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Review

Brewers' spent grain: generation, characteristics and potential applications

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Abstract

Brewers' spent grain (BSG) is the major by-product of the brewing industry, representing around 85% of the total by-products generated. BSG is a lignocellulosic material containing about 17% cellulose, 28% non-cellulosic polysaccharides, chiefly arabinoxylans, and 28% lignin. BSG is available in large quantities throughout the year, but its main application has been limited to animal feeding. Nevertheless, due to its high content of protein and fibre (around 20 and 70% dry basis, respectively), it can also serve as an attractive adjunct in human nutrition. Recently, attempts have been made to use BSG in biotechnological processes, such as in cultivation of mushrooms and actinobacteria, as a source of value-added products, such as, ferulic and *p*-coumaric acids, xylose, arabinose, or as raw material for xylitol and arabitol production. The main characteristics and potential applications of BSG are reviewed focussing on these alternative uses of this agro-industrial by-product as a raw material in foods, in energy production and in biotechnological processes.

Keywords: Brewers' spent grain; Chemical composition; Animal and human nutrition; Biotechnological processes

1. Introduction

Nowadays, there is great political and social pressure to reduce the pollution arising from industrial activities. Almost all developed and underdeveloped countries are trying to adapt to this reality by modifying their processes so that their residues can be recycled. Consequently, most large companies no longer consider residues as waste, but as a raw material for other processes.

The brewing industry generates relatively large amounts of by-products and wastes; spent grain, spent hops and yeast being the most common. However, as most of these are agricultural products, they can be readily recycled and reused. Thus, compared to other industries, the brewing industry tends to be more environmentally friendly (Ishiwaki et al., 2000).

Spent grain is the most abundant brewing by-product, corresponding to around 85% of total by-products generated (Reinold, 1997). According to Townsley (1979), spent grain accounts, on average, for 31% of the original malt weight, representing approximately 20 kg per 100 l of beer produced (Reinold, 1997). Brewers' spent grain (BSG) is available at low or no cost throughout the year, and is produced in large quantities not only by large, but also small breweries.

For example Brazil, the world's fourth largest beer producer, ~8.5 billion litres/year, exceeded only by the United States of America (23 billion), China (18 billion) and Germany (10.5 billion) (Berto, 2003), in 2002 generated around 1.7 million tonnes of spent grain.

2. Generation of brewers' spent grain

Barley is the world's most important cereal after wheat, maize and rice, and is used mainly as an animal feed or as a raw material to produce beer (Kendal, 1994). Barley grain is rich in starch and proteins and consists of three main parts: the germ (embryo), the endosperm (comprising the aleurone and starchy endosperm) and the grain coverings. The last

Abbreviations: BSG, Brewers' spent grain; GBF, germinated barley foodstuff; VOC, volatile organic compounds.

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may be divided into three fractions: the seed coat, the innermost layers surrounding the aleurone; overlying the seed coat are the pericarp layers, which, in turn, are covered by the husk (Fig. 1) (Kunze, 1996). Because the pericarp is waxy and somewhat waterproof, and the seed coat acts as a semi-permeable membrane, the pericarp–seed coat interface effectively defines the exterior and the interior of the kernel. The husk provides external protection to the grain, and is a multilayered, dead tissue composed mainly of lignocellulosic cell walls, but also contains small quantities of proteins, resins and tannins (Lewis and Young, 1995; Venturini Filho and Cereda, 2001).

In preparation for brewing, harvested barley is cleaned and graded according to size. Plump and medium grades, retained on sieves with 2.5, 2.4, 2.2 and 2.0 mm apertures, are malted separately. After a dormancy period of 4–6 weeks, barley is malted in a controlled germination process, which serves to increase the enzymatic content of the grain. Malting is performed in three steps: (1) steeping, (2) germination and (3) drying or kilning.

During steeping, cleaned barley grains are placed in tanks with water at between 5 and 18 °C for approximately 2 days. Water enters the embryo through the micropyle and eventually the moisture content of the grain reaches between 42 and 48%. The steeping water is changed every 6–8 h, and is not recycled. Hydration during steeping initiates germination and leads to activation of aleurone metabolism. After steeping, the barley is conveyed to a germination vessel, where it is turned by screw turners, and maintained in contact with a humid air stream flowing through the grain bed maintaining the temperature between 15 and 21 °C. The germination step promotes the synthesis and activation of enzymes in the aleurone and starchy endosperm, including amylases, proteases, β-glucanases and others. The action of these enzymes modifies the structure of the starchy endosperm. At the end of the germination process, which normally lasts 6 or 7 days, the endosperm is fully and evenly modified. The malted barley is dried (kilned) at 40–60 °C to a 4–5% moisture content, to avoid microbial contamination and to generate flavour components. After this step, the dried malt is stored for 3 or 4 weeks, to reach homogeneity and equilibrium (Kendal, 1994; Tschope, 2001; Venturini Filho and Cereda, 2001).

In the brewery, malted barley is milled, mixed with water in the mash tun and the temperature slowly increased (from 37 to 78 °C) to promote enzymatic hydrolysis of malt constituents, primarily starch, but also other components such as proteins, (1→3, 1→4)-β-glucans and arabinoxylans, and to solublise their breakdown products. During this process, starch is converted to fermentable sugars (mainly maltose, and maltotriose) and non-fermentable sugars (dextrins), and proteins are partially degraded to polypeptides and amino acids. This enzymatic conversion stage (mashing) produces a sweet liquid known as wort. The insoluble, undegraded part the malted barley grain is allowed to settle to form a bed in the mash tun and the sweet wort filtered through it (lautering). The filtered wort is used as the fermentation medium to produce beer (Dragone et al., 2002; Linko et al., 1998). The residual solid fraction is known as 'brewers' spent grain' (BSG). Fig. 2 is a schematic representation of the process resulting in the production of brewers' spent grain from barley grain.

