Brewers’ spent grain: bioactivity of phenolic component, its role in animal nutrition and potential for incorporation in functional foods: a review

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Brewers’ spent grain (BSG) is a low-value co-product of the brewing industry produced in large quantities annually. This paper reviews the existing evidence regarding the phenolic component of BSG, focusing on composition, extraction and biofunctions such as antioxidant, anti-atherogenic, anti-inflammatory and anti-carcinogenic activities. Furthermore, the incorporation of BSG in foodstuffs will be discussed, including the use of BSG as an animal feed supplement and the potential of BSG to be incorporated into foods for human consumption. BSG contains hydroxycinnamic acids including ferulic acid, p-coumaric acid and caffeic acid; which have shown bioactivity in the pure form (antioxidant, anti-inflammatory, anti-atherogenic and anti-cancer). Phenolic extracts from BSG have also shown antioxidant potential, by protecting against oxidant-induced DNA damage, possibly by Fe chelation. Studies show that BSG has many benefits when used as a supplement in animal feed, such as increasing milk yield, milkfat content and in providing essential dietary amino acids. The incorporation of BSG in human foods such as cookies and ready-to-eat snacks has resulted in increased protein and fibre contents of the products, where the changes in organoleptic properties are controllable. It can be concluded that the phenolic component of BSG has potential bioactive effects, which are worth pursuing given that the inclusion of BSG into human foodstuffs is viable and beneficial.

Brewers’ spent grain: Phenolic acids: Animal nutrition: Functional foods: Bioactivity

Abbreviations: BSG, brewers’ spent grain; COX-2, cyclooxygenase-isoform 2; DPPH, 1,1-diphenyl-2-picrylhydrazyl.
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of both protein and fibre. BSG is an excellent feed ingredient for ruminants, providing all the essential amino acids when combined with inexpensive N sources such as urea. However, with the increased cost of disposal of the solid fraction, alternative uses are highly sought-after and it has been shown that BSG can be effectively integrated into ready-to-eat snacks to increase dietary fibre, crude protein and fat levels. Other areas of successful research include the blending of BSG with flour for incorporation into cookies and the addition of BSG to dough to improve the dietary fibre content in bread.

This review details the existing evidence regarding BSG. A specific focus is placed on the potential bioactivities of phenolic compounds (particularly ferulic acid and p-coumaric acid) present in BSG, and the incorporation of BSG into foodstuffs, for both human and animal consumption.

Composition of brewers’ spent grain

Many studies have reported on the approximate composition of BSG, which contains protein, fat, cellulose, hemicellulose and lignin (Table 1). As shown, there is good consistency with regard to the composition of BSG. However, variations can arise due to differences in barley variety, harvesting time, characteristics of hops added and brewery technology. BSG predominantly consists of the husk-pericarp-seed coat layers that are rich in cellulose, non-cellulosic polysaccharides, lignin, protein and fat. This is reflected in the composition of BSG (Table 1), and thus BSG can be regarded as a lignocellulosic material. In addition to the components detailed in Table 1, it has been shown that BSG is also a valuable source of vitamins, minerals and amino acids, particularly for animal feeding. The vitamins present in BSG are biotin, folic acid, niacin, choline, riboflavin and thiamine, pantothenic acid and pyroxidine. BSG is also reported to contain minerals such as Ca, Cu, Fe, Mn, K and Na and both essential (including lysine, histidine, methionine, phenylalanine, tryptophan) and non-essential (including alanine, serine, glycine, proline) amino acids. When combined with inexpensive N sources, such as urea, BSG can provide all the essential amino acids to ruminant animals.

