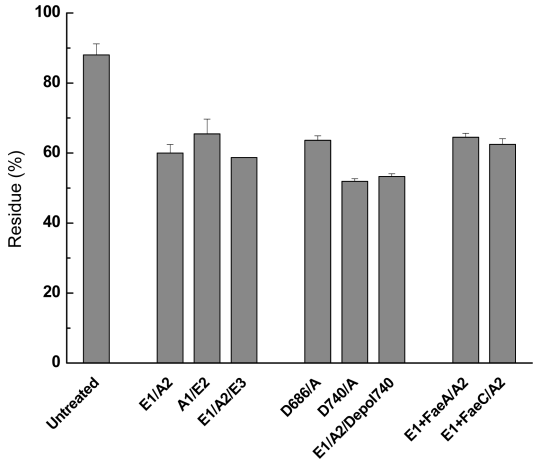

Figure 2. Dry matter recovery from BSG pre- and postenzyme digestion treatments. Results are % dry matter relative to the untreated original BSG.

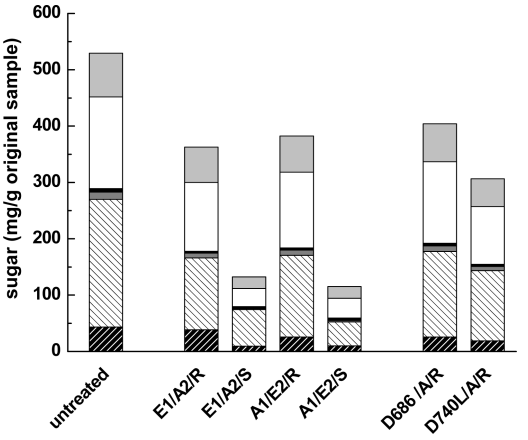

Figure 3. Polysaccharide content and profile of component sugars in BSG residues following enzyme treatments, relative to the untreated BSG: Ara, light gray; Xyl, white; Man, black; Gal, dark gray; Glc, white with black lines; uronic acid, black with white lines.

RESULTS AND DISCUSSION

Enzyme Treatments. Aqueous extraction of BSG removed 12% of the initial dry weight. The insoluble residue recovered and available for enzymatic digestion contained ~50% carbohydrate (Table 1).The proportion of soluble material was less than the 20% removed during preparation of AIR[1] and probably reflects the more severe extraction conditions and solubilization of some constituent alcohol-soluble hordeins[24] during AIR preparation. The aqueous extract contains mainly glucan (~6%) and protein (~2.6%) and with only a trace of arabinoxylan.[14] All enzyme treatments resulted in a significant digestion of BSG (Figure 2), though the proportion varied with enzyme preparation. Digestion was greatest with the carbohydrase treatment involving Depol 740L, followed by the protease, Alcalase, treatment [D740L/A].

Sequential additions of Econase and Alcalase (E1/A2 or A1E2) digested ~26% of BSG, much less than the D740L/A combination. However, the extent of digestion was similar irrespective of the order of enzyme application. Further treatment of the E1/A2 residues with either Econase or Alcalase had negligible effect. Econase digested ~15% of the BSG (Figure 3), producing a hydrolysate which was ~95% carbohydrate and rich in arabinose, xylose and ferulic acid (FA). In the absence of detectable feruloyl esterase activity in Econase,[6] some FA and related diferulate cross-links will be present still esterified to arabinose in the oligosaccharides released. Some dextrins/dextrans from residual starch were also present.[1] Similarly, Alcalase, with no detectable carbohydrase activity,[5] digested ~15% of the BSG. This hydrolysate included some dextrin/dextran, but only a trace of other carbohydrate, which was presumed to have been trapped in the BSG residue. Subsequent treatment of the E1/A2 residue with Depol 740L resulted in a limited further degradation, due to either extra activities within the Depol 740L preparation or higher activity of the *Humicola insolens* enzymes over those in the Econase *Trichoderma reesei*. The extent of digestion using Econase/Alcalase was similar to that achieved with Depol 686/Alcalase, with feruloyl esterase activity (~3 U/mL) present in Depol 686, but was significantly less than with Depol 740L/Alcalase, with feruloyl esterase activity ~36 U/mL (t = 3.429; p < 0.05). Depol 740L/Alcalase treatments gave the apparent limit to the extent of digestion using enzyme cocktails and was below the limits of potential digestion according to the polysaccharide content of the residues. This suggests that while hydroxycinnamic acids can influence the digestion of BSG, they do not limit the overall extent of digestion, which appears contrary to the reported limiting effects of phenolics on forage degradation.[10,15,25] However, feruloyl esterases in Depol 740L have a recognized low activity against diferulates,[26] and it may be that these components contribute more significantly to the limitation on degradation of lignified plant cell walls. The supplementation of Econase with monocomponent feruloyl esterases AnFaeA and TsFaeC, known to partially deferuloylate BSG and wheat bran,[27] increased biomass

