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REVIEW

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Preservation techniques and their effect on nutritional values and microbial population of brewer's spent grain: a review

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Abstract

The most prevalent by-product produced by the brewery factory is brewer's spent grain (BSG). A total of 70%, 10%, and 20% of the BSG produced are used for animal feed, biogas production, and landfills, respectively. Feeding wet brewery spent grain can avoid the cost of drying. Wet brewery spent grain is used as a replacement for forage in the diets of animals. The high moisture content and ease of deterioration of wet brewery leftover grain as a fresh feed are drawbacks (3–5 days). BSG is provided as a low-cost feed despite its greater perishability and microbiological instability. There are two significant challenges brought on by the BSG's higher moisture content (80%). First, transportation is expensive. Second, the abundance of proteins and polysaccharides in BSG promotes microbial development and deterioration. Therefore, these problems can be solved by utilizing various preservation methods, including drying (solar, freeze, and oven drying), freezing, ensiling (both alone and in combination with other animal feeds), and additives (Silo-King GPX preservatives, xylanase, carbohydrase (econase) and protease (alcalase), urea and lime, sodium formate, calcium propionate, formic and propionic acids, acetic acid, NaCl, NaOH, HCl, and H2SO4).

Keywords: Brewer's spent grain, Preservation, Drying, Freezing, Ensiling, Additives

Introduction

Brewer-spent grain is the major by-product of the brewing industry, representing around 85% of the total by-product. It became a potential source of income for the brewery (Russ et al. 2005). BSG contains cellulose (17%), non-cellulosic polysaccharides (28%), and lignin (28%. BSG can be used for animal feed and agro-industrial by-products as a raw material in foods, energy production, and biotechnological processes. A large amount of BSG is available throughout the year, but its application is limited to animal feeding (Mussatto et al. 2006b). Currently, the higher production (70%) is used for animal feed while the remaining by-product (30%) used for biogas and landfills (Mitri et al. 2022). BSG from ten breweries have thermophilic aerobic bacteria ($< 10^7$ g^{-1} fresh weight). This population is susceptible to rapid change, but the point of production can be considered microbiologically stable. Due to the seasonal production patterns of breweries, delivery of BSG is making long-term anaerobic storage challenging (Schneider et al. 1995). Under environmental conditions, the higher moisture and fermentable sugar in BSG produce rapid deterioration and environmental problems after 7–10 days of storage (Russ et al. 2005; Mussatto et al. 2006). Improper handling of the by-product causes mold and mycotoxin growth, high dry matter losses, unpleasant odor, lowered nutritional value, and reduced palatability of the feed. Mutwedu et al. (2022) reported that lack of storage and conservation facilities causes BSG spoilage and adoption problems. BSG must be transported to animal farms within a short period (El-Shafey et al. 2004). Feeding wet brewery spent grain could avoid the cost of drying. Considering this advantage, it is conceivable that the by-product could

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serve as a replacement for forages in the diets of animals. To prevent easy deterioration between 3 and 5 days, some attempts have been made to ensile BSG resulting in poor quality with characteristic offensive odors and high dry matter losses (Allen and Stevenson 1975). Emerging safety issues for BSG used as a food and feed additive considering the presence of a mycotoxin. Information on the microbial population in BSG is the beneficial for minimizing health risks (Bianco et al. 2020). The evolution of the fungal and bacterial communities during the 51 days of pre-treatment (vermicompost) in BSG accelerates the decomposition of the waste (Bianco et al. 2022). To avoid the limitation of BSG application in the feed and food industry, BSG needs a strong and sustainable pre-treatment (Zeko-Pivač et al. 2022). A rapid feeding of ensiled material is a means of preventing deterioration (Marston 2007). However, ensiling BSG may not be worth the added cost. The products that will decrease the spoilage of aerobically stored BSG are necessary (Marston 2007). To minimize, the spoilage of BSG during long-term and short-term storage various preservation methods was studied previously. This review intends to highlight the impact of various conservation strategies of BSG on microorganism development and their nutritional values.

