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Biotechnological, food, and health care applications

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CHAPTER 9

BIOTECHNOLOGICAL, FOOD AND HEALTH CARE APPLICATIONS

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Abstract:

The processing of cereal grains has evolved over the years and a substantial wide range of commodities are produced globally. Primary and secondary processing of cereals results in a wide range of waste material entrapping resources that