Table 1. The approximate chemical composition of brewers’ spent grain

<table>
<thead>
<tr>
<th>Study</th>
<th>Protein</th>
<th>Lipid/Oil</th>
<th>Ash</th>
<th>Cellulose</th>
<th>Hemicellulose</th>
<th>Lignin</th>
<th>Starch</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beldman et al.</td>
<td>23.8–3.5</td>
<td>0.12–2.0</td>
<td>5.1</td>
<td>15.1</td>
<td>24.8</td>
<td>2.0</td>
<td>2.0</td>
</tr>
<tr>
<td>Valverde</td>
<td>24.0–0.1</td>
<td>6.0–1.7</td>
<td>17.0</td>
<td>25.4</td>
<td>39.0</td>
<td>4.0</td>
<td>4.0</td>
</tr>
<tr>
<td>Kanauchi et al.</td>
<td>24.0–0.6</td>
<td>10.6–3.9</td>
<td>2.4</td>
<td>25.4</td>
<td>21.8</td>
<td>11.9</td>
<td>11.9</td>
</tr>
<tr>
<td>Santos et al.</td>
<td>24.2–3.9</td>
<td>3.9–1.9</td>
<td>3.4</td>
<td>3.4</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>El-Shafey</td>
<td>26.7–8.9</td>
<td>8.9–3.9</td>
<td>3.9</td>
<td>–</td>
<td>–</td>
<td>5.3</td>
<td>5.3</td>
</tr>
<tr>
<td>Mussatto &amp; Roberto</td>
<td>15.3</td>
<td>4.6–1.6</td>
<td>16.8</td>
<td>28.4</td>
<td>27.8</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Xiros</td>
<td>14.2–3.3</td>
<td>13.3–3.3</td>
<td>12.0</td>
<td>40.2</td>
<td>11.5</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Treimo</td>
<td>23.4–1.2</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>12.6</td>
<td>7.8</td>
<td>–</td>
</tr>
</tbody>
</table>

*Values expressed as % dry matter, which has been documented in two studies to be 20.4 and 20.9.

Phenolic component of brewers’ spent grain

Phenolics present in brewers’ spent grain

Phenolic acids, particularly hydroxycinnamic acids and hydroxybenzoic acids are secondary plant metabolites found extensively in plant foods. Phenolic acids are currently the focus of much attention due to their potential to act as antioxidant, anti-inflammatory and anti-carcinogenic compounds.

As previously mentioned, BSG consists predominantly of the husk-pericarp-seed coat and is largely made up of cell walls. Since most of the phenolic compounds of the barley grain are contained in the husk and hydroxycinnamic acids accumulate in the cell walls, BSG is a potentially valuable source of phenolic acids.

There is evidence to suggest that ferulic acid and p-coumaric acid (as shown in Fig. 1) are present at relatively high concentrations in BSG. Some of the existing literature regarding the presence of ferulic and p-coumaric acid is detailed in Table 2. Ferulic acid was found to be the most abundant hydroxycinnamic acid being present at concentrations ranging from 1860 to 1948 mg/g, while the p-coumaric levels ranged from 565 to 794 mg/g. More recent evidence shows that BSG consists of 1.16% monomeric and dimeric phenolic acids, with 53% of the monomeric phenolic acids accounted for by ferulic acid. The vast majority of phenolic acids were also found to be in the bound form. It has been reported that following ferulic and p-coumaric acids, the next most abundant phenolic acids in BSG were found to be sinapic, caffeic and sinapinic acid.
syringic acids (22). A summary of the phenolic acids present in BSG is given in Table 3.

### Table 3. The most abundant phenolic acids present in brewers' spent grain modified from Szwajgier et al. (22)

<table>
<thead>
<tr>
<th>Phenolic acid</th>
<th>Free acid concentration (mg/100 g dry matter)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ferulic acid</td>
<td>336.3 ± 16.0</td>
</tr>
<tr>
<td>p-Coumaric acid</td>
<td>64.4 ± 4.6</td>
</tr>
<tr>
<td>Sinapic acid</td>
<td>42.0 ± 1.1</td>
</tr>
<tr>
<td>Caffeic acid</td>
<td>9.9 ± 0.7</td>
</tr>
<tr>
<td>Syringic acid</td>
<td>6.5 ± 0.1</td>
</tr>
<tr>
<td>4-OH-benzoic acid</td>
<td>1.2 ± 0.0</td>
</tr>
<tr>
<td>Chlorogenic acid</td>
<td>0.6 ± 0.2</td>
</tr>
<tr>
<td>Protocatechuic acid</td>
<td>0.5 ± 0.1</td>
</tr>
</tbody>
</table>

With 1–4 M NaOH solution) has been widely used to extract hydroxycinnamic acids from BSG (3, 23, 29).