Chemical composition and microorganism count

The literature-based chemical composition of BSG is presented in Table 1. The higher nutritional value of brewery spent grain has many potential uses in the functional food industry (Malu et al. 2014). BSG is composed of 70% lignocellulosic material, 20% proteins, and 10% lipids; additionally, it also consists of vitamins, minerals, amino acids, and phenolic compounds. Using BSG as a substrate in submerged and solid-state fermentation, different microorganism leads to the production of various value-added compounds such as organic acids, amino acids, volatile fatty acids, enzymes, vitamins, second-generation biofuels, and other products (Mitri et al. 2022). Different researchers studied that after collecting the by-products, BSG has limited microbial contamination, and it can be considered microbiologically stable and within acceptable limits for food use. Marston (2007) reported that wet brewery spent grain has 4.57,7.53,5.23 and 1.6 log10cfu/g FW, LAB, yeast, mold, and clostridia contamination, respectively. Hatungimana et al. 2021 and Kitaw et al. 2022a, b reported different proportions of microbial contamination in BSG. Cultivable microflora was isolated in the raw grain of durum wheat variety 'Senatore Cappelli' and the isolated bacterial strains were rhizospheric (Kocuria rhizophila, Microbacterium aerolatum, and Bacillus pumilus) and associated with the microbiota of wheat (Staphylococcus spp.). The dominant filamentous fungus genera were Alternaria and Rhizopus. Low levels of mycotoxigenic Fusarium spp, Aspergillus spp., and Penicillium spp. were isolated. The microorganisms including deoxynivalenol, T2-HT2, fumonisin, aflatoxin, and ochratoxin identified from malt and grain were below the thresholds defined by European law (Bianco et al. 2019).

Preservation techniques of BSG

The literature-based preservation techniques of BSG are presented in Fig. 1. BSG cannot be stored for long periods, and it is challenging for transportation. BSG is not economically sustainable and commercially viable products to overcome these problems different conservation methods were applied by Santos et al. 2003; Russ et al. 2005; Mussatto et al. 2006. The influence of introducing a new stage in the life cycle of the BSG for ultimate use in human nutrition is very significant on the global

Table 1 Chemical composition and microbial populations of wet brewery spent grain

Nutrients	1	2	3	4	5	6	7	8
Dry matter	23.36	24.15	21–28	24.1	25.75	22.6	24.27	24.2
Crude protein	32.57	27.35	26.5–37	28.6	27.80	32.1	22.48	26.5
Neutral detergent fiber	45.47	–	42.9–61	53.7	62.91	43.5	26.4	63
Acid detergent fiber	21.20	–	26–32	28	20.5	22.5	12.1	25.2
Acid detergent lignin	–	–		–	–	–	–	6.66
Microbial population (log10cfu g^{-1} FW)								
Lactic acid bacteria	4.57	–	–	4.97	–	–	4.57	–
Aerobic bacteria		–	–	6.61			4.62	–
Yeast	7.53	–	–	7.13	3.79	8.05	4.69	4.24
Mold	5.23	–	–	–	–	7.33	–	4.08
Clostridia	1.6	–	–	–	–	–	–	–

(1) = Marston 2007, (2) = Souza et al. 2012, (3) = Young et al. 2015). (4) = Wang et al. 2020, (5) = Lv et al. 2020, (6) = Hatungimana et al. 2021,(7) = Dai et al. 2022, and (8) Kitaw et al. 2022a, b