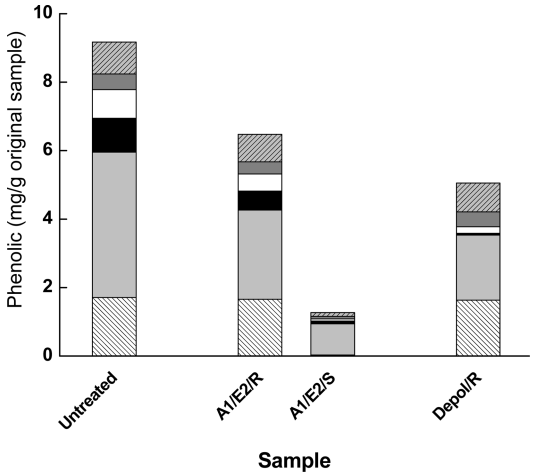

Figure 4. Cell wall-bound phenolic acid in residues recovered following enzyme treatments, relative to the untreated BSG: coumaric, white with black lines; ferulic, light gray; 8-8′, black; 8-5′, white; 5-5′, dark gray; 8-*O*-4′, gray with black lines.

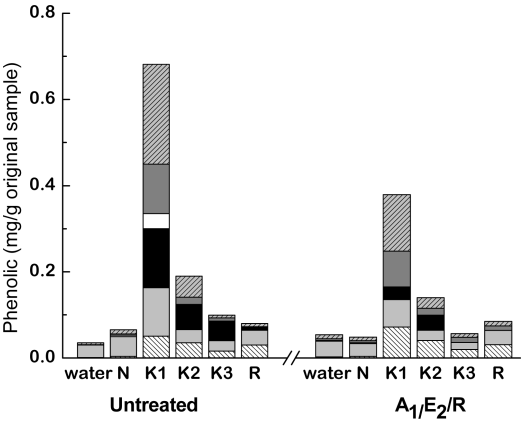

Figure 5. Cell wall-bound phenolic acids released in fractions during the controlled chemical fractionation of the original untreated BSG and enzyme treated BSG [A1E2/R/...]: coumaric, white with black lines; ferulic, light gray; 8-8′, black; 8-5′, white; 5-5′, dark gray; 8-*O*-4′, gray with black lines.

digestion by only 1−2%. Also, little further digestion of the E1/A2 residue resulted from repeat treatment with Econase, confirming that the initial high content of protein in BSG did not influence polysaccharide degradation by Econase.[27] Thus, with enzyme cocktails there are apparent defined limits on the extent of BSG digestion, presumed related to the spectrum of enzyme activity available and the structural constraints imposed by the BSG cell wall matrix. The enzyme-resistant residues remained rich in polysaccharide, and there was no indication of any preferential loss or concentration of particular carbohydrates due to a specific enzyme treatment.