Extraction methods similar to those used for BSG, have also been utilised with other materials such as wheat bran extracts (30) and apple waste extracts (31).

### Potential health benefits of phenolic component of brewers’ spent grain

As previously mentioned, ferulic and p-coumaric acids are the phenolic acids at highest concentrations in BSG (Table 3). A lot of research has been conducted looking at the antioxidant activity of hydroxycinnamic acids, particularly ferulic and p-coumaric acids. A commonly used method for quantification of antioxidant activity is the 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay, which measures the ability of the test compound to scavenge the DPPH radical. Chen and Ho (32) have shown the antioxidant potential of ferulic acid using the DPPH and Rancimat methods, but ferulic acid was a less potent antioxidant than caffeic acid and α-tocopherol (33). Caffeic acid has been shown to act as an antioxidant in vitro and scavenged radicals including DPPH and the superoxide anion (34). It has also been shown, using the DPPH assay, that a number of hydroxycinnamic acids act as antioxidants, scavenging DPPH in the order caffeic acid > sinapic acid > ferulic acid > ferulates > p-coumaric acid (35). Similarly, but using an alternative method, a study investigating the phenolic compounds in wheat bran extract and their antioxidant activity again found that ferulic acid was one of the strongest antioxidants using the β-carotene linoleic acid model system. The β-carotene linoleic acid model system assay is based on the principle that at a high temperature the oxidation of linoleic acid produces peroxides that decolourise β-carotene. The wheat bran extracts with highest ferulic acid concentrations (following alkaline hydrolysis) also exhibited higher antioxidant activity (30). Ferulic acid and caffeic acid have been reported to have excellent antioxidant potential at low concentrations, with the ability to scavenge a range of free radicals. Both phenolic acids scavenge the reactive oxygen species and reactive nitrogen species, with concentration-dependent scavenging of NO, superoxide and 2,20-azino-bis-3-ethylbenzthiazoline-6-sulfonic acid radical. In line with the previously discussed evidence, caffeic acid was a stronger scavenger of the DPPH radical, but ferulic acid was better...
at scavenging 2,20-azino-bis-3-ethylbenzthiazolone-6-sulfonic acid radical and NO(36). In a recent study on beers, a direct correlation was found between ferric-reducing antioxidant power and a number of phenolic acids including ferulic, p-coumaric, caffeic, sinapic and vanillic acids(37). A second study also showed that some of the phenolic acids present in beer correlate with the antioxidant activity measured by the DPPH radical and superoxide anion scavenging, metal chelation and reducing power; these include syringic and caffeic acids(38). Since ferulic acid is so well recognised as an antioxidant, it is approved for use as a food additive in some countries to prevent oxidation(39,40). In addition, it is important to note that while phenolic compounds can have an antioxidative effect, they have also been shown to act as pro-oxidants under certain conditions, thus inducing oxidative stress. Recent literature suggests that at low concentrations, many phenolics exhibit pro-oxidant behaviour, whereas the synthetic antioxidants, including α-tocopherol, do not(41). For caffeic acid and ferulic acid to act as pro-oxidants, higher concentrations are required(36). It has also been found by using the comet assay that at high concentrations p-coumaric acid enhanced DNA breakage induced by H2O2. This may be due to the production of reactive oxygen species by p-coumaric acid as a result of its pro-oxidant activity(42). It has been suggested that this pro-oxidant effect is related to the presence of metal ions in the body (for example due to tissue injury releasing Fe and Cu) and is of relevance for the bioactivity of phenolic compounds in vivo(43). It is clear from a small number of in vivo studies that the hydroxycinnamic acids have antioxidant properties. Such studies are essential to understand the biological role of these phenolic acids(44).