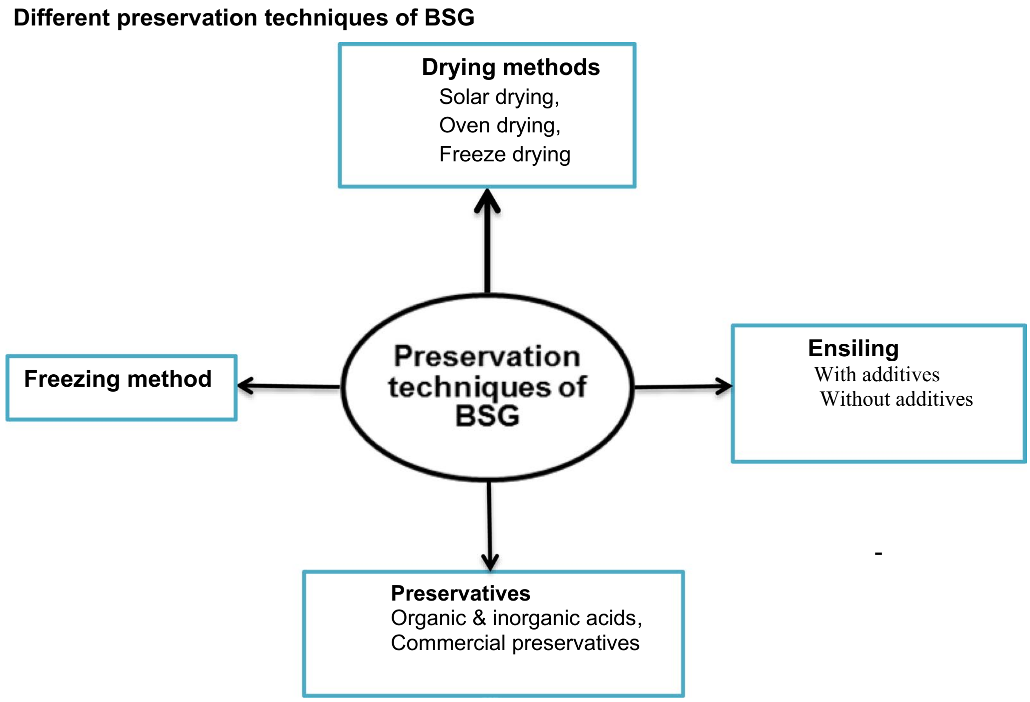

Fig. 1 Overview of preservation techniques

environmental impact of the product. Lacto-fermentation, freeze-drying, and refrigeration have an energy consumption reduced to the functional unit and not negligible compared to the other stages of the life cycle of the spent grain (Petit et al. 2020).

Drying

Drying is the most widely used preserving method of BSG. Many breweries have plants used for BSG processing by using a two-step drying technique, where the water content is first reduced (< 60%) by pressing, followed by drying to ensure the moisture content (< 10%) (Santos et al. 2003; Lynch et al. 2016). Factory drying is the most effective method of preserving BSG. However, the traditional process for drying BSG is the basis on the use of direct rotary-drum driers. BSG preservation by oven drying, freeze-drying and solar drying reduces the volume of the product and declining transport and storage costs, does not alter its composition (Bartolomé et al. 2002; Santos et al. 2003; Faccenda et al. 2021). But, in terms of economic cost, oven drying is preferred over freezing (Bartolomé et al. 2002). Preserving BSG through the freezing technique is not appropriate as it affects some sugar (arabinose) components of BSG (Faccenda et al. 2021). To protect against unpleasant flavors, BSG must be dried in the oven at temperatures (< 60 °C). In oven drying, the risks of the grain temperature near the dryer rise leading to the burning of the dried grains. After 16 h of sun exposure to BSG and storing the dry by-product for 180 days, It was not affecting the nutritional and microbiological quality (Faccenda et al. 2021). Brewery factories are discouraged from using the drying technique (at 60 °C) of BSG due to the higher energy costs compared with the different methods of BSG preservation concerning microbial proliferation, from the fresh material at 20 °C to that refrigerated at 4 °C, autoclaved at 120 °C for 1 h and frozen (Aliyu and Bala 2011). According to El-Shafey et al. (2004), a membrane filter press is used for high drying levels of brewery spent grain cake. BSG is mixed with water and filtered at a feed pressure of 3–5 bar and washed with hot water (65.8 °C), membrane-filtered and vacuum-dried is used to reach moisture levels between 20 and 30%. After 6 months of storage, the cake in the open-air bacterial activity does not occur. An alternative drying method that is important to save the cost of energy is to use superheated steam with attracted additional advantages such as reduction in environmental impact, improved drying efficiency, elimination of fire or explosion risk, and enhanced recovery of valuable organic compounds (Tang et al. 2005). The circulation of superheated steam occurred in a closed-loop system; this reduces the energy wastage that occurs with hot-air drying. The exhaust steam produced from the evaporation of moisture from the BSG can be used for other operations.

The superheated steam method has several advantages, such as the reduction in the environmental impact, an improvement in drying efficiency, the elimination of fire or explosion risk, and the recovery of valuable volatile organic compounds. In the storage of wet brewery spent grain under 4 °C (> 16 days), the numbers of aerobic bacteria in the by-product remained < 10^6 CFU g^{-1}, while in the frozen and autoclaved samples; there was no evidence of microbial activity. BSG is stored under 4 °C and 20 °C, resulting in sugar loss and ascribable to the activities of microbial enzymes, such as xylanases, esterases, and cellulases (Anderson et al. 2015). Autoclaving is the effective long-term storage of BSG (Lynch et al. 2016). The chemical composition of BSG makes it susceptible to microbial attack and chemical deterioration. Rapid colonization of microbes and nutritional loss in BSG occurred at 20 °C storage temperature, refrigeration gave a similar but lower-level response. Freezing BSG does not change chemical composition but the solubilisation of polysaccharides and phenolic compounds occurred in autoclaving BSG. Changes are related to the temperature profile determined during autoclaving and also partially due to the breakdown of residual starch. The storage stabilization of brewery spent grain and the methods selected for stabilization can lead to a substantial modification (Aulds and Aldron 2010).

