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Brewer's Spent Grain: its Value as Renewable Biomass and its Possible Applications

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Climate change, depletion of fossil resources, constant population growth and increasing energy demand are issues of great concern. The idea of valorising tonnes of industrial waste produced on the planet every year appears as a possible pathway for a sustainable growth. Among different alternatives, lignocellulosic biomasses (LCBs) are the most important, abundant and cheap resource available. They are obtained from crops and organic agricultural or industrial waste as bio-degradable by-products. Therefore, LCBs represent a versatile, renewable, widely available and low-cost raw material that can be potentially used to produce biofuels, new materials and chemical compounds, satisfying the industry's desire to move towards a green transition. The brewery's spent grain (BSG) is the main by-product of the brewing industry (about 85% of total by-products), generated in massive amounts throughout the year. It represents a raw material with an interesting chemical composition that has shown to have the potential to be used in different industrial exploitations. This study summarises some of the relevant research on BSG valorisation starting from the simplest case of its use as an ingredient for animal feed up to the production of biofuels and the recovery of value-added compounds.

1. Introduction

In recent years there has been a growing awareness of human actions and how they impact the environment. In a world increasingly affected by environmental issues and unprecedented population growth, the possibility of recycling industrial wastes as resources of raw material is becoming more and more popular. The main principle is to valorise wastes and exploit them in a wide range of industrial sectors such as the production of biochemicals, food ingredients, nutraceuticals and biofuels. Significant quantities of waste are produced every day worldwide, creating environmental and social problems. The recovery and conversion of these wastes would be an attempt to achieve a greener policy oriented towards a sustainable circular economy. In this way, the problem of disposing of by-products, which would otherwise be landfilled or incinerated, would be solved. In this scenario, lignocellulosic biomass is undoubtedly one of the most important wastes generated by human activity every year or easily available in the environment. It was estimated that lignocellulosic biomass production reaches 200 billion tons/year (Mankar et al., 2021). This estimate, combined with the fact that it is a natural and cheap raw material, are important factors encouraging its use in different applications such as the manufacturing of biofuels, new materials and chemical compounds. Brewer's spent grain is the main insoluble solid by-product generated during beer production and after wort extraction. It represents about 85% of the total by-products. Ikram et al. (2017) reported that the amount of BSG produced annually by the brewing industry in the European Union is about 3.4 million tons, while global production reaches 39 million tons (Birsan et al, 2019). 100 kg of malt gives approximately 100-130 kg of fresh BSG, with a moisture content of 70-80%, equivalent to around 20 kg of BSG per 100 L of brewed beer. BSG is composed of the barley malt husk, pericarp and seed coat, containing nutrients that are not extracted during the mashing and malting processes. Factors such as the type and quality of grain used can influence its composition, but also the malting and mashing conditions and the harvest time. Despite this, its composition generally includes high levels of dietary fiber, protein, essential amino

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acids, as well as minerals, polyphenols, vitamins and lipids. Bianco et al. (2020) reported that 70% of the BSG produced is used for animal feed as this is the main and the quickest alternative, 10% for biogas production, and the remaining 20% is landfilled. Today, with the new agricultural and eco-political circumstances aimed at solving the logistical problem of the disposal of this by-product, there is a growing interest in possible alternative methods of using it. These include the production of biofuels, but also its use in human food, as ingredient or using value-added components extracted from it, or in non-food products such as pharmaceuticals, cosmetics, construction of food packaging (Chetrariu and Dabija, 2020). In this study, relevant literature on BSG has been reviewed, highlighting its properties in terms of composition and its potential applications. In particular, the focus is on its use as food or food additive in both animal and human diet, in the production of bioethanol, for the recovery of phenolics compounds, and as energy source. The most recent studies are reported and discussed.

Table 1: Chemical composition of brewer's spent grain (BSG) on a dry weight basis.

Components [g kg^{-1}]	(Mussatto and Roberto, 2006)	(Xiros et al., 2008)	(Waters et al., 2012)	(Meneses et al., 2013)
Cellulose	167.8	120	260	217.3
Hemicellulose	284.2	402	222	192.7
Xylan	199.4	NR	NR	136.3
Arabinian	84.8	NR	NR	56.4
Lignin	277.8	115	NR	194.0
Proteins	152.5	142	221.3	246.9
Ashes	46.0	33	11	41.8

*NR = Not Reported.