In addition to their antioxidant potential, there is increasing evidence to suggest that phenolic acids can have an anti-carcinogenic effect. Caffeic acid exhibits an anti-proliferative effect on several cancer cells including mammary gland, adenocarcinoma, lymphoblastic leukaemia(45) and cervical cancer cell lines as assessed using the MTT assay(45,46). The cyclooxygenase-isoform 2 (COX-2) pro-oxidant activity(42). It has been suggested that this prooxidant effect is related to the presence of metal ions in the body (for example due to tissue injury releasing Fe and Cu) and is of relevance for the bioactivity of phenolic compounds in vivo(43). It is clear from a small number of in vivo studies that the hydroxycinnamic acids have antioxidant properties. Such studies are essential to understand the biological role of these phenolic acids(44).

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Table 4. DNA damage in U937 cells following 24 h incubation with 0.5% (v/v) pale (P1–P4) or black (B1–B4) brewers’ spent grain phenolic extracts or 0.1 µg/ml ferulic acid (adapted from McCarthy et al. (69))

<table>
<thead>
<tr>
<th>Sample</th>
<th>DNA damage (% tail DNA) oxidative – H2O2</th>
<th>DNA damage (% tail DNA) oxidant – SIN-1</th>
<th>DNA damage (% tail DNA) oxidant – 4-NQO</th>
<th>DNA damage (% tail DNA) oxidant – t-BOOH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SE</td>
<td>Mean</td>
<td>SE</td>
</tr>
<tr>
<td>Control</td>
<td>3.2†</td>
<td>0.5</td>
<td>4.3†</td>
<td>0.4</td>
</tr>
<tr>
<td>Oxidant control</td>
<td>40.3†</td>
<td>0.3</td>
<td>40.0†</td>
<td>3.9</td>
</tr>
<tr>
<td>P1</td>
<td>30.3†</td>
<td>7.3</td>
<td>32.8†</td>
<td>4.9</td>
</tr>
<tr>
<td>P2</td>
<td>29.0†</td>
<td>6.0</td>
<td>22.7†</td>
<td>1.9</td>
</tr>
<tr>
<td>P3</td>
<td>39.9†</td>
<td>3.9</td>
<td>33.8†</td>
<td>2.9</td>
</tr>
<tr>
<td>P4</td>
<td>23.1†</td>
<td>3.2</td>
<td>32.2†</td>
<td>6.1</td>
</tr>
<tr>
<td>B1</td>
<td>25.8†</td>
<td>7.5</td>
<td>33.6†</td>
<td>2.9</td>
</tr>
<tr>
<td>B2</td>
<td>9.6†</td>
<td>1.1</td>
<td>6.9†</td>
<td>1.0</td>
</tr>
<tr>
<td>B3</td>
<td>16.0†</td>
<td>2.0</td>
<td>18.1†</td>
<td>0.3</td>
</tr>
<tr>
<td>B4</td>
<td>14.0†</td>
<td>3.8</td>
<td>24.1†</td>
<td>2.2</td>
</tr>
<tr>
<td>Ferulic acid</td>
<td>17.1†</td>
<td>0.8</td>
<td>30.8†</td>
<td>3.0</td>
</tr>
</tbody>
</table>

SI-N-1, 3-morpholinosydnonimine hydrochloride; 4-NQO, 4-nitroquinoline oxide; t-BOOH, tert-butylhydroperoxide.

†Denotes a significant difference in DNA damage (P<0.05), relative to oxidant control. Statistical analysis by ANOVA followed by Dunnett’s test.