Ensiling

One of the efficient and alternative methods of preserving spent grain is its silage. This method is complicated by the low dry matter content (about 12–25%) and low (about 1% calculated on the dry matter) sugar content, which can ferment relatively quickly during silage to form lactic acid. According to established views, the following conditions are most important for obtaining high-quality silage—a rapid decrease in the pH value of the juice of the silage mass to 4.0–4.2 and below; the presence of dry matter in the silage mass not less than 30% and ensuring the temperature of the silage mass in the range of 20–30 °C, and during storage of the silo—not above 15 °C. Besides, to prevent the development of mold and putrefactive bacteria in the silage mass, oxygen should be excluded. Ensiling requires the material to be compacted and sealed quickly to minimize losses associated with aerobic deterioration from undesirable bacteria and molds and rapid lactic acid production must occur to lower pH, thus inhibiting clostridia growth. Unless stored under anaerobic conditions, feeds rapidly deteriorate and promote mold and mycotoxin growth. Allen and Stevenson (1975) proposed that bacterial inoculants may improve the availability of BSG as they observed a rapid increase in the Lactobacilli population during the first 2 days of ensiling wet brewery spent grain, followed by a decline over the remaining 16 days of the experiment. The addition of lactic acid bacteria was beneficial for fermentation, as they aided in lowering the pH, increased the initial concentration of lactate, and consequently, decreased the initial concentrations of acetate and butyrate. In addition, including a high-moisture grain inoculant and beet pulp pellets to wet brewery spent grain lowered pH, acetate, and NH_3N concentrations and increased the content of lactic acid under long-term storage. However, including a high-moisture grain inoculant for short-term storage was not beneficial (Schneider et al. 1995). After 30 days of storage, different strains of microorganisms such as Aspergillus, Fusarium, Mucor, Penicillium, and Rhizopus are isolated in BSG (Sodhi et al. 1985). BSG was stored at 20 °C for 5 days, and microbial populations such as microaerophilic, anaerobic, aerobic, mesophilic, and thermophilic bacteria were increased to 10^6 CFU g^{-1}. Filamentous fungi such as Aspergillus spp., Fusarium spp., Mucor spp., Penicillium spp., and Rhizopus spp were isolated in the BSG after storage at room temperature (Anderson et al. 2015). Wang et al. (2014) is examined the effect of different storage duration (0, 1, 2, and 3 days) and storage temperatures (5, 15, 25, and 35 °C) on chemical composition and microorganism development of BSG and indicated that surface spoilage is apparent at higher temperatures (25 and 35 °C). Nutrient contents in BSG decreased concomitantly with prolonged storage times and increasing temperatures. The amount of yeast and mold populations in BSG increased with increasing storage times and temperatures. However, when storage temperature exceeds 35 °C, BSG is used within a day to prevent the impairment of rumen fermentation in the subtropics such as Southeast China, where the temperature is typically above 35 °C during summer. Similarly, recent research looked at the effects of storage periods (2, 4 and 6 days) and storage temperatures (15, 20, and 25 °C) on the characteristics of BSG as ruminant feeds. Extensive mold growth in BSG has detected on the sixth day at 25 °C. Changes in nutrients, yeast, and mold colony count in BSG are significantly affected by the interaction of storage temperatures and durations (Kitaw et al. 2022a, b). BSG can be ensiled with the combination of dry feeds or alone depending on the interest of farmers (Gustavsson et al. 2013). BSG ensiled for 28 days can be ready for feeding of cows. The key feature of ensiling BSG is that it can be done easily by farmers after the commencement of the training and all the required materials are available locally. Moreover, the low-cost silage-making technique was tested and found to be applicable by farmers in developing countries due to the low cost and use of locally available materials and its improvement in milk production and income of farmers. The preservation of BSG packaged under aerobic conditions is not appropriate due to the development of fungi and yeasts microbial populations; however, storage of BSG under anaerobic conditions is evidenced to be an effective conservation process (de Souza et al. 2012). Combination of BSG (25% − 50%) with whole-plant maize in silage making with a different level of mixing improved fermentation quality and stability against aerobic deterioration (Koc et al. 2010). With the rise in feed cost and shortage of animal feeds, it is essential to substitute common vetch with BSG (up to 20%) in the ensiled total mixed ration which does not negatively affect fermentation quality, nutrient composition, aerobic stability, in vitro gas production kinetics and digestibility (Wang et al. 2020). Including BSG (up to 20%) in corn stalk, apple pomace sweet potato peel-based total mixed ration is important to improve fermentation quality and in vitro digestibility. Nevertheless, the production of propionic and butyric acid in brewery spent grain-treated total mixed ration produce the potential clostridia fermentation. The brewery spent grain-based silage has higher lactic acid bacteria, lower pH, and NH3-N content (Dai et al. 2022). It ensures anaerobic fermentation for lactic acid, reduction in pH, and preservation of the quality of ensiled material (Souza et al. 2012). The use of wet distiller grains from tapioca and rice up to 20% (as-fed basis) in total mixed ration did not show any effect on the performance of Hanwoo steers in the initial to the mid-fattening period (Young et al. 2015). The combination of soy hulls (15% − 30%) in BSG silage-making produces a higher total DM loss and variation in nutrient composition (Moriel et al. 2015). The minimal dry matter loss, lower fungal, yeast, and mold colony count, and higher digestion kinetics (CP) were investigated in ensiling techniques of BSG preservation techniques (Kitaw et al. 2022a).