2. BSG chemical composition

Brewery's spent grain consists of a heterogeneous mixture of cereal grain husks, pericarp, residual amounts of endosperm depending on the brewing regime applied, and the original barley grain coating layer (Steiner at al., 2015). These outer layers contain nutrients not extracted during the malting and mashing processes, making the spent grain an attractive raw material. Table 1 summarises the chemical composition of BSG. Independently of the variations that can be observed in its composition in terms of the individual component concentration, this residue is predominantly composed of fiber (cellulose, hemicellulose and lignin) since barley malt husk is a lignocellulosic material. Hemicellulose and cellulose are fractions composed of sugars, of which xylose, arabinose and glucose are the most abundant mono-glycans as stated by Mussatto and Roberto (2006), comprising around 50% (w/w) of the BSG composition. Lignin and proteins are also significant constituents of BSG. In particular, lignin is a polyphenolic macromolecule responsible for maintaining the structural integrity and rigidity of the cell wall. Moreover, it contains several phenolic compounds, the most important of which are ferulic, p-coumaric, syringic, vanillic and p-hydroxybenzoic (Mussatto et al., 2007). Protein generally comprises 15% to 25% of the composition of BSG. The study conducted by Santos et al. (2003) showed that the protein fraction in oven-dried BSG can be 15% to 24.2% and includes globulins, albumins, glutelins and hordeins. BSG also contains significant amounts of minerals, the most abundant being calcium, followed by magnesium, phosphorus and sodium (Meneses et al., 2013). Still, it is possible to find also iron, copper, potassium and manganese (Mussatto, 2009). Vitamins are also present in noteworthy quantities, in particular biotin, niacin, folic acid, choline, thiamine, pantothenic acid, riboflavin and pyridoxine (Ikram et al., 2017; Chetrariu and Dabija, 2020). Finally, there are extractives formed by waxes, resins, tannins, essential oils, and lipids, among which triglycerides and fatty acids are the most abundant (Mussatto 2009; Ikram et al., 2017; Chetrariu and Dabija, 2020).

3. Potential applications for BSG

Being a low-cost raw material, many efforts have been made to investigate BSG possible application in the field of animal and human nutrition, as feedstock for biofuels production, and in biotechnological processes through the extraction of value-added compounds. The most relevant studies in the literature are presented and discussed in the following paragraphs.

3.1 BSG in animal feed

Due to BSG low cost, availability, high protein and fiber content, spent grain has always been mainly used for different animals (cattle, fish, chickens, pigs and ruminants) feed, either in wet or dried form (Mussatto, 2014), creating an output for this material and solving the problem of its disposal (Aliyu and Bala, 2011). It has been demonstrated that the combination of BSG with nitrogen sources such as urea can provide ideal nutrition for ruminants with all essential amino acids (Huige, 2006). As reported by Tang et al. (2009), the consumption of BSG or its derivatives by rats is responsible for beneficial effects on intestinal digestion, which are related to the glutamine-rich protein content, to a high content of non-cellulosic polysaccharides and small amounts of β-glucans. Other important benefits have been observed in other animals such as fish. A study conducted by Kaur and Saxena (2004) revealed that the substitution of rice with spent grain resulted in greater body weight gain attributed to the high-quality protein and essential amino acid content of a BSG-based diet. Aliyu and Bala (2011) examined the effect of BSG on the nutritional value of milk yield in dairy cattle observing that, compared to a maise-based diet (45% w/w), an integration of BSG (45% w/w) leads to an increase in current milk yield, milk total solids content and milk fat yield. Although the major nutritional benefits of BSG consumption have been observed especially in dairy cattle, several efforts have been made to exploit all or part of this raw material in mixed forms and in different animals. In fact, as reported in Mussatto (2014), it is used as part of the poultry diet. However, most of the polysaccharides in the BSG cell walls, such as arabinoxylan and β-glucans cannot be digested by these animals. This problem is due to the lack of enzymes required for hydrolysis of the polymer chains and has been solved simply by adding the missing enzymes (xylanase and β-glucanase) to the feed.

3.2 BSG in human diet

Several beneficial effects have been observed in the use of BSG or its components in the human diet, including accelerated transit time, increased faecal volume, reduced cholesterol and postprandial glucose levels (Mussatto, 2014). Further positive effects are associated with a significant reduction in contracting type II diabetes, obesity, diverticulitis, cardiovascular problems, or colorectal cancer (Steiner et al., 2015). This is due to the presence of biologically active compounds such as β-glucans which play an important physiological role in the body (Chetrariu and Dabija, 2020). This has led to increased interest in developing BSG fortified foods, which are considered functional food offering health benefits when combined with a balanced diet. The incorporation of spent grain in the manufacturing of bakery products such as bread, biscuits and snacks was also evaluated in order to increase the fiber content. To make this possible, BSG is usually converted into flour as its grainy nature may alter the final product's physical properties. In this way, the particle size is reduced, which is important to make the final product acceptable to the consumer (Mussatto, 2014). BSG incorporation in food products was analysed by Lynch et al. (2016) at different levels (10-40% on a dry weight). The main effects observed were an increase in protein, fiber and amino acids content and a decrease in the starch level and calories. However, it is important to consider the possible sensory alterations that may occur. In fact, since it has a brown colour, it is recommended to use BSG only in colored products (bread, biscuits, etc.) to prevent colour alteration of the product. For a BSG addition higher than 20%, the colour is not the only property to be affected, but also the product structure, the volume and the texture. According to Steiner et al. (2015), these problems can be prevented by replacing only 10% of normal flour with BSG. Besides bakery products, Özvural et al. (2009) investigated the production of Frankfurters sausages using BSG as a fat substitute to produce a meat product with high fiber and low-fat content.