Our research group is the first, to our knowledge, to look specifically at phenolic extracts from BSG. Four extracts from pale BSG (P1, P2, P3 and P4) and four extracts from black BSG (where the grain is roasted to 200°C before brewing; B1, B2, B3 and B4) were analysed. Each extract results from a different step in the extraction process and hence contains different phenolic acid compositions. Extract 1 (P1, B1) contains free phenolics, extract 2 (P2, B2) contains bound phenolics, extract 3 (P3, B3) contains the remainder of bound phenolics and extract 4 (P4, B4) contains phenolics extracted with 110 mM NaOH (69). The ability of the phenolic extracts to protect against oxidant-induced DNA damage was determined using the comet assay. In the U937 cell line, oxidative DNA damage was induced by a range of oxidants; H2O2, 3-morpholinosydnonimine hydrochloride, 4-nitroquinoline oxide and tert-butylhydroperoxide. Ferulic acid and the black BSG extracts significantly reduced the DNA damage induced by H2O2, while P2, B2, B3 and B4 significantly reduced the percent tail DNA induced by 3-morpholinosydnonimine hydrochloride (69). The four oxidants used have different mechanisms of action; damage induced by both H2O2 and 3-morpholinosydnonimine hydrochloride involve the Fenton reaction which is an Fe-dependent reaction, 4-nitroquinoline oxide mimics the action of UV and Cu2+ plays an essential role, whereas Fe does not (70). tert-Butylhydroperoxide causes lipid peroxidation and acts in a Ca2+-dependent manner and Fe plays less of a role than in H2O2-induced damage (71). Therefore, BSG phenolic extracts may provide protection against oxidant-induced DNA damage by Fe chelation (69).

In summary, there is increasing evidence to suggest that phenolic acids, including those found at highest concentrations in BSG, can confer potential health benefits including anti-inflammatory, antioxidant, anti-carcinogenic and anti-atherogenic effects. Recent data suggest that BSG has antioxidant potential and therefore further research on the phenolic compounds extracted from BSG is warranted.

Incorporation of brewers’ spent grain into feed/foodstuffs

Animal food

As previously mentioned, BSG contains approximately 20% and 70% protein and fibre, respectively, and it is due to this favourable chemical composition that it has great potential for use as a raw material/food ingredient (4). BSG is an ingredient of significant importance for ruminants.
When administered with low-cost N sources such as urea, BSG can supply all the essential amino acids to the ruminants. The effect of BSG on milk yield and composition and the blood components of dairy cattle has also been studied\(^{(72)}\). The cattle received a diet consisting of ground maize, maize silage, soya bean meal and wheat bran, with the latter three being substituted with wet brewer’s grain in the treatment group. The study showed the treatment group had an increased milk yield, milkfat and milk total solids content. Blood components such as glucose, cholesterol, Na and TAG were not affected. While the main outlet for BSG is currently as a feedstuff for dairy cattle, research has also been conducted looking at the benefits of BSG for use as a feed for poultry and fish. The effect of replacing rice bran in a fish diet with 10–40% brewery waste grains has been investigated\(^{(73)}\). The brewery waste used contained 19% crude protein, 18–20% crude fibre and had a good amino acid profile. It was found that carp (oily freshwater fish) had better growth performance on diets containing brewery waste than the control group. The authors attributed this enhanced growth performance to the high-quality protein contained in the waste grains. A more recent study showed that biodegraded BSG contained cysteine, lysine and methionine in addition to fourteen other amino acids\(^{(74)}\). Depending on the microbe used to degrade the BSG, different amino acid concentrations were found, with alanine consistently at highest concentrations. This composition was noted to be of particular importance for poultry as cysteine, lysine and methionine are the main amino acids required in poultry nutrition. For convenience, the possibility of producing dry BSG cakes suitable for long-term storage was examined using membrane filter press technology\(^{(75)}\). These dry cakes could be used as an animal feed at any time, or as a starting material for the production of other products using BSG.

In summary, evidence suggests that whole BSG, fed as part of a total mixed ration, has many nutritional benefits for a range of animals, particularly dairy cattle. This has resulted in the routine use of BSG as an animal feed for cattle.