Sugars. Following each enzyme treatment the BSG insoluble residues showed a decrease in arabinose and xylose, presumed to represent mainly arabinoxylans (AX) and glucose (Figure 3). Glucose content in particular dropped from ∼225 to ∼125 $mg \cdot g^{-1}$ in all treated residues. The composition of the soluble fractions recovered from the combined Econase and Alcalase treatments was similar, indicating that order of enzyme treatment had no apparent effect. Soluble glucose is derived mainly from starch (53.7 ± 15.6 $mg \cdot g^{-1}$). The other significant components present were arabinose and xylose. The presence of galactose may indicate the presence of some arabinogalactan, though a small proportion may be associated with AX.[29] The solubilized material had an A:X ratio of 0.62 ± 0.04, compared to an initial ratio of 0.48, typical of BSG,[6,28] and indicates the release of more highly branched AX. Whether the AX and glucose are released as polymeric, oligomeric or monomeric sugars is the subject of a more detailed analysis of the enzyme-solubilized fractions for feruloylated oligosaccharides ((F)AXOS).[8]

Profile of Phenolic Acids. The enzyme digested fraction from Econase and Alcalase treatments contained phenolic acids, mainly ferulic acid but also some diFA (Figure 4). The phenolic acid content of the untreated BSG was 9.2 mg/g, with ferulic acid predominant. The Alcalase/Econase treatment resulted in a 30% enzyme-related release, leaving 6.5 mg/g in the undigested residue. Depol 740L treatment released 45% of the phenolic acids, leaving 5 mg/g in the residue. *p*-Coumaric acid was not affected by any of the enzyme treatments, consistent with it being associated mainly with lignified tissues.[30] The 8-*O*-4′ diFA persisted in residues during the enzyme treatment and the 5-5′ dimer was less affected than the other diFA and FA. This may reflect some selectivity by esterases present or steric constraints within the cell wall which limit enzyme accessibility.[15,17] Although there was a decrease in FA and diFA in the residue, the level of arabinose substitution, at ∼1 FA+diFA/18 arabinose units, was similar between the Econase and untreated samples and similar to the extent of substitution in the enzyme digested fraction. The arabinose substitution of FA+diFA on arabinose increased to ∼26 following treatment with Depol 740L. This reflects the esterase activity in Depol 740L but also that de-esterification did not promote an increase in the xylanase activity required to degrade the AX.[6] Hence, factors other than the extent of AX esterification and cross-linking are important in restricting AX degradation.

Table 2. Effect of Enzyme Treatments and Delignification on Polysaccharide Sugar Content in Residue(s) ($mg \cdot g^{-1}$ Original BSG Residue Dry Weight)[a]

sample	untreated	enzyme treated
R1	372	328
R1 sugars	309 (184)	248 (159)
R1 delignified	258	225
R1 delignified sugars	223 (185)	192 (161)
R2/E1 residue	170 ± 6	167 ± 6
R2/A1/E2 residue	172 ± 2	174 ± 8
R2/Depol 740L residue	176 ± 9	179 ± 14

[a] R1 = alkali-insoluble residue; values in parentheses = Glc. R2 = alkali-insoluble residue after delignification; E = Econase; A = Alcalase; values for R2 residues shown as mean + SD.

Chemical Fractionation. Fractionation using alkali with untreated BSG and following enzyme treatment resulted in a de-esterification, but gave an indication of phenolic acids present but resistant to enzyme attack in residues (Figure 5). As noted previously,[19] the 0.5 M KOH (K1) residue is richest in phenolic acids. However, the concentration of phenolic acids in the K1 residue represents only around 10% of the phenolics, as measured in the original BSG. Fractions arising from residues subsequent to 0.5 M treatment each retain less than ∼2% of the original level of