According to Townsley (1979), the brewing process is selective, removing only those nutrients from the malt necessary to produce the wort, leaving washed, water insoluble proteins and the cell wall residues of the husk, pericarp and seed coat within the spent grain. Depending on the type of beer to be produced, BSG may consist of the residues from malted barley, or those from malted barley and adjuncts (non-malt sources of fermentable sugars), such as wheat, rice or maize added during mashing (Reinold, 1997).

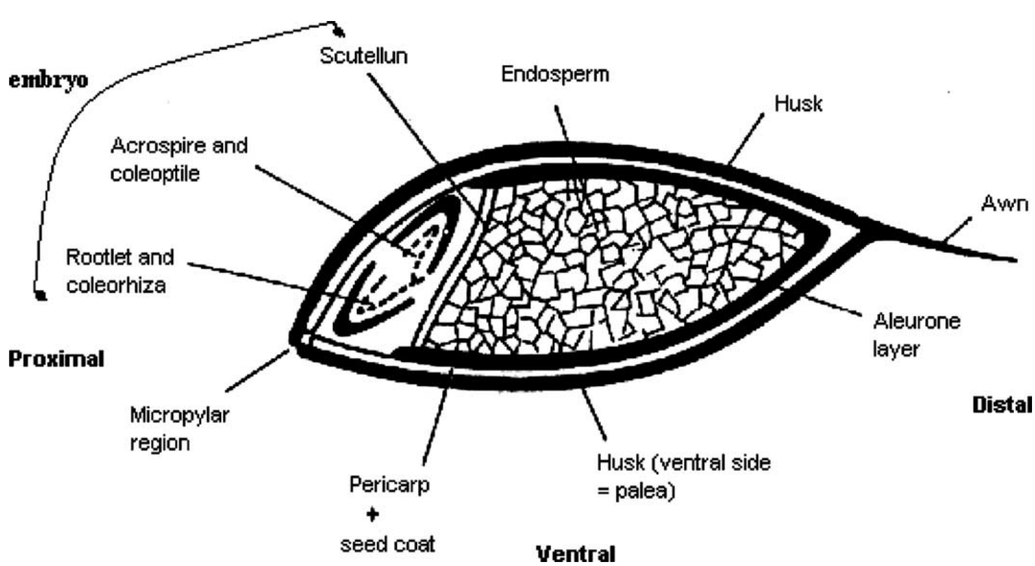

Fig. 1. Schematic representation of a barley kernel in longitudinal section (adapted from Lewis and Young, 1995).

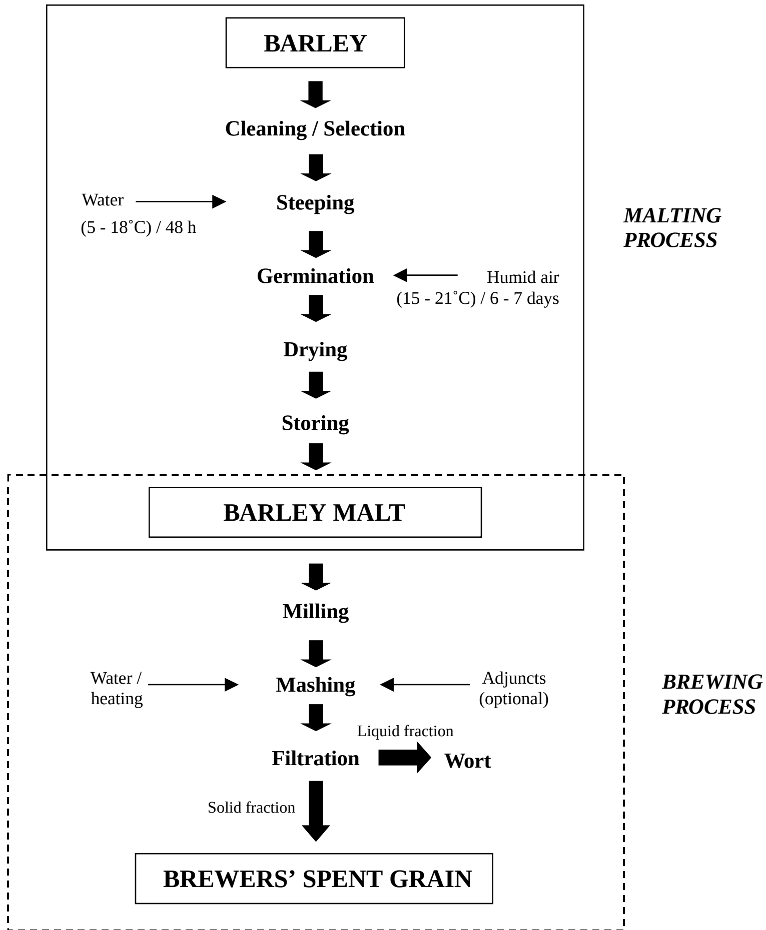

Fig. 2. Schematic representation of the process to obtain BSG from natural barley.

3. Characteristics of brewers' spent grain

3.1. Chemical composition and physicochemical properties

BSG basically consists of the husk–pericarp–seed coat layers that covered the original barley grain. Depending on the evenness of malting more or less starchy endosperm and walls of empty aleurone cells may also remain. The starch content will be negligible, and some residues of hops introduced during mashing will be present depending on the brewing regime used. Thus, the major components of BSG will be the walls of the husk–pericarp–seed coat, which are rich in cellulose and non-cellulosic polysaccharides and lignin, and may contain some protein and lipid. The husk also contains considerable amounts of silica and much of the polyphenolic components of the barley grain (Macleod, 1979). According to Kunze (1996), 25% of the minerals present in barley are present as silicates. Fig. 3 shows the appearance of a BSG particle by scanning electron microscopy. The bright points in the external portion of the husk are silicates.