Other than a direct application of BSG as a substitute for other ingredients in food preparation, the extraction of phenolic compounds as additives represents a feasible valorisation route. As suggested by McCarthy et al. (2013), the addition of BSG extracts to food drinks such as fruit juices and smoothies can significantly improve the total phenolic content in the case of important differences with the originals. Further studies were conducted on the use of polyphenols and flavonoids extracted from BSG for the preparation of fish burgers using various bioactive powders (Spinelli et al., 2016a), and on the possibility of strengthening food products such as pasta and infant formulas using the extracted proteins (Nazzaro et al., 2018).

3.3 BSG for ethanol production

Biofuels obtained from the conversion of lignocellulosic materials are a viable alternative to fossil fuels since they are considered carbon neutral. Cellulosic bioethanol has received considerable global attention as a transport fuel for several benefits, among which its potential to reduce greenhouse gas emissions by 86% certainly stands out (Wang et al., 2007). Several studies were performed to obtain ethanol from the biological conversion of SBG using different pre-treatment techniques and fermentation conditions, leading to significantly different results in terms of ethanol yield. White et al. (2008) compared the results obtained from acid pre-treatment with HNO_3, H_2SO_4 and HCl at different concentrations and found the former to be the most suitable for performing this step. The hydrolysate pretreated with 0.16 N HNO_3 and subjected to enzymatic hydrolysis, was subsequently fermented for 48 h at 30°C using two different yeast strains, *Pichia stipitis* NCYC 1540 and *Kluyveromyces marxianus* NCYC 1425, obtaining respectively 8.3 and 5.9 g L^{-1} ethanol, which correspond to 0.32 and 0.23 g ethanol (g substrate^{-1}) and 63% and 45% in terms of theoretical conversion. Liguori et al. (2015) obtained a higher concentration of ethanol, which combined an acid pre-treatment with H_2SO_4 with an alkaline treatment, resulting in a hydrolysate containing 75 g/L glucose. The fermentation step was performed using

Saccharomyces cerevisiae NRRL YB 2293 and comparing the ethanol yield obtained using the BSG hydrolysate as a growth substrate with and without a supplement of yeast extract. In the first case, an ethanol concentration of 12 gL^{-1} corresponding to 0.26 g ethanol (g $substrate^{-1}$) was obtained, compared to 12.79 gL^{-1} ethanol and 0.28 g ethanol (g $substrate^{-1}$) when the hydrolysate was enriched with nutrients. Therefore, the authors demonstrated that in both cases, similar concentrations of ethanol were achieved. The only difference is that enriching the medium with a nitrogen source improves performance in reducing fermentation time. Mata et al. (2015) optimised the acid and enzyme BSG pre-treatments carried out sequentially by evaluating different combinations of reaction times and enzyme/BSG ratio values but keeping the acid amount and concentration constant. The maximum total sugar conversion obtained was 22.24%. Fermentation was then conducted at 30°C for 72 h using both a synthetic growth medium and BSG hydrolysates in order to evaluate the potential presence of inhibitors from the previous pre-treatment steps. Two different strains were used in this case as well, *Pichia stipitis* NCYC 1541 and *Kluyveromyces marxianus* NCYC 2791. In both cases the results showed that the fermentation efficiency is much higher in the case of synthetic medium (around 80%), and only 45.10% for *P.stipitis* and 36.58% for *K.marxianus*. The theoretical ethanol yield was 0.27 and 0.19 g ethanol (g $substrate^{-1}$) respectively for *P.stipitis* and *K.marxianus,* while the actual 0.0856 and 0.0308 g ethanol (g $substrate^{-1}$). The low values of these yields compared to those obtained by White et al., 2008, were justified as a consequence of the presence of fermentation inhibitors form the pre-treatment steps.

Overall, BSG represents a valuable raw material with potential for ethanol production. However, research efforts are still necessary to optimise the process conditions, develop green technologies for the pre-treatment steps, develop cheap enzymatic routes, and invest in genetically modified strains to improve the sugar-ethanol conversion.