Additives/preservatives

Organic and inorganic acids

Organic and inorganic acids are one of the most effective BSG silage additives (Filya et al. 2004; Koc and Coskuntuna 2003). Acids such as lactic, acetic, formic, benzoic, hydrochloric, and sulphuric acids are essential BSG preservatives, with benzoic and formic acids being particularly effective; however, the use of such chemicals can be at odds with the consumers' desire for more natural food ingredients (Sodhi et al. 1985; Mussatto et al. 2006a, Kazemi et al. 2014). A low level (0.5%) of sulphuric acid additive is an effective mechanism to improve the nutritional value of ensiled BSG (Kazemi et al. 2014). The supplementation of organic acid or SÝLOFARM® LIQUID, Farmavet KOCAELI (the mixture of 60% formic acid, 20% sodium formate, and 20% water) in BSG ensiled for up to 45 to 90 days can prevent mold growth and improve nutritional values. Warmer silages are more susceptible to aerobic deterioration, especially if the ambient temperature is high (> 37 °C). The efficacy of organic acid for silage making is affected by high ensiling temperatures. Formic acid, as a silage additive, has an anti-bacterial effect on many bacteria species, including lactic acid bacteria; thus, the addition of formic acid into silage results in limited fermentation and reduction in the organic acid content (Coskuntuna et al. 2010). Allen and Stevenson (1975) studied the effect of formic acid and propionic acid as a preservative of BSG. Formic acid is the only additive that eliminates the decline in lactic acid during storage. Formic acid is the most effective treatment for improving silage quality. A low level of propionic acid is produced in BSG silages, while isobutyric, butyric, and isovaleric acids are not detected in any samples. The BSG silage without additives is poorly preserved which contains high levels of acetic and butyric acids and ammoniacal nitrogen. The BSG preserved with different rates of formic acid (0.50 and 0.75%) and the higher rate of the formic–propionic mixture (0.75%) is important to prevent microbial contamination and nutritional deterioration. Including either formic acid (0.20 and 0.40%) or propionic acid (0.40%) is effective in reducing subsurface deterioration, but is not effective in surface deterioration. However, a 0.40% mixture of the two acids maintained the quality of BSG during the 14-day storing. Characteristically, weak-acid preservatives do not kill microorganisms but inhibit their growth, causing much-extended lag phases. Preservatives are more effective at low pH values where solutions contain increased concentrations of undissociated acids. Inhibition by weak acids involves rapid diffusion of undissociated molecules through the plasma membrane; dissociation of these molecules within cells liberates protons, thus acidifying the cytoplasm and preventing growth (Lambert and Stratford 1999).