The chemical composition of BSG varies according to barley variety, harvest time, malting and mashing conditions, and the quality and type of adjuncts added in the brewing process (Huige, 1994; Santos et al., 2003); but in general, BSG is considered as a lignocellulosic material rich in protein and fibre, which account for around 20 and 70% of its composition, respectively. Microscopic examination shows the presence of numerous fibrous tissues from the surface layers the original barley grain (Fig. 3). The main components of these fibrous tissues are arabinoxylan, lignin (a polyphenolic macromolecule) and cellulose (a linear homopolymer of glucose units). Analyses of BSG are shown in Table 1. Santos et al. (2003) found, besides fibre, 24.2% protein, 3.9% lipid and 3.4% ash in oven-dried BSG. Table 2 shows the protein, apparent starch and non-starch polysaccharide composition, and monosaccharide analyses of the non-starch polysaccharide fraction of BSG from pilot scale trials with a malting barley and a feed barley. Protein and fibre are highly concentrated in spent grain because most of the barley starch is removed during mashing (Kissel and Prentice, 1979).

Minerals, vitamins and amino acids are also found in BSG. The mineral elements include calcium, cobalt, copper, iron, magnesium, manganese, phosphorus, potassium, selenium, sodium and sulphur, all in concentrations lower than 0.5% (Huige, 1994; Pomeranz and Dikeman, 1976). The vitamins include (ppm): biotin (0.1), choline (1800),

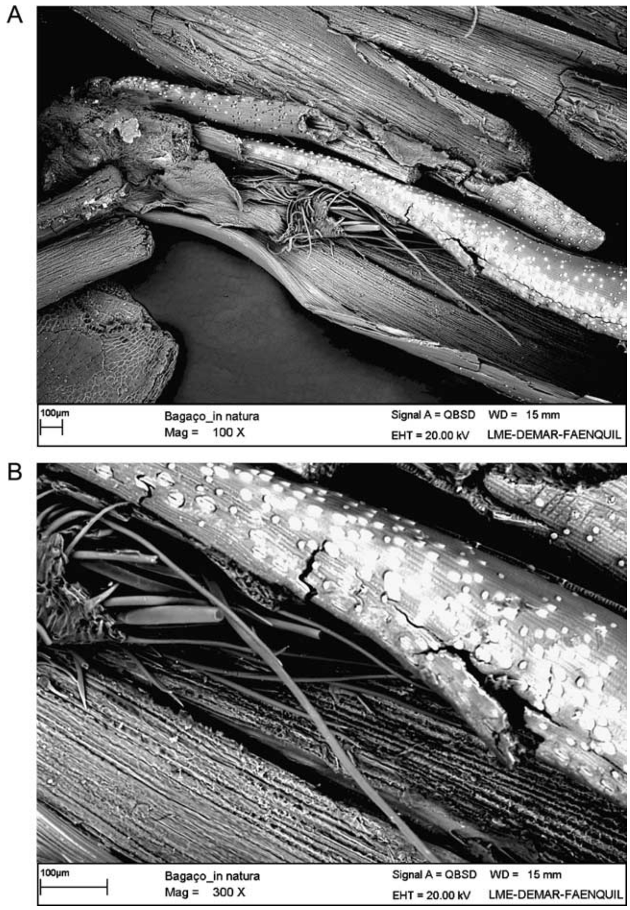

Fig. 3. Scanning electron microscopy of BSG particles. (A) Magnification 100 fold; (B) magnification 300 fold.

folic acid (0.2), niacin (44), pantothenic acid (8.5), riboflavin (1.5), thiamine (0.7) and pyridoxine (0.7); protein bound amino acids include leucine, valine, alanine, serine, glycine, glutamic acid and aspartic acid in the largest amounts, and tyrosine, proline, threonine, arginine, and lysine in smaller amounts. Cystine, histidine, isoleucine, methionine, phenylalanine, and tryptophan can also be present (Huige, 1994; Mariani, 1953).

Table 1
Chemical composition of brewers' spent grain (BSG) and germinated barley foodstuff (GBF)

Component (% dry wt)	BSG[a]	BSG[b]	GBF[a]	GBF[c]
Cellulose	25.4	16.8	8.9	9.1
Arabinoxylan	21.8	28.4	17.0	19.2
Lignin	11.9	27.8	8.2	6.7
Protein	24.0	15.2	46.0	48.0
Lipid	10.6	Nd	10.2	9.2
Ash	2.4	4.6	2.0	2.0

[a] From Kanauchi et al. (2001).
[b] From Mussatto and Roberto (2005).
[c] From Fukuda et al. (2002). nd, no determined.

Hernández et al. (1999) reported that a sample of BSG as received from a Cuban microbrewery had an average density of 0.45 kg/l, a water content of 3.1 g/g dry basis and a triglyceride absorption value of 1.6 g/g dry basis. The net and gross calorific values of BSG (Russ et al., 2005) were 18.64 MJ/kg and 20.14 MJ/kg of dry mass, respectively.

3.2. Spoilage and techniques for BSG preservation

Wet BSG from a lauter tun contains 77–81% (w/w) water (Huige, 1994; Russ et al., 2005). Due to its high moisture and fermentable sugar contents, BSG is a very unstable material and is liable to deteriorate rapidly due to microbial activity. After storage in gunnysacks for 30 days, Sodhi et al. (1985) found eight isolates of *Aspergillus*, *Fusarium*, *Mucor*, *Penicillium*, and *Rhizopus*. Several methods have been proposed to prolong BSG storage time. Lactic, formic, acetic or benzoic acid–water–BSG mixtures packed in plastic containers and held for three summer months, all effectively preserved BSG quality and nutritional value. Of the acids, benzoic and formic acids were especially effective (Al-Hadithi et al., 1985). Potassium sorbate is also effective in preserving pressed BSG (Kuntzel and Sonnenberg, 1997).

