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ARTICLE

Effect of Storage Conditions on the Microbial Ecology and Biochemical Stability of Cell Wall Components in Brewers' Spent Grain

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The composition of brewers' spent grain (BSG) makes it susceptible to microbial attack and chemical deterioration. This can constrain its appeal as an industrial feedstock. The current study has monitored the effects of BSG storage as fresh material (20 °C), refrigerated and autoclaved, measured against frozen material in relation to microbial proliferation and modification to plant cell wall polysaccharides and component phenolic acids. At 20 °C there was a rapid colonization by microbes and an associated loss of components from BSG. Refrigeration gave a similar but lower level response. When stored frozen, BSG showed no changes in composition but autoclaving resulted in a solubilization of polysaccharides and associated phenolics. Changes were associated with the temperature profile determined during autoclaving and were also partially due to the breakdown of residual starch. Losses of highly branched arabinoxylan (AX) and the related decrease in ferulic acid cross-linking were also found. The results confirm the need for storage stabilization of BSG and demonstrate that the methods selected for stabilization can themselves lead to a substantial modification to BSG.

KEYWORDS: Brewers' spent grain; storage; stabilization; polysaccharide; microbiology; starch; cell wall; phenolic acid

INTRODUCTION

Malted barley is the primary ingredient used for brewing beers. The residue remaining following malt extraction, or mashing, is termed brewers' spent grain (BSG) and can represent more than 25% (w/w) of the starting malted barley. In 2008, 1.811×10^6 hL of beer was produced worldwide (*1*), and this represents the generation of 32.6×10^6 tons of BSG. Although enriched in polysaccharides, protein, and phenolics, BSG is considered a low-value product and, until now, has been sold as cattle feed, composted, or disposed to landfill. Due to legislative drivers (*2*) related to the cost for the disposal of BSG and other brewery wastes, there is increasing awareness that economic and environmental sustainability needs to be developed within the industry (*3*). As this awareness has increased, so has the drive for alternative value-added commercial outlets (*4*).

The high water content, organic nature, and presence of fermentable sugars in BSG (*3*) make it prone to microbial attack and chemical modification; hence, it appears to be an unstable material, liable to deteriorate rapidly. Harvested cereal grain has a resident microflora comprising bacteria and molds, arising from various sources (*5*), which can cause problems during grain storage and processing. For example, after 30 days Sodhi and co-workers found eight isolates of *Aspergillus*, *Fusarium*, *Mucor*, *Penicillium*, and *Rhizopus* present in stored BSG (*6*). The propensity for microbial activity reduces the potential for the exploitation of BSG as a food-grade material. Several methods have been proposed to stabilize and store BSG: washing with water to neutral pH and mild drying to 10% water content (*7*) and treatment with lactic, formic, acetic, or benzoic acid to preserve BSG quality and nutritional value (*8*). Potassium sorbate has also been shown to preserve pressed BSG (*9*).

Drying as a possible alternative for preservation has the advantage that the product volume is reduced, and therefore transport and storage costs can be reduced (*10*). However, the drying process cannot be considered effective for destroying microbial contaminants (*11*) and energy requirements for drying add expense, especially since the traditional process for drying BSG is based on the use of direct thin-layer rotary-drum dryers, a procedure considered to be very energy intensive (*12*, *13*). Three methods evaluated for preserving spent grain, freeze-drying, oven drying, and freezing (*14*), have been considered as inappropriate because large volumes must be stored. Refrigeration may be an alternative, unless very long term storage stability is required. Freeze-drying is considered economically unacceptable, and for oven drying, temperatures below 60 °C are required, to avoid

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Table 1. Presumptive Groups of Bacteria, Yeasts, and Molds Enumerated from BSG

presumptive group	plating medium	incubation conditions
aerobic mesophilic bacteria	plate count agar (PCA)	in air at 20 °C
aerobic thermophilic bacteria		in air at 55 °C
Pseudomonas spp.	cephaloridine,−fucidin−cetrimide agar (CFC)	in air at 25 °C
yeasts and molds	oxytetracyline−dextrose−yeast extract agar (ODY)	in air at 20 °C
microaerophilic bacteria	De Man−Rogosa−Sharpe Agar (MRS)	gaseous atmosphere of 90% H_2 and 10% CO_2 at 25 °C
strictly anaerobic bacteria	reinforced clostridial medium (RCM)	gaseous atmosphere of 90% H_2 and 10% CO_2 at 25 °C

unpleasant flavours which can be generated at higher temperatures (*15*, *16*) and the risk of toasting or burning of the dried BSG. Superheated steam has been proposed as an alternative drying method (*13*, *17*, *18*), claiming additional advantages such as reduction in environmental impact, improved drying efficiency, elimination of fire or explosion risk, and enhanced recovery of valuable organic compounds. Superheated steam has the advantage of reducing or eliminating microbial contamination during drying (*19*). A membrane filter press with hot water (65 °C) has also been used to dry BSG to moisture levels of between 20 and 30% (*20*). The product remained free of bacterial activity for up to 6 months in the open air.