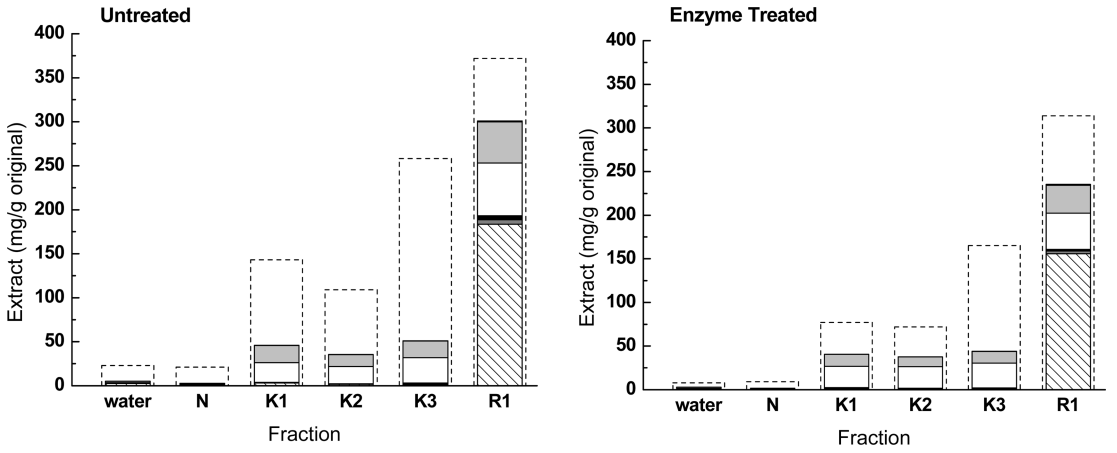

Figure 6. Dry matter distribution in fractions recovered during controlled chemical fractionation of BSG residues and the polysaccharide content and composition in fractions extracted, relative to the untreated BSG: total dry matter, dashed outline; Ara, light gray; Xyl, white; Man, black; Gal, dark gray; Glc, white with black lines.

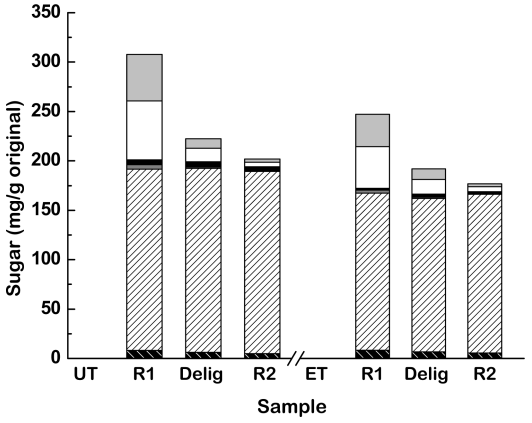

Figure 7. Delignified samples (R1 delig) recovered during controlled chemical fractionation of BSG residues, and the polysaccharide content and composition in fractions extracted, relative to the original BSG. UT = untreated; ET = enzyme treated [A1E2/R/...]: Ara, light gray; Xyl, white; Man, black; Gal, dark gray; Glc, white with black lines; uronic acid, black with white lines.

phenolic acids. Hence it is presumed that the bulk of esterified phenolic acids are released at relatively low concentration of alkali (<0.5 M KOH equivalent). The profile of phenolic acids recovered from each residue following sample pretreatment with enzymes (A1/E2) indicates that the enzyme treatment did not selectively degrade polysaccharide fraction containing esterified phenolic acids.