Drying is a possible alternative for BSG preservation with the advantage that it also reduces the product volume, and therefore, decreases transport and storage costs (Santos et al., 2003). The traditional process for drying BSG is based on the use of direct rotary-drum driers, a procedure considered to be very energy-intensive. Three methods for preserving spent grain: freeze-drying, oven drying and freezing were evaluated by Bartolomé et al. (2002). Freezing is inappropriate because large volumes must be stored and alterations in the arabinose content may occur. Preservation by oven drying or freeze-drying reduces the volume of the product and does not alter its composition. However, freeze-drying is economically unacceptable. Oven drying of BSG must be conducted at temperatures below 60 °C, because at higher temperatures unpleasant flavours are generated (Hernández et al., 1999; Prentice and D'Appolonia, 1977). In oven drying, there is risk that the grain temperature near the dryer exit may rise leading to toasting or burning of the dried grains. In addition, smoke emerging from dryer stacks causes odour pollution problems (Huige, 1994). An alternative drying method which could save energy is to use superheated steam (Tang et al., 2004, 2005) with claimed additional advantages including reduction in environmental impact, improved drying efficiency, elimination of fire or explosion risk, and enhanced recovery of valuable organic compounds.

In a pilot plant study, El-Shafey et al. (2004) used a membrane filter press to achieve high drying levels of BSG cake. In the process, BSG was mixed with water and filtered at a feed pressure of 3–5 bar, washed with hot water (65 °C), membrane-filtered, and vacuum-dried, to reach moisture levels of between 20 and 30%. No bacterial activity was observed after storing the cake in the open air for 6 months.

Table 2
Composition of spent grain from two barley varieties, Triumpf and Golf (from Vietor et al., 1993)

	Triumpf			Golf		
	Top	Middle	Bottom	Top	Middle	Bottom
Protein	24[a]	16	16	27	24	20
Apparent starch[b]	26[a]	13	14	5	6	7
Non-starch polysaccharides	24[a] (0.67)[c]	38 (0.74)	38 (0.89)	37 (0.61)	44 (0.60)	47 (1.38)
Ara/Xyl	0.71	0.47	0.48	0.48	0.43	0.42
Arabinose	25[d]	22	22	21	19	19
Xylose	35	47	46	44	44	45
Mannose	3	1	1	2	1	1
Galactose	5	2	3	3	2	2
Glucose	34 (3)[e]	27 (2)	29 (2)	31 (2)	34 (1)	34 (3)

[a] Values are expressed in % w/w on dry matter.
[b] Apparent starch = glucose, maltodextrins and residual starch fragments.
[c] Values in parentheses are (1→3,1→4)-β-glucan content in % (w/w on dry matter).
[d] mol % of non-starch polysaccharides.
[e] Values in parentheses are (1→3,1→4)-β-glucan as a fraction of total monosaccharides (in mol%).

4. Potential applications for brewers' spent grain

Although BSG is the main by-product of the brewing process, it has received little attention as a marketable commodity, and its disposal is often an environmental problem. Nevertheless, due to its chemical composition (Table 1), it can be of value as a raw material. Some possible applications for this agro-industrial by-product are described below.

4.1. Food ingredient

4.1.1. Animal nutrition

Until now, the main application of BSG has been as an animal feed (mainly for cattle), due to its high content of protein and fibre. As an animal feed, BSG can be employed either as a wet residue, shortly after separation from the wort at lautering, or as a dried material (Öztürk et al., 2002; Townsley, 1979). According to Huige (1994) BSG is an excellent feed ingredient for ruminants since it can be combined with inexpensive nitrogen sources, such as urea, to provide all the essential amino acids. In addition to its high nutritional value, BSG is reported to promote increased milk production without affecting animal fertility (Belibasakis and Tsirgogianni, 1996; Reinold, 1997; Sawadogo et al., 1989). When BSG was incorporated into the diet of cows, milk yield, milk total solid content and milk fat yield were increased. On the other hand, blood plasma concentrations of glucose, total protein, albumin, urea, triglycerides, cholesterol, phospholipids, sodium, potassium, calcium, phosphorus and magnesium were not affected (Belibasakis and Tsirgogianni, 1996).

Currently, the primary market for BSG is dairy cattle feed, but as the BSG provides protein, fibre and energy, its consumption has also been investigated for a range of animals, including poultry, pigs and fish (Table 3). Kaur and Saxena (2004) evaluated BSG as a replacement for rice bran in a fish diet, and observed that fish fed with a diet containing rice bran and 30% spent grain had a superior body weight gain when compared with fish fed with rice bran only. According to these authors, the better growth performance was due to the increased content of proteins and essential amino acids provided by the spent grain.

4.1.2. Human nutrition

Due to its relatively low cost and high nutritive value, BSG has been evaluated for the manufacture of flakes, whole-wheat bread, biscuits and aperitif snacks. However, BSG is too granular for direct addition in food and must first be converted to flour (Hassona, 1993; Miranda et al., 1994a,b; Öztürk et al., 2002).

Table 3
Range of animals investigated for Brewers' spent grain consumption

Animals	Reference
Rats	Hassona, 1993; Kanauchi and Agata, 1997; McIntosh et al., 1993, 1996
Hamsters	Zhang et al., 1990, 1992
Chicken	Gondwe et al., 1999; Kratzer and Earl, 1980; McIntosh et al., 1995; Oh et al., 1991
Cows	Batajoo and Shaver, 1994; Belibasakis and Tsirgogianni, 1996; Chiou et al., 1998; Cozzi and Polan, 1994; Dhiman et al., 2003; Firkins et al., 2002; Gallo et al., 2001; Karikari et al., 1995; Sawadogo et al., 1989; West et al., 1994
Fish	Kaur and Saxena, 2004; Muzinic et al., 2004; Shimeno et al., 1994; Yamamoto et al., 1994
Pigs	Dung et al., 2002; Yaakugh et al., 1994

A high protein flour prepared from BSG was successfully incorporated into a number of bakery products, including breads, muffins, cookies, mixed grain cereals, fruit and vegetable loaves, cakes, waffles, pancakes, tortillas, snacks, doughnuts and brownies (Huige, 1994; Townsley, 1979). Nevertheless, there are some limitations in the use of the flour as a protein additive or as a partial replacement for presently used flours, due to its colour and flavour. BSG is brownish in colour when moist and thus can only be used in off-white products, such as light coloured cookies, cakes, bread, or spaghetti that are made entirely from wholemeal flour. Moreover, because of alterations in the flavour and physical properties (e.g. texture) of the final products, only relatively small quantities (5–10%) can be incorporated (Hassona, 1993; Miranda et al., 1994a,b; Townsley, 1979).