The methods previously tested for BSG stabilization all tend to have high energy input requirements, which is recognized as not economically desirable for handling large-volume materials, and at low moisture content undesirable flavor components may be generated. Also, in previous studies the time of storage is often indefinite, whereas in practice, storage time may be relatively short term to satisfy the requirements for a continuous processing facility. In the current study we have compared the effects of storage at ambient temperature against refrigeration for short-term storage and with autoclaving and freezing for longer term storage.

MATERIALS AND METHODS

Materials. Brewers' spent grain (BSG) was obtained as one bulk sample from a local brewery, as the residues resulting from wort preparation. The BSG was supplied fresh from the mash tun while still hot (~60 °C) and allowed to cool in sealed polythene bags. Samples were subsequently stored aerobically at temperatures of −20, +4, and +20 °C. A fresh sample was also autoclaved (120 °C, 1 h) and then stored at +20 °C. A further sample was freeze-dried and stored in a sealed container prior to analysis. All chemicals used were at least Analar quality.

Sample Preparation. Samples of BSG were stored aerobically in a controlled temperature cabinet at +4 and +20 °C, and samples at −20 °C were stored in a laboratory freezer with a temperature monitor. For the storage treatments, samples were recovered for analysis on days 0, 1, 2, 7, and 16, except for the frozen sample, which was only sampled at day 16. One sample from each storage regime was processed immediately using appropriate microbiological techniques to enumerate the predominant components of the microflora; corresponding samples were used to prepare alcohol-insoluble residues (AIR) for chemical analysis.

Sample Heat Treatment. Samples of BSG which had been stored frozen (−20 °C) were thawed to room temperature, sealed in 15 mL Soveril tubes, and heated at preset temperatures in a hot block for temperatures between room temperature (20 °C) and 120 °C. The time at each temperature was determined from the temperature log of the autoclave (see **Figure 5**; inset). Samples were then used to prepare alcohol-insoluble residues for starch analysis, as polymeric starch.

Methods. *Microbiology*

(*a*). *Estimation of Numbers of Viable Micro-Organisms Present*. Estimation of numbers of viable micro-organisms was carried out as described previously (*21*). In brief, a subsample (40 g) from each treatment on each test day (i.e., day 0, 1, 2, 7, and 16) was diluted 1/10 w:v with peptone salt dilution fluid (PSDF (*22*), 400 mL) and blended in a Stomacher Lab-blender 400 (Seward Laboratory, London, U.K.) for 30 s using a Stomacher filter bag (Seward Ltd., London, U.K.). The blended and filtered material was diluted in PSDF. Counts on solid media were made by inoculation of duplicate plates with suitable dilutions by means of a spiral plate maker (Don Whitley Scientific, Shipley, U.K.) and incubated as described below.

(*b*). *Media*. The culture media, incubation conditions, and the presumptive identification of micro-organisms enumerated on each medium are as shown in **Table 1**,: i.e. plate count agar (PCA, Oxoid CM325) at 20 °C for aerobic mesophilic bacteria and at 55 °C for aerobic thermophilic bacteria, oxytetracycline−dextrose−yeast extract agar (ODY) (*23*) for yeasts and molds, cephaloridine−fusidin−cetrimide agar (CFC) (*24*) for pseudomonads, reinforced clostridium agar (RCM) (*25*) for strict anaerobic bacteria, and MRS (Oxoid, Basingstoke, U.K.) for microaerophilic bacteria.

Plates of ODY and PCA were incubated in air for 5 days at 20 °C. PCA plates for enumeration of thermophiles were incubated in air at 55 °C for 24 h. CFC plates were incubated in air at 25 °C for 5 days. RCM plates were prereduced prior to inoculation in H_2/CO_2, 9/1 v/v, for 24 h at room temperature, and then both RCM and MRS plates were incubated at 25 °C under H_2/CO_2, 9/1 v/v, for 5 days.