Despite an extensive de-esterification during alkaline extraction, alkali-mediated release of polysaccharides from BSG was limited and comprised mainly AX in fractions K1, K2 and K3. The profile of polysaccharide release in each fraction was similar for both the untreated and enzyme treated BSG. The R1 aqueous wash was a very minor fraction and contained only a trace of polysaccharide. A significant proportion of AX remained associated with the unextracted residues (R1), with glucose being the major component. Most notable during alkali treatment was the extraction of non-polysaccharide material. In the K1, K2 and K3 fractions in particular, a large proportion of each fraction was non-carbohydrate. In both the untreated and enzyme treated residues the cumulative polysaccharide in K1, K2 and K3 accounted for ~150 mg/g whereas the corresponding solubilized material accounted for ~550 mg/g in the untreated residue and ~350 mg/g in the enzyme treated residue. Alkali solubility of hordeins[24] can account for a proportion of the non-polysaccharide material, since around 50% of the initial protein content in BSG will remain undigested by Alcalase treatment.[27] At an expected protein content of ~150 mg·g^{-1} for the BSG,[1] non-protein and non-polysaccharide material must be present in each fraction. That a significant proportion of lignin-like material from wheat straw can be solubilized in alkali[31] would account for the lower than expected recovery of non-polysaccharide material in R1, from BSG with a Klason lignin content ~150 mg·g^{-1}.[1] The persistence of non-polysaccharide material in fractions after protease treatment at pH 8.5[14] would also support the extraction of a significant component of alkali-solubilized lignin. The chemical identity of this material remains unknown, but a component may correspond to material present in what is classed as Klason lignin. It may be noncore lignin, extracted as ligno-cellulosic complexes[32] or cutin-like material,[33,34] but it can be identified as a fraction distinct from alkali-insoluble lignocellulosic residues.

Delignification. Chemical delignification of the alkali-insoluble residue(s), R1, resulted in a significant loss of material (Table 2). AX accounted for the major loss of sugars (Figure 7). Also, the proportion of material lost during delignification, at 70−80 mg·g^{-1} (Figure 7), was similar to the proportion of non-polysaccharide material persistent in the R1 residues (Figure6). This suggests that the non-polysaccharide component in R1 residues corresponds to alkali-resistant lignin, as distinct from the alkali-soluble lignin released previously. Subsequent alkali fractionation treatment of the delignified insoluble residues removed all but a trace of the noncellulosic sugars. Cellulose persisted through the delignification process, similar to the effect found through ammonia treatment of barley hulls.[32] A further chemical fractionation of the delignified BSG residues, using alkali as previously, had no effect on cellulose. Cellulose remained at 170 ± 14 mg·g^{-1} original sample, which is typical for cellulose content in BSG.[35] This also indicates there was little effect of enzyme treatments on cellulose, despite the presence of endoglucanase and β-glucanase activity. Delignification was apparently complete insofar as the delignified residues showed no phloroglucinol staining or trace of phenolic acids. However, a trace of lignin may remain since wood lignin has been reported to persist after repeated cycles of chlorite treatment.[36] When delignified residues (R2) were treated with Econase, Econase/Alcalase, or

Depol 740L, some further digestion was observed: ~33% for the original untreated source sample and ~25% for the original A1/E2 source sample, but with no difference between enzyme treatments. For each R2 residue this represented ~7% of the original untreated BSG and 4% for the original A1/E2 treated BSG. In each case the residue represented ~17% of the original BSG and was composed of over 90% cellulose (Table 2).

GENERAL DISCUSSION

Enzymatic treatments can be used to digest a significant proportion of BSG (25−30%), but the release of polysaccharide components is limited. Controlled fractionation using alkali can enhance the solubilization of AX from BSG, suggesting that the alkali treatment removes structural constraints on AX release. However, alkali treatment affects more the non-polysaccharide components and the extent of solubilization of non-polysaccharide material cannot be accounted for by residual protein alone. This indicates that a significant solubilization of alkali-soluble lignin material may occur and also that structural features in the alkali-soluble lignin material can constrain enzymatic digestion of associated polysaccharides. Delignification treatment of residues resulted in a further and almost complete release of residual AX, indicating, as expected, that lignification can restrict the release of AX. Further chemical fractionation left a residue which corresponded to mainly cellulose. Whether the apparent absence of cellulose breakdown was due to there being an insufficient level of cellulase activity present or due to structural restraints remains to be tested.

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