Prentice and D'Appolonia (1977) made high-fibre bread containing BSG and evaluated its consumer acceptance. BSG was finely milled and heat treated at 45, 100 or 150 °C, and replaced white flour in a conventional bread formula at 5, 10 and 15% levels. Bread containing heat-treated (45 °C) BSG, at 5 and 10% flour replacement levels, was accepted favourably. Breads with BSG treated at 100 or 150 °C were not acceptable due to undesirable flavours caused by heating to these.

BSG addition improves the nutritional value of breads (Hassona, 1993). The addition of 10% spent grain increased the protein and essential amino acid content by 50 and 10%, respectively, and doubled the fibre content compared with traditional breads without BSG. In addition, the breads had about 7% less calories than traditional breads. The caloric density of BSG is about half that of most cereals (Huige, 1994). Some properties of BSG flour in foods are shown in Table 4.

The ingestion of BSG, or derived products, provides benefits for health, which are associated with increased fecal weight, accelerated transit time, increased cholesterol and fat excretion and decrease in gallstones (Fastnaught, 2001). The addition of spent grain to rat diets is beneficial to intestinal digestion, alleviating both constipation and diarrhoea. Such effects were attributed to the content of glutamine-rich protein, and to the high content of non-cellulosic polysaccharides (arabinoxylan, 20–47%) and smaller amounts (less than 1%) of (1 → 3, 1 → 4)-β-glucans (McCleary and Nurthen, 1986; Vietor et al., 1993, Table 2). Incorporation of spent grain in rat diets prevented an increase in plasma total lipid as well as of cholesterol (Hassona, 1993; Ishiwaki et al., 2000). It was suggested that such an effect might be due to the increased stool bulk, which leads to decreased lipid absorption or increased bile acids, fat and sterol excretion. Soluble dietary fibre, which includes (1 → 3, 1 → 4)-β-glucan, appears to be important in lowering plasma cholesterol and postprandial serum glucose levels (reviewed by Brennan and Cleary, 2005). However, large quantities of barley-derived-(1 → 3, 1 → 4)-β-glucan are required ($>$10 g/d) for clinically significant effects (Keogh et al., 2003) and brewers' spent grain is specifically depleted in this polysaccharide.

Table 4
Properties of BSG flour in foods (Huige, 1994)

1. Ease of blending
2. Calorie content is about half that of most cereal flours
3. High water absorption capacity
4. Provides valuable minerals such as Ca, P, Fe, Cu, Zn and Mg
5. Low-fat absorption (beneficial for batters and coating)
6. Uniform tan colour, bland flavour, and mildly roasted aroma
7. High fibre content
8. High protein content

BSG has been converted to a newprotein-rich fibrous foodstuff by separating the husk fraction by milling and sieving. The product, germinated barley foodstuff (GBF), contains the aleurone layer, scutellum, and germ fractions of germinated barley, and is composed mainly of non-cellulosic polysaccharides and glutamine-rich protein and is low in lignin (Kanauchi and Agata, 1997). GBF feeding is considered a potentially new attractive pre-biotic treatment in patients with ulcerative colitis, since when administered to patients with mild to moderate ulcerative colitis it gave significant improvement in their clinical condition and endoscopic score, which was associated with an increase in stool butyrate concentration (Bamba et al., 2002; Kanauchi et al., 2001). GBF is considered to be a mild anti-inflammatory through increases in colonic butyrate concentration and the number of *Bifidobacterium* sp. and butyrate producing *Eubacterium limosum* (Kanauchi et al., 1999). The bacterial butyrate production from GBF improved the intestinal mucosal function, resulting in mitigation of colitis. Butyrate serves as the primary energy source for the colonic mucosa, and its administration improves the proliferation of colonic epithelial cells. Furthermore, GBF has a high water-holding capacity compared with other water insoluble dietary fibre sources and this feature might contribute to a conspicuously high stool-forming ability in the colon (improvement of bowel movement) (Bamba et al., 2002). GBF also appears to be safe and well tolerated.

On the whole, BSG is a cheap source of protein and fibre that may provide a number of benefits when incorporated in human diets. For this reason, it is a potentially important food ingredient, especially in developing countries where poor malnutrition exists.

4.2. Energy production

Another proposed use for BSG is in energy production, either through direct combustion or by fermentation to produce biogas (a mixture of 60–70% methane, carbon dioxide and small proportions of hydrogen, nitrogen and carbon monoxide) (Ezeonu and Okaka, 1996; Okamoto et al., 1999; Reinold, 1997).

The combustion process needs pre-drainage of the spent grain to ≤55% moisture, and problems arise from NOx and dust particle emissions (Meyer-Pittroff, 1988). The toxic gases emitted during combustion of dried BSG contain nitrogen and SO_2 at approximately 1000–3000 and 480 mg/m^3, respectively (Keller-Reinspach, 1989).

An alternative possibility for energy production from BSG is by anaerobic fermentation, which is efficient only if it is divided into a hydrolytic and a methanogenic step. The hydrolysis of the fibre material in BSG is the limiting step for complete degradation of the material. Nevertheless, there are several different pre-treatment possibilities for enhancing the rate of fermentation, including chemical–thermal treatment with 0.2 M NaOH at 70 °C, crushing by wet rotor grinding, or ball milling, as well as enzymatic treatment with cellulase-producing fungi, with BSG as the sole substrate (Rieker et al., 1992). In the methanogenic step, acidogenic microorganisms convert complex macromolecules to the volatile fatty acids, acetate, butyrate and propionate, and subsequently, methanogenic bacteria convert these volatile acids to methane (Ezeonu and Okaka, 1996).