Alcohol-Insoluble Residues (*AIR*). AIR were prepared prior to analysis for cell wall sugars, cell wall bound phenolic acids, and starch (*21*). Wet BSG (100 g; ~22% dry weight) was homogenized for 30 s and then added to absolute ethanol (300 mL: 80% ethanol final concentration) and boiled for 1 h. The insoluble residue recovered (GF/C paper using a Buchner funnel) was extracted (×2) in boiling 70% ethanol (each 300 mL; 2 h) and then in boiling absolute ethanol (300 mL; 5 min) and washed with cold absolute ethanol (150 mL). The insoluble residue (AIR) was dried by solvent exchange using acetone, and the final residue was then dried to constant weight at 40 °C in a force draft oven.

Sugars. Sugars were released from the sample AIR through Saeman hydrolysis (72% H_2SO_4, 3 h room temperature) followed by 1 M H_2SO_4 for 1 h at 105 °C. Hydrolysates were derivatized as their alditol acetates and analyzed by GLC using a flame ionization detector (*26*). The total uronic acid content in samples was determined in the sugar hydrolysates, spectrophotometrically, using glucuronic acid as a standard (*27*).

Phenolic Acids. Phenolic acids were determined as total alkali-extractable cell wall bound phenolic acid, assuming that any water-soluble phenolics in the original BSG have been removed during wort production. The assay involved saponification with 4 M NaOH (~5 mg L^{-1}, 18 h, room temperature in the dark). An aliquot of the supernatant (0.8 mL) recovered following centrifugation was dosed with 10 μg of *trans*-cinnamic acid as internal standard and acidified using concentrated HCl (end pH ~2). Following extraction with ethyl acetate (3 × 3 mL) the extracts were reduced to dryness. After resuspension in methanol/H_2O (50/50, v/v), constituent alkali-soluble phenolic acids were separated and quantified using HPLC (LUNA C18 reverse phase HPLC column; Phenonomex, Macclesfield, U.K.) (*28*), using *trans*-cinnamic acid as internal standard.

Starch Determination. Starch was determined as total starch (K-TSTA; Megazyme International Ireland Ltd.), assayed as glucose following sample dispersion in DMSO, digestion using a thermostable α-amylase followed by amyloglucosidase treatment, with colorimetric quantification of glucose released. The assay conditions for starch assumed the starch content < 100 mg g^{-1} in a 10 mL reaction volume (*21*). Starch was assayed in the original freeze−thawed samples, to determine total starch (starch + dextrins), and then in the corresponding AIR preparations to distinguish changes in polymeric starch during autoclaving procedures.

Moisture. Samples of fresh BSG from each treatment and at each sampling point were dried to constant weight (16 h) at 104 °C in a forced draft oven to determine dry weight.

Statistical Analysis. Results from microbiology were expressed as the range of organisms obtained from replicate samples ($n = 3$). Composition

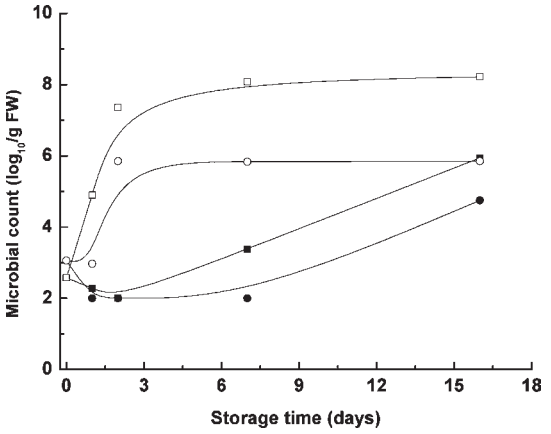

Figure 1. Profiling thermophilic and mesophilic populations of microbes during storage of BSG for up to 16 days at 4 and 20 °C: (−□−) mesophilic, 20 °C; (−○−) thermophilic, 20 °C; (−■−) mesophilic, 4 °C; (−●−) thermophilic, 4 °C.