3.4 Extraction of value-added compounds from BSG

As already mentioned, BSG represents an important and inexpensive source of value-added components such as carbohydrates, proteins, and lipids but it contains also phenolic compounds that have recently gained considerable interest for their valuable health-benefiting properties. In particular, due to their role in the prevention of chronic disorders, cancer and intracellular oxidative stress their recovery is attractive for manufacturers and scientists (Ikram et al., 2020). Different levels of phenolic compounds have been found in BSG, but usually the hydroxybenzoic acids (HBA) content of BSG is lower than hydroxycinnamic acids (HCA). The most abundant among HBA is the syringic acid but their quantity can vary depending on the barley variety, the harvesting time, and the characteristics of the growing region. Regarding the HCA, those present in higher quantities are ferulic acid (FA) and *p*-coumaric acid (*p*-CA) (Mussatto et al., 2007), which are both contained in the outer layers of grain and remain in BSG after the entire brewery's process. The recovery process of phenolic compounds can be summarised in the following steps: pre-treatment, extraction, isolation, and purification. The pre-treatment can include maceration, homogenisation, grinding and milling leading to the disruption of the cellular structure improving the recovery of bioactive compounds and increasing the mass transfer between the solvent and the biomass. In addition to the pre-treatment techniques already mentioned, other methods such as autohydrolysis, acid, alkaline, and enzymatic pre-treatments can be useful in the breakdown of the biomass structure and access to the cell vacuole where the phenolics are contained. Among the different extraction techniques used to recover phenolic compounds, a differentiation can be made between conventional and non-conventional methods. The solid-liquid extraction (SLE) together with Soxhlet (SE) are the most common and well-established techniques used due to their simplicity, efficiency and wide industrial applicability. The former is usually combined with alkaline hydrolysis to increase extraction efficiency by degrading lignin and cellulose and releasing unbound HCA (FA and *p*-CA). Although this technique ensures a good recovery of phenolics (Meneses et al., 2013), there are drawbacks such as the important amounts of solvent required, the long extraction time but also the possible need for additional procedures to remove unwanted non-phenolic compounds thus increasing the process cost, which led to consider alternative methods. Alternative extraction methods, also called green techniques because they follow the standards set by the Environmental Protection Agency (USA), have recently been proposed. Supercritical fluid extraction and pressurised fluid extraction belong to this category. In recent decades, they have caught the interest of researchers due to the reduced extraction time, reduced solvent use, higher extraction yield and enhanced quality of extracts. Ultrasound and microwave-assisted extraction can be considered in the same way if combined with a conventional method using inorganic solvents or with pressure liquid extraction. The advantage of their use is the obtainment of cleaner extracts without presence of residues, which would occur when extracting with organic solvents. Although there are still few works involving their application on BSG, a significant progress has been made (Wang et al., 2013; Spinelli et al., 2016b; Herbst et al., 2021) and, they are very encouraging, offering an interesting starting point for further research into possible a possible scale-up.

3.5 Energy production from BSG

As an agri-food biomass, BSG represents a valid raw material that can be used effectively in a waste-to-energy process due to its ability to produce energy. This approach includes technologies such as aerobic digestion and thermochemical conversion processes (combustion, pyrolysis and gasification). Important requirements for a valid energy valorisation are calorific values over 15 MJ kg^{-1} and humidity in the range 10-15 % (Gil-Castell et al., 2022). Considering the BSG composition, a drying phase is certainly necessary in order to reach the desired moisture value. Following the circular economy principle, it is worth noting the possibility of reusing the spent grain energy content within the beer industry, allowing a reduction in energy consumption. However, mature strategies are not yet available and further studies in this direction are required.

4. Conclusions

Waste reuse is an increasingly common practice that allows tackling the issue related to their management while exploiting their potential as raw materials. This review discussed a summary of the composition and possible applications of brewer's spent grain. BSG represents a valuable agro-industrial by-product. Due to its chemical composition and wide availability at low cost, it can be reused in different applications spanning feed and food and recovery of target compounds. In the last years, several efforts have been made to use it in an alternative way, not only as animal feed. Important results have been obtained from its integration in the human diet, which have confirmed the value of its chemical composition. Its applications in the chemical and biotechnology sector are also noteworthy, and in particular as a fermentation substrate for the production of biofuels and as an extraction matrix of valid and useful bioactive components for the manufacturing of chemicals, pharmaceutical and cosmetic compounds. However, further studies are needed to optimise the production process in the different areas. In particular, the development of unit operations sequence that can exploit BSG potential and case studies devoted to understanding the economics behind the process and the market potential for the obtainable products is still missing. Future works are called to address the process cost minimisation and yield maximisation with the aim of implementing industrial reuse of BSG an ever-closer reality.

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