Behmel et al. (1993) optimised hydrolytic pre-treatment of BSG to improve the economic feasibility of biogas production. The hydrolysis was assisted by an alkaline treatment and 86% conversion of organic dry matter was obtained during a total reactor residence time of eight days. Ezeonu and Okaka (1996) evaluated the process kinetics and digestion efficiency of biogas anaerobic batch fermentation of BSG, and obtained a total yield of 3476 cm^3/100 g of spent grain, after fifteen days digestion. The operational efficiency of the digester was 26%. Other studies by Okamoto et al. (1999) showed that the conversion of BSG to gas for use as potential alternative energy source has few adverse environmental effects.

Combustion and biogas energy production from BSG has been evaluated for reuse in the brewery. Petricek and Fort (1998) concluded that biogas technology was especially suitable for obtaining thermal energy in breweries. Zanker and Kepplinger (2002) evaluated the combustion of spent grains in a brewery-integrated system. BSG was concentrated by pressure and the wastewater recycled after purification. The concentrated BSG was combusted and the heat generated partly supported the energy demand of the plant.

Considering the energy crises that the world experiences, energy production from BSG provides an interesting alternative use of this industrial by-product.

4.3. Charcoal production

Recently, Okamoto et al. (2002) developed a process for producing charcoal bricks from BSG, and evaluated their physical and chemical properties. In the process, BSG (67% water content) was dried, pressed and carbonised in a low oxygen atmosphere. The charcoal bricks, so produced, contained various minerals such as calcium, magnesium and phosphorus, and had a high calorific value (27 MJ/kg), which compared favourably with the calorific value of charcoals produced from other raw materials (Table 5). Sato et al. (2001) also produced charcoal from BSG and evaluated the physical properties (moisture and ash contents, volatile matter, fixed carbon and specific surface area) and the burning properties of the charcoals. According to these authors, the BSG charcoal contains 81% fixed carbon and 12% ash (which is composed of 47% P, 22% Ca, 14% Mg, 13% Si, among others). The thermal analysis showed that the BSG charcoal is inferior to sawdust charcoal in burning properties: ignition temperature and burning period, because the ignition temperature is higher and the burning period is longer.

4.4. As a brick component

The low ash content of BSG and the high amount of fibrous material (cellulose, non-cellulosic polysaccharides and lignin) make it suitable for use in building materials (Russ et al., 2005). When BSG was used to increase the porosity of bricks, it also improved their dry characteristics, but did not influence colour, or compromise brick quality, and did not require any alteration in production operations. Thus, BSG could substitute for sawdust, which is commonly used in brick-making to increase porosity.

4.5. Paper manufacture

The fibrous nature of BSG has led to its investigation as a raw material for paper production (Ishiwaki et al., 2000). BSG was used to prepare paper towels, business cards and coasters and was reported to confer a high-grade texture on the products.

4.6. Adsorbent

Adsorption processes must be fast, efficient, and use cheap adsorbents to compete with other techniques. Due to its low cost and easy availability, BSG has been tested as an adsorbent for several types of compounds. Pyrolysed spent grain was used as an adsorbent for removing volatile organic compounds (VOCs) from waste gases (Chiang et al., 1992). The adsorption capacity of VOCs on pyrolysed BSG was similar to that of coconut-shell charcoal. BSG adsorbs cadmium and lead from aqueous solutions (Low et al., 2000) with maximum sorption capacities of 17.3 and

Table 5
Calorific value of charcoals produced from different raw materials

Raw material	Charcoal calorific value (MJ/Kg)	Reference
Wood	25.5	Ffoulkes et al., 1980
BSG	27.0	Okamoto et al., 2002
Sugarcane	29.3	Ffoulkes et al., 1980
Grape bagasse	30.0	Encinar et al., 1996
Olive bagasse	31.0	Encinar et al., 1996
Hazelnut shell	32.0	Demirbas, 1999

35.5 mg/g, respectively. Treatment of BSG with NaOH greatly enhanced metal sorption compared with water-washed spent grain. BSG also successfully removed chromium from an aqueous solution, with a maximum sorption capacity of 18.94 mg/g (Low et al., 2001). The sorption capacities of BSG for cadmium, lead and chromium compares favourably with reported values for other low-cost biological materials (Table 6).

Acid Orange 7 dye (AO7), a monoazo acid dye currently used in paper and textile industries whose presence in effluents causes environmental problems, is removed by BSG (Silva et al., 2004a,b) with a maximum adsorption capacity of 30.5 mg AO7/g BSG, at 30 °C. The high level of colour removal (>90%) was achieved with a low contact time between adsorbent/dye (<1 h) and did not require any previous treatment of BSG (such as milling and/or sieving, incineration or chemical modification). Thus, BSG could be an alternative to more costly adsorbents used for dye removal in wastewater treatment processes.

4.7. Biotechnological processes

BSG is rich in polysaccharides and as well as in associated proteins and minerals and thus is a substrate of high biotechnological value. In this respect, several possible applications of BSG in biotechnological processes have been evaluated recently, and are described in the next sections.

Table 6
Sorption capacity of some low-cost biological materials for cadmium, chromium and lead

Ion	Material	Sorption capacity (mg/g)	Reference
Cadmium	Coconut shell	11.1	Budinova et al., 1994
	Pine bark	14.2	Al-Asheh and Duvnjak, 1998
	Tree fern	16.3	Ho and Wang, 2004a
	BSG	17.3	Low et al., 2000
	Wheat bran	21.0	Farajzadeh and Monji, 2004
Chromium	Sugarcane bagasse	13.4	Sharma and Forster, 1994
	Sugar beet pulp	17.2	Sharma and Forster, 1994
	Hazelnut shell	17.7	Cimino et al., 2000
	Casurina equisetifolia leaves	18.6	Ranganathan, 2000
	BSG	18.9	Low et al., 2001
Lead	Coconut shell	26.5	Sekar et al., 2004
	Sphagnum moss peat	30.7	Ho et al., 1996
	BSG	35.5	Low et al., 2000
	Groundnut husks	39.4	Okieimen et al., 1991
	Tree fern	40.0	Ho et al., 2004b

4.7.1. Substrate for cultivation of microorganisms

BSG has been successfully used as substrate for cultivation of species of *Pleurotus*, *Agrocybe* and *Lentinus* (Schildbach et al., 1992). BSG had a good biological efficiency and high nutritional value as a substrate for *Pleurotus ostreatus*, especially when water-rinsed BSG was used (Wang et al., 2001). It has been proposed that BSG favours the growth of these mushrooms not only due to its high protein content (Townsley, 1979) but also to its high moisture content and physical properties such as particle size, volume weight, specific density, porosity and water-holding capacity (Wang et al., 2001).