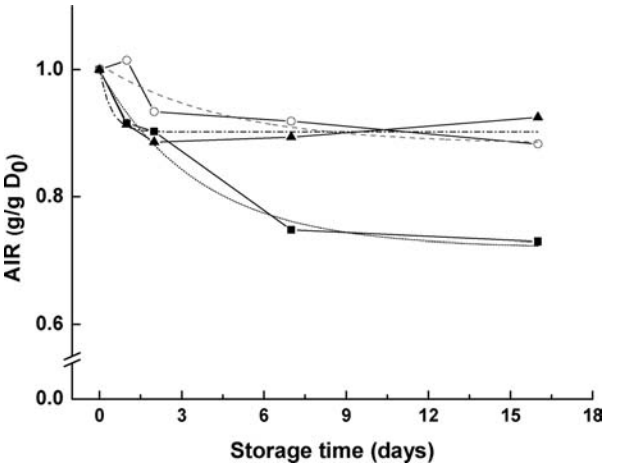

Figure 2. Change in the proportion of alcohol-insoluble residue (AIR) recovered from BSG during storage for up to 16 days at 4 and 20 °C and after autoclave treatment: (−■−) observed, 20 °C; (−○−) observed, 4 °C; (−▲−) observed, autoclaved 20 °C; (· · ·) fitted, 20 °C; (− − −) fitted, 4 °C; (− · −) fitted, autoclaved 20 °C. AIR values were measured as g g^{-1} relative to the original BSG (day 0). Data were fitted to a first-order exponential decay model ($y = y_0 + Ae^{-x/t}$), where y = total substrate remaining at time t, y_0 = asymptote of the extent of degradation, and A = potentially degradable substrate.

analysis data for polysaccharides and phenolics are the mean and standard deviation of triplicate samples. Trend fitting of data points used Origin 7 Scientific Graphing and Analysis Software (OriginLab Corp., Northampton, MA).

RESULTS AND DISCUSSION

Microbiology. At the point of production the fermentable sugar content of BSG and its high moisture content (~80%) make it susceptible to microbial deterioration postproduction (*20*, *30*). Hence, it is important to consider the persistence and prognosis of microbial contaminants postmashing in relation to the storage and potential exploitation of BSG. In the fresh BSG (day 0) low numbers of aerobic mesophilic and thermophilic bacteria were detected (10^2−10^3 g^{-1}). Other micro-organisms remained below the limit of detection (10^2 g^{-1}). This is consistent with a low level of microbial contaminants, as has been noted in other cereals: e.g., wheat grains (*5*). The concentration of bacteria can also be considered low in relation to the 10^5−10^6 cfu/g concentration noted for wheat kernel (*5*), implying that a significant washout of bacteria occurs during mashing. The major zone of microbial and fungal contamination in cereal grains is the outer pericarp (*5*). In its original state, barley grain, like most lignocellulosic material, is relatively resistant to microbial attack. However, extensive changes in structure which occur during malting and mashing allow accessibility to hydrolytic enzymes (*29*), thus making BSG an easier substrate for microbial attack.

Throughout storage at 4 °C the numbers of aerobic bacteria remained below 10^5 g^{-1}, and in frozen and in autoclaved samples there was no evidence of microbial activity (limit of detection 10^2 g^{-1}). At 20 °C microbial populations increased 1000-fold, to ~10^6 g^{-1}, by day 5 (**Figure 1**). This was paralleled by an almost 100-fold increase in the aerobic mesophilic bacteria, to around 10^8 g^{-1}. At the maximum microbial population density, achieved by day 5, the aerobic mesophilic bacteria represented the predominant naturally associated microflora. At 4 °C, proliferation was much slower and there was little change during the first week of storage, but by day 16 the numbers of aerobic mesophilic bacteria had reached ~10^6 g^{-1} and were not yet into the stationary phase (**Figure 1**). Given that opportunities for external contamination were restricted during the experiment, it is apparent that spoilage of BSG can potentially occur through the persistence and proliferation of relatively low numbers of micro-organisms in BSG. While at 20 °C this can result in a significant population of spoilage organisms within a few days, the effect is reduced through refrigeration and this presents refrigeration as a suitable method for short-term storage of BSG. However, it should be noted that it is critical to stabilize and control the microecology if opportunities to exploit BSG are to be realized. Although the resident microflora may effect some desirable modifications or component release from the BSG, as spoilage organisms the resident microflora will compromise the microbial safety of the BSG for food-related exploitation. However, realizing the potential for proliferation of a resident microflora has implications when considering exploitation of BSG through use of specific microbial inocula to control structural modifications or component loss from the BSG matrix.