**Human food**

In addition to its use as an animal feed, BSG has been incorporated into foodstuffs for human consumption. Given its low cost and high nutritional value, BSG makes an ideal ingredient for human foods such as biscuits and ready-to-eat snacks, particularly where there is a need to increase fibre content. In 1978, the possibility of preparing cookies with flour containing BSG at levels ranging from 5 to 60% was examined\(^{(8)}\). At 40% BSG addition, the physical qualities of the cookies were sustained. This supplementation level gave a 74% increase in N and increased crude fibre ten-fold. These results were supported by work published in 2002, where the authors looked at the effect of BSG (at levels of 5–25%) on the fibre content and quality of cookies\(^{(76)}\). As the level of addition of BSG increased, there was a significant increase in dietary fibre content. Another documented foodstuff suitable for the inclusion of BSG is ready-to-eat snacks\(^{(4)}\). BSG was added to the formulation mix (consisting of ingredients such as corn starch and wheat flour) at levels ranging from 10 to 30%. The incorporation was successful, increasing dietary fibre and crude protein levels. Similarly, the addition of BSG into extruded snack food has been studied\(^{(70)}\). The maize flour of chickpea snacks was replaced with BSG at levels of 10, 20, 25 and 30%. The parameters measured included the effect of BSG supplementation on texture, colour, moisture, fat, fibre, starch, protein, phenolic compounds and antioxidant capacity. With increasing levels of BSG addition, the percent protein, fat and fibre content increased, while starch decreased. It was suggested that foods fortified with BSG be considered as functional foods. In a further study by Stojceska and Ainsworth\(^{(77)}\), BSG was incorporated at different levels (0–30%) into wheat flour breads treated with four different enzymes and the bread quality was then evaluated\(^{(77)}\). Similar to the previous study, it was found that the fibre content of the breads was significantly increased by BSG addition. The change in fat content was significantly linked to the addition of BSG. When addition of BSG is combined with the appropriate use of enzymes, the shelf life, texture and loaf volume can also be improved. Initially, it was thought that BSG was too granular for direct addition to food and that it would have to first be converted to flour before use. However, a study in 2009 demonstrated that BSG of various particle sizes could be effectively used in the production of frankfurters\(^{(78)}\). The control frankfurter had the highest score for acceptability, but the other products also had high scores, with the score decreasing with increase in particle size and reduction in fat levels. The authors suggested that BSG be used to produce low-fat high-fibre meat products. In addition to particle size, there are a number of points for consideration with the incorporation of BSG into foodstuffs. Firstly, there are concerns about appearance. When moist, BSG is brown in colour, thus it can only be effectively integrated into off-white products. Such foods include cookies and cakes. More importantly, it is imperative that the organoleptic properties of the foodstuff remain acceptable to consumers and are similar to the current commercially available products. The study by Prentice et al.\(^{(8)}\), demonstrated that BSG addition at a level of 15% was the upper limit for organoleptic acceptability\(^{(7)}\). At this level, the organoleptic quality was lowered but still remained acceptable to consumer panels. Similarly, Stojceska et al.\(^{(4)}\) found that there was a limit to acceptability\(^{(7)}\). At a level of addition of 30%, physicochemical characteristics (such as texture, colour and hardness) remained acceptable. However, the authors concluded that addition of BSG at 20% level was optimal to maintain properties of commercially available snack foods. Where the protein hydrolysates are to be extracted and incorporated into foodstuffs, there is concern over the bitter taste of some peptides, due to the hydrophobic amino acid content\(^{(79)}\).

**Conclusion**

The literature shows that phenolic compounds including ferulic, caffeic and p-coumaric acid can have antioxidant, anti-cancer, anti-atherogenic and anti-inflammatory effects. Given that these phenolic acids are some of the major phenolics in BSG, it is expected that phenolic extracts
from spent grain may also exhibit similar properties and have the potential to be developed for a range of bioactivities. BSG currently functions as an animal feed, having many nutritional benefits. While some attempts have been made to incorporate the bioactive components of BSG into foodstuffs, further research in this area is needed. Given the potential bioactive nature of the phenolic extracts from BSG, and the large amounts of BSG produced annually as a low value co-product, it is imperative that an alternative use be explored.

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