Recently, Szponar et al. (2003) used a protein fraction from BSG as a medium for enhanced growth and sporulation of soil actinobacteria, especially *Streptomyces*.

4.7.2. Substrate for enzyme production

Cereal brans with compositions and physical structures comparable with BSG have been used extensively as substrates for the production of commercial enzymes in so called, koji, or solid-state fermentations (Aikat and Bhattacharyya, 2000; Chou and Rwan, 1995; Sangeetha et al., 2004). For this reason, BSG has also been evaluated as an alternative substrate for enzyme production. BSG is a suitable nitrogen and energy source for production of xylanase by *Aspergillus awamori* (Bhumibhamon, 1978), however, for protease production an additional nitrogen source, such as proteose peptone had to be supplied. BSG is an efficient substrate for xylanase production by a Streptomyces isolate from Brazilian cerrado soil (Nascimento et al., 2002), and for production of xylanase and feruloyl esterase by *Streptomyces avermitilis* (Bartolomé et al., 2003).

Alpha-amylase production by *Bacillus subtilis* (Duvnjak et al., 1983) and *B. licheniformis* (Okita et al., 1985) cultivated on BSG also has been reported. *Alpha*-amylase yield was higher when BSG was the nitrogen source than with corn steep liquor or $(NH_4)_2SO_4$ (Duvnjak et al., 1983). BSG was also a good substrate for production of *alpha*-amylase by *Aspergillus oryzae* NRRL 6270 in solid-state fermentation (Francis et al., 2002, 2003). BSG was a better substrate than corn fibre for *alpha*-amylase production by *Aspergillus oryzae* (Bogar et al., 2002).

BSG can also be used as substrate without pre-treatment or further adjustment for the production of enzymes of cellulase complex by *Trichoderma reesei* (Sim and Oh, 1990).

4.7.3. Additive or carrier in brewing

The reuse of BSG in the brewing process could be attractive from the point of view of brewery economics. Roberts (1976) showed that a BSG extract (a spent grain pressing concentrate) was effective as an antifoaming agent in the fermentor, additionally, hop utilisation was improved and the properties of the final beer were not affected when the BSG extract was added. Addition of untreated BSG to wort enhanced the fermentation performance of yeast (Kado et al., 1999), but the flavour and taste of the resultant beer was not satisfactory. However, addition of a neutralised acid extract of BSG to wort enhanced yeast performance and produced beer of quality equal to that of beer fermented without spent grain.

BSG sequentially pre-treated with HCl and NaOH solutions has been evaluated as a carrier for immobilising brewers' yeast (*Saccharomyces uvarum*) (Brányik et al., 2001, 2002, 2004a,b). The cellulose-based carrier obtained was very efficient, due to its high yeast loading capacity, determined by the physicochemical and biochemical characteristics of both cell and carrier surfaces. BSG is irregular in shape and non-homogeneous in chemical composition, providing 'active sites' that are readily colonised by yeasts. BSG is considered as a promising alternative for yeast immobilisation when compared with other materials normally employed as supports. It also presents advantages from an economic viewpoint, due to its ease of preparation, reusability, availability and its inert, non-toxic nature.

BSG preparations have also been evaluated for cell immobilisation in other biotechnological processes, such as production of pectinase by *Kluyveromyces marxianus* CCT 3172 (Almeida et al., 2003, 2005).

4.7.4. Source of added-value products

The cell walls of the barley grain residues in BSG are rich in cellulose and non-cellulosic polysaccharides, in particular arabinoxylans, but also some residual (1→3, 1→4)-β-glucan and some unconverted starch (See Section 3.1), which, depending on cell type, are associated either covalently or non-covalently with lignins. The cell wall polysaccharides can be degraded into their corresponding constituents by hydrolytic procedures (hydrothermal, enzymatic or acidic). On hydrolysis, cellulose yields glucose and the non-cellulosic polysaccharides xylose, mannose, galactose and arabinose as well as acetic and hydroxycinnamic acids (Mussatto and Roberto, 2004; Palmqvist and Hahn-Hägerdal, 2000) and some of these products are of industrial significance as precursors of food grade chemicals or as energy sources in microbial fermentations.

Hydrothermal hydrolysis (autohydrolysis by acetic acid released from its esterified form on the arabinoxylans) treatment of BSG with water at 150 °C, for 60 and 120 min (Kabel et al., 2002) gave a wide variety of arabino-oligoxylosides with different structural features. The arabinofuranosyl side-branches on the xylan backbone are readily hydrolysed and are easily removed by this treatment. Nevertheless, the molecular weights of the oligosaccharides released depend on the autohydrolysis temperature (150–190 °C) and reaction time (25–450 min) employed (Carvalheiro et al., 2004a). The higher thermal sensitivity of the arabinose components compared to xylose, leads to release of large amounts of free arabinose when the temperature of the process is increased; and to major amounts of xylo-oligosaccharides.