Moisture. The dry weight of the BSG as supplied was 226 mg/g fresh weight. During storage at +20 °C the mean dry weight over the storage period was 224 mg/g fresh weight ±16. Corresponding values for samples at 4 °C were 180 mg/g fresh weight ±24 and for autoclaved samples were 264 mg/g fresh weight ±7. While there may be some effect of sample treatment on moisture content, there was no evidence of change during storage. The high moisture content of all samples also indicated that a high water activity would be expected and hence a high susceptibility to microbial growth, as noted from the profiling of the microbiology.

AIR Profiles. The yield of AIR from BSG, as delivered from the brewery (day 0), was 836 mg g^{-1} dry weight, and this value was used as the reference (100%) AIR used to determine relative AIR yield for the different sample storage treatments. For stored samples maintained at 20 or 4 °C or autoclaved prior to storage at 20 °C, there was a loss of AIR in comparison to day 0 (**Figure 2**). When it was fitted to a first-order exponential decay model (SigmaPlot), the asymptote for the observed limit of degradation (y_0) was ~720 mg g^{-1} for samples stored at 20 °C and ~880 mg g^{-1} for samples either autoclaved or stored at 4 °C. Hence, up to 28% of BSG would be expected to be lost during storage at 20 °C but refrigeration at 4 °C would also give a loss of up to 12%. No loss in AIR was found for samples stored either frozen or freeze-dried (data not shown). The loss in AIR at 20 °C was similar to that which can be achieved using in vitro enzyme digestions (*31*, *32*), but over a much longer time scale. This reflects the initial relatively

low level microbial population attached to BSG and presumably relatively low levels of endogenous and exogenous hydrolytic activity present. Interestingly, for equivalent losses in AIR, day 1 at 20 °C and day 16 at 4 °C, the microbial loading of mesophilic bacteria was similar (~10^5 cfu/g). However, the reasons an asymptote for samples treated at 4 °C was reached at ~880 mg g^{-1} rather than a projected ~720 mg g^{-1} are unclear. Psychrophilic microbes may be active at 4 °C (*33*), with a similar but more limited hydrolytic activity compared to the microbial population at 20 °C. The decay profile of AIR and differences due to temperature also indicate that it is microbial enzyme activity which is mainly responsible for loss of AIR. This activity will include a broad spectrum array of plant cell wall degrading enzymes, including xylanases, esterases, and cellulases. These activities were not measured in this current study. While low temperature can reduce activity, freezing is required to prevent significant microbial activity. Autoclaved samples showed no microbial activity and no loss in AIR after day 1. Loss in AIR up to day 1 may be due to residual enzyme activity in samples prior to reaching enzyme inactivation temperatures during the autoclave cycle (see below) or some loss of starch, similar to that found during critical steam drying (*13*).

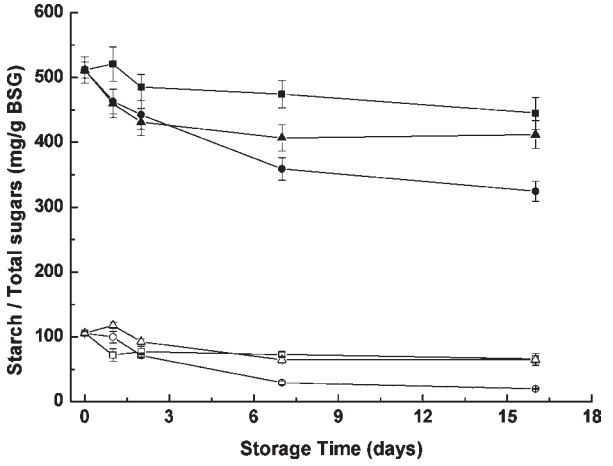

Figure 3. Changes in starch and total sugars measured during storage of BSG for up to 16 days at 4 °C, at 20 °C and after autoclave treatment: (−○−) starch, 20 °C; (−□−) starch, autoclaved 20 °C; (−△−) starch, 4 °C; (−●−) total sugars, 20 °C; (−■−) total sugars, autoclaved 20 °C; (−▲−) total sugars, 4 °C. Starch and sugars (mg g^{-1} relative to the original BSG (day 0)) were measured as the mean ±1sd of determinations made in triplicate.