Commercial preservatives and others

Commercial preservatives and other additives are essential in BSG storage to prevent spoilage. Dixon and Combellas (1983) stated that there is no beneficial effect of NaCl or NaOH as preservatives of BSG under aerobic conditions. However, treating BSG with salt is essential to suppress microbial growth (fungal, yeast, and mold colony counts), improving in situ dry matter and protein degradability of BSG (Hatungimana and Erickson 2019; Hatungimana et al. 2021; Getu et al. 2022). Urea and lime are essential to conserving BSG as silage for 70 days and there is no mold growth and to increase in vitro dry matter digestibility (Soriano et al. 1991).

Potassium sorbate prolongs the shelf life of foods by stopping the growth of mold, yeast, and fungi and it is an effective BSG preservative (Küntzel and Sonnenberg

1997).The Silo-King GPX preservative is the dry and free-flowing product that contains lactic acid-producing bacteria Lactobacillus plantarum, Enterococcus faecium, and Pediococcus pentosaceus and fermentation extracts from Aspergillus oryzae, Trichoderma longibrachiatum and Bacillus subtilis. This product also contains the preservative and anti-oxidant butylated hydroxytoluene, along with anti-fungal agents such as potassium sorbate, sodium benzoate, propionic acid, and acetic acid, benzoic acid, and sorbic acid. Monosodium phosphate is included as a nutrient and acidulant, while sodium silico aluminate acts as a moisture scavenger. The application of Silo-King GPX preservatives in BSG at different rates (0.45 kg/900 kg BSG, and 0.9 kg/900 kg BSG) is important to protect the spoilage or extent of microorganism development for 28 days. Using Silo-King GPX as preservatives of BSG is also important to increase fat, acetic, and butyric acids and quadratic decreases starch, Ca, Mg, K, and Mn concentrations. In Silo-King treated brewery grain, the log CFU counts of yeast and mold microbial populations and crude protein content are decreased linearly, while lactic acid concentration is increased. Therefore, the suggested inclusion level of Silo-King GPX preservatives is 0.45 kg/900 kg of BSG (Marston 2007)**.** The addition of calcium propionate (3 g/kg fresh weight) in BSG does not affect the pH and lactic acid concentrations and the concentration of propionic acid is affected positively. The addition of sodium formate (3 g/kg fresh weight) has the lowest pH and acetic acid, butyric acid, ammonia nitrogen contents, and the highest lactic acid concentration. After fermentation, through the addition of sodium formate the contents of dry matter, water-soluble carbohydrates, and neutral detergent fiber in BSG are improved. Both sodium formate and calcium propionate additives are used to improve the silage quality of BSG for short-term storage (20 days). BSG ensiled with sodium formate is important to improve effective in situ crude protein degradability. The analysis of the genus level of the bacterial flora (Lactobacillus) in BSG treated with sodium formate is higher and the content of Clostridium is lower. Therefore, the addition of sodium formate in BSG silage is important to suppress the undesirable microorganisms and enhance fermentation qualities. During short-term storage of high-moisture feed, sodium formate has a more beneficial preservation effect than an equivalent dose of calcium propionate (Lv et al. 2020). Bioprocessing BSG with xylanase and lactic acid bacteria strains enhanced anti-oxidant potential, radical scavenging activity, long-term inhibition of linoleic acid oxidation, and protective effect toward oxidative stress on human keratinocytes NCTC 2544 (Verni et al. 2020). Commercial carbohydrase (econase) and protease (alcalase) enzymes are essential to convert BSG carbohydrate and protein components into a more valuable product, respectively (Forssell et al. 2011).

Conclusions

Brewer's spent grain (BSG), which makes up around 85% of the entire product, is the most prevalent by-product produced in the beer-brewing business. A large fraction of BSG has been used as animal feed, and only a small amount of BSG is produced into biogas and dumped in landfills. BSG is an excellent animal feed because it has high protein content, breaks down gradually in the rumen, has a high P/Ca ratio, and has a comparatively low amount of water-soluble carbohydrates. BSG's increased levels of polysaccharides, proteins, and moisture make it more prone to microbial development and deterioration, which increases its transportation costs (per unit of dry matter mass) and makes it more difficult to store. Different preservation methods, including as drying, freezing, ensiling, and commercial additives, should be used to address these problems.

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Conceptualization, investigation methodology, and writing—original draft by writing—review and editing by GT. The author read and approved the final manuscript.

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Declarations

Competing interests

The authors declare no conflict of interest.

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