Enzymes can be used to hydrolyse polysaccharides in lignocellulosic materials, but the results vary with the type of preparation employed. Khan et al. (1988) using mixtures of polysaccharide hydrolases from *Aspergillus japonicus*, *A. versicolor* and *Trichoderma ressei*, hydrolysed >42% of the total polysaccharides in BSG to sugars in one day. Beldman et al. (1987) hydrolysed up to 47% of the polysaccharides in untreated BSG, using a commercial enzyme preparation. Moreover, pre-treatment of BSG with NaOH or H_2SO_4 before enzymatic hydrolysis doubled the release of soluble sugars. This pre-treatment would de-esterify arabinoxylans and disrupt any ester–ether linked hydroxycinnamic acid bridges between arabinoxylan and lignin (Iiyama et al., 1990, 1994) making the polysaccharide hydrolases more accessible to their substrates. The effectiveness of alkaline treatment prior to enzymatic hydrolysis with cellulases from *Trichoderma reesei* grown on untreated spent grains has also been observed. Up to 77% saccharification of available cellulose was achieved in 24 h, from BSG pre-treated with mild alkali (Sim et al., 1989). According to Macheiner et al. (2003), a combination of extrusion cooking and enzymatic hydrolysis is a very promising procedure for recovery of soluble carbohydrates from BSG.

Another treatment reported as able to hydrolyse BSG polysaccharides with 80% efficiency is heating by microwave radiation at 160 °C in the presence of 0.1 M HCl (Macheiner et al., 2003).

Hydroxycinnamic acids (ferulic and *p*-coumaric acids) present in BSG (Section 3.1) have potential uses in the food industry (Bartolomé and Gómez-Cordovés, 1999; Bartolomé et al., 2002; Beldman et al., 1987). Bartolomé et al. (1997) used an esterase from *Aspergillus niger* to release ferulic acid from BSG and observed that 3.3% of the total ferulic acid was released but in the presence of a xylanase from *Trichoderma viride* increased the extraction up to 30%. The synergystic action of ferulic acid esterase and xylanase is responsible for this effect. The actinomycete, *Streptomyces avermitilis* CECT 3339, produces both feruloyl esterase and xylanase in the culture supernatant when grown on BSG (Bartolomé et al., 2003). Similarly, an enzyme preparation from the thermophilic fungus, *Humicola insolens*, solubilized more than half of the BSG biomass, concomitantly releasing almost all the ferulic acid and 9% of the *p*-coumaric acid (Faulds et al., 2004).

Acid hydrolysis of BSG with dilute sulphuric acid produced a sugar rich hydrolysate, which when neutralised supported high biomass yields when fermented by the yeast *Debaryomyces hansenii* (Carvalheiro et al., 2004b). A broth consisting of acid-hydrolysed BSG when fermented by *Saccharomyces cerevisiae* produced ethanol at 12.6 g/l, corresponding to 82.4% of the maximum theoretical yield

(Laws and Waites, 1986). A molasses-BSG-coconut water medium fermented by *Lactobacillus plantarum* produced lactic acid at a maximum fermentation efficiency of 93–95% in 4 days (Cabacang et al., 1997). BSG was the most economical nitrogen source, although yeast extract produced a slightly higher yield, however, the molasses-BSG-coconut water medium eliminated the use of inorganic salts.

Xylitol, an important alternative to sucrose as a sweetener with many applications in the food industry (Mussatto and Roberto, 2002), can be produced by fermentation from xylose in acid hydrolysates of BSG. A fermentable pentose-containing hydrolysate was produced by BSG autohydrolysis (converting non-cellulosic polysaccharides into oligosaccharides) followed by sulphuric acid-catalysed post-hydrolysis (converting the oligosaccharides into monosaccharides). The pentose-rich liquor was then fermented by *Debaryomyces hansenii* to produce xylitol and arabitol as the major fermentation products together with some ethanol and glycerol (Carvalheiro et al., 2005; Duarte et al., 2004). Detoxification of the hydrolysate by pH adjustment combined or not with adsorption into activated charcoal or ion exchange resins, did not improve biomass yield or productivity when compared with non-detoxified hydrolysate (Carvalheiro et al., 2005). After optimisation the BSG acid hydrolysis conditions, a xylitol yield and productivity of 0.70 g/g and 0.45 g/l h, respectively, could be attained during fermentation the hydrolysate by *Candida guilliermondii* yeast (Mussatto and Roberto, 2005).

5. Conclusions

Increasing efforts are being directed towards the reuse of agro-industrial by-products, from both economic and environmental standpoints. BSG is an abundant by-product that can be obtained from brewing companies worldwide. However, in spite of all the possible applications described, its use is still limited, being basically used as animal feed or simply as a land fill. For this reason, the development of new techniques to use this agro-industrial by-product is of great interest; since spent grain is produced in large quantities throughout the year.

Numerous attempts have been made to recycle the constituents of spent grain into the brewing process. Due to the large continuous supply, relative low cost and potential nutritional value, BSG can be considered as an attractive adjunct for human food. If BSG were used, for example, to make rich-protein breads, which could be very useful in the poorer regions of the world, where food is scarce. Besides, the ingestion of BSG or derived products provides several health benefits. Its use as neutraceutical, especially for the treatment of ulcerative colitis seems to be an important possible future medical strategy. On the other hand, considering that carbohydrates are the major components, more attention should be paid to its conversion into soluble and fermentable sugars. Currently, a number of added-value bioproducts such as organic acids, amino acids, vitamins, ethanol, butanediol, among others, are produced by fermentation using glucose or xylose as substrates. The cellulosic and non-cellulosic fractions of lignocellulosic materials in BSG are rich source of these monosaccharides.

Another possibility that should be considered is the production of polymers and resins from BSG. A large number of these compounds are produced from chemicals such as ethylene, propylene, benzene, toluene or xylene. The aromatic compounds (benzene, toluene and xylene) could be produced from BSG lignin, whereas the low molecular weight aliphatic compounds (ethylene and propylene) could be derived from ethanol produced by fermentation of sugars generated from BSG cellulose and arabinoxylan.

Although there are a number of feasible uses for BSG, the biggest impediment to its use is the cost of transport (especially of the wet form) and/or drying. More efforts must be directed to finding alternative economically sustainable drying methods.

A consequential benefit of the use of industrial by-products such as BSG as raw materials is the generation of more jobs. Additionally, from an environmental viewpoint, the elimination of industrial by-products represents a solution to pollution problems.

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