Sugars. The sugar content of AIR represented around 500 mg g^{-1} of the original BSG (**Figure 3**). The major sugars, as glucose, xylose, and arabinose, confirmed that the major polysaccharides present were cellulose and arabinoxylans, and the ara:xyl ratio of 0.50 ± 0.05 was similar to previously reported ratios (*21*, *34*). A loss of sugars was noted in samples treated at 4 and 20 °C and autoclaved, which mirrored the profile for the loss of AIR during each storage treatment. Overall, the loss in total sugars represented around 190 mg g^{-1} of the original BSG dry matter at 20 °C, 60 mg g^{-1} at 4 °C, and 100 mg g^{-1} for the autoclaved samples. The loss at 20 °C was greater than could be accounted for by the 104 mg g^{-1} total starch (starch + maltodextrins) measured in the fresh BSG. However, a reduction in total starch, to around 19 mg g^{-1} at 20 °C, 50 mg g^{-1} at 4 °C, and 72 mg g^{-1} for autoclaved samples, was found. Since the measured starch included maltodextrins, the reduction represented a loss of glucose from samples during storage, and since mixed linkage β-glucans represent only a minor component of the BSG (*21*), the loss of glucose is presumed to be through starch fermentation at 20 and 4 °C.

A detailed analysis showed no selectivity in the loss of sugars from AIR during storage (**Figure 4**). The losses at 20 and 4 °C gave a first-order decay, but for autoclaved samples, sugar losses were confined to the period of autoclaving (day 0−1). Such losses, prior to microbial and enzyme inactivation, can represent a significant change in the composition of BSG postproduction and may be more significant on an industrial scale. BSG delivered to the laboratory took several hours to cool to ambient temperature (20 °C), and during this time microbial and surviving endogenous enzyme activity would persist. On an industrial scale, the high bulk volume would reduce the rate of cooling in comparison to the small volumes used in the laboratory; thus, postproduction of BSG, changes will be expected to be greater.

Heat Treatment and Starch Loss. Residual starch in BSG, as maltodextrins and polymeric starch, represented 105 mg g^{-1} dry weight. After AIR preparation the starch content was reduced to around 70 mg g^{-1}: i.e., around 30% of the original total starch was maltodextrin, and the residual starch was enriched in amylose (*21*). During autoclaving a regelatinizing of the residual amylose makes it susceptible to amylase attack. The profile of the autoclave cycle temperature (**Figure 5**, inset) shows a window of around 5 min at 60−70 °C available for starch gelatinization and amylase digestion. Simulation of the autoclave cycle, with recovery of samples at set temperatures, showed that polymeric starch was depleted during autoclaving (**Figure 5**). Over the temperature range 20−120 °C a significant decrease in polymeric starch occurred ($y = 78.938 - 0.190x$; $p < 0.001$). However, regression analysis on starch content below 60 °C ($y = 72.800 - 0.005x$; $p > 0.05$) and above 70 °C ($y = 69.760 - 0.104x$; $p > 0.05$) confirmed the

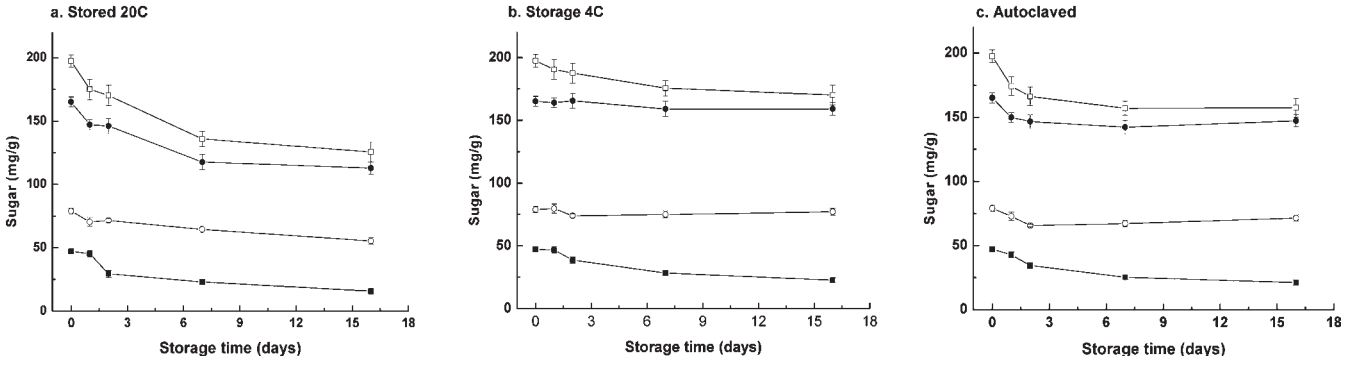

Figure 4. Profile of loss of constituent polysaccharide sugars (mg g^{-1} relative to the original BSG (day 0)), measured as the mean ±1sd of determinations made in triplicate, during storage of BSG for up to 16 days: (a) 20 °C; (b) 4 °C; (c) 20 °C after autoclave treatment. Legend: (−○−) arabinose; (−●−) xylose; (−□−) glucose; (−■−) uronic acid.

change was confined to temperatures between 60 and 70 °C and thus related to a degradation by residual amylase activity in the BSG. That BSG showed no microbial activity or further loss of components analyzed following autoclave treatment is testament to the effectiveness of autoclaving to stabilize BSG, but this must be considered against any modifications which may result during autoclaving.

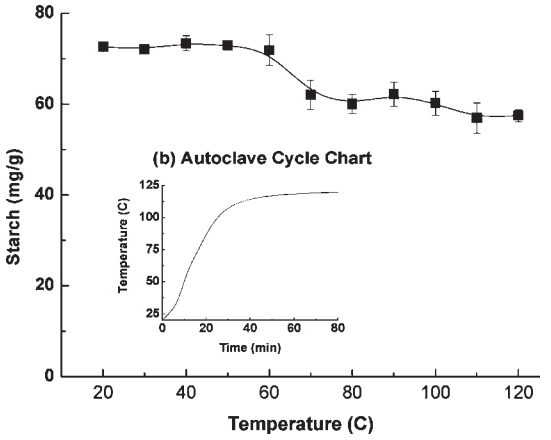

Figure 5. Change in starch content measured during (simulated) autoclave treatment. Inset b: temperature change profile recorded during autoclave treatment of BSG. Starch was measured as mg g^{-1} of dry matter in BSG.

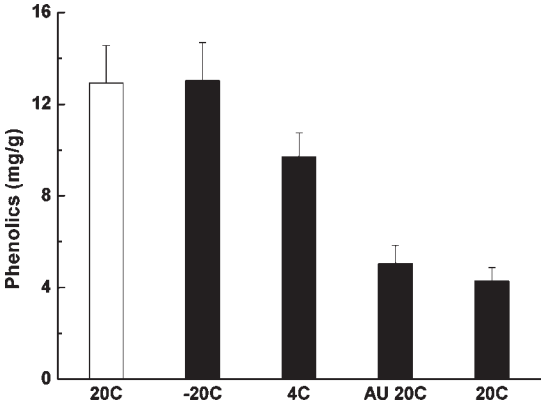

Figure 6. Changes on total phenolic acid measured after storage of BSG for 16 days at −20, 4, and 20 °C and after autoclave treatment: (□) day 0; (■) day 16. Phenolic acids were measured as mg g^{-1} relative to the original BSG.

Phenolics. Bound phenolics are a minor component in BSG, their concentration representing around 13 mg g^{-1} of the original BSG (**Figure 6**). However, their role in intra- and interpolymeric interactions is important for the structural integrity of the cell wall, and they are key targets in the dynamics of wall opening during microbial attack. The loss of phenolic acids was highest during storage at 20 °C (67%) but was similar to that for autoclaved samples (60%). Loss of phenolics at 4 °C was around 25%, and in samples frozen at −20 °C there was no change from that in the original BSG. The loss in phenolic acids is presumed to be through microbial rather than endogenous enzyme activity, since levels of bound phenolic acids in BSG were similar to concentrations which can be estimated from a range of barley varieties (*35*, *36*). Loss of phenolic acids from BSG can be expected to occur through microbial enzyme activity, to release free phenolic acids, but also as feruloylated oligosaccharides, released through xylanase activity during the degradation of component arabinoxylans (*37*−*39*). The loss from autoclaved samples was unexpected, since extensive degradation of NSP can occur during malting (*40*, *41*) but less during mashing (*42*). A detailed analysis of phenolic acids (**Figure 7**c) indicates that the loss was confined to the period of autoclave treatment. This may be related to enzyme activity affecting AX and phenolic release, akin to the effects of the further starch digestion noted during autoclaving (**Figure 5**), but for endogenous xylanases in particular, inactivation will occur above 65 °C (*43*).

Losses in both ferulic acid and its dehydrodimers occurred (**Figure 7**). The profiles of loss were similar to those found for sugars in the corresponding storage treatment. That a high proportion of phenolic acids can be lost even at relatively low levels of microbial colonization indicates the high lability of many phenolic acids in BSG, as also noted for the breakdown of plant cell walls in the gut (*44*). However, there is also a component of the phenolic acids which remains very resistant to degradation. A change in the relative proportion of ferulate and its dehydrodimers, particularly up to day 7, implies that there has been a decrease in cross-linking. This was confirmed when cross-linking was expressed as the ratio of xylose to dehydrodimers (*45*), with the gross index of cross-linking at 20 °C showing a linear increase from around 27 at day 0 to around 95 by day 16. Since the degree of cross-linking has hitherto been considered a control point restricting the disassembly of BSG and other cell wall matrices (*46*), the implication is that while specific cross-links in muro may restrict disassembly, the relative lability of many diferulates makes overall cross-linking a less critical consideration in the restriction of disassembly.

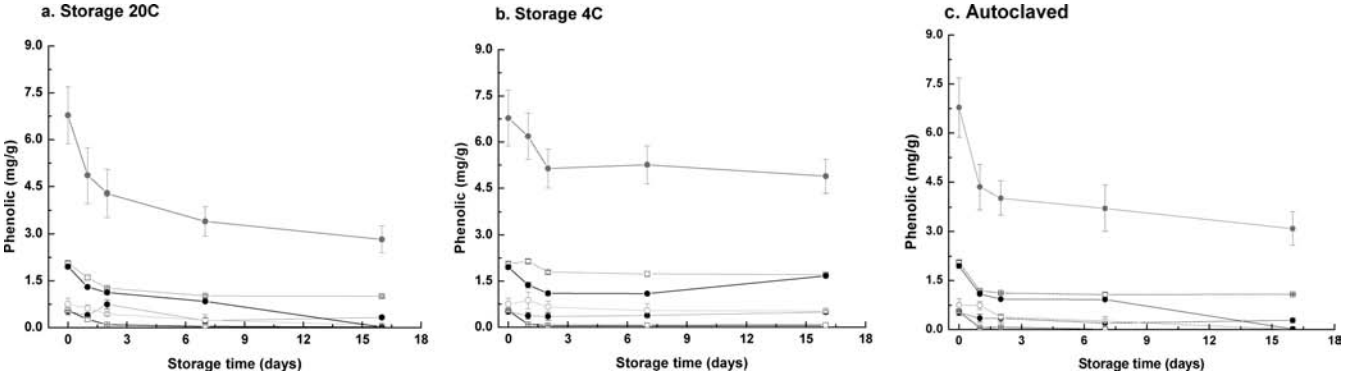

Figure 7. Profiles of loss of constituent phenolic acids during storage of BSG for up to 16 days: (a) 20 °C; (b) 4 °C; (c) 20 °C after autoclave treatment. Legend: (···□···) coumaric; (—○—; grayscale) ferulic; (···●···) 8,8′DiFA; (—□—) 8,5′DiFA; (···○···) 5,5′DiFA; (—●—) 8-O-4′DiFA. Phenolic acids (mg g^{-1}) relative to the original BSG (day 0)) were measured as the mean ±1sd of determinations made in triplicate.

Estimation of losses to phenolic acids was confined to the analysis of the insoluble residue; hence, the relative contribution to the losses from either esterase activity or degradation of arabinoxylan was not determined. However, regression analysis of the change in arabinose against feruloyl derivatives, at 20 °C, gave a regression coefficient of 0.293 ($P < 0.01$), or one feruloyl residue was being lost for every 3 arabinose units lost. Simultaneously the ratio of feruloyl units to arabinose in the residue changed from ~1:7 at day 0 to ~1:19 at day 16, indicating a preferential loss of the more highly branched AX during storage. Together this results in a disproportionate loss of feruloylated substrate from BSG during storage. Similar effects but at lower levels were observed for refrigerated samples.

Overall the results show that stabilization of BSG may be achievable short-term through refrigeration and that autoclaving can give longer term stability. During stabilization, the treatment process used can also lead to a loss of component material but also a modification to BSG, through loss of polysaccharide components and cross-linking agents. Modification to either of these components can affect the interlocking polymer network of polysaccharides, proteins, and polyphenolics in the cell wall matrix, each of which is susceptible to breakdown by the large array of microbial enzymes present, through the proliferation of a resident "spoilage" microflora, during inappropriate storage regimens to stabilize BSG. This has implications for downstream processing, where associated losses of components, such as phenolic acids, can represent a significant loss in any potential bound antioxidant activity being considered through exploitation of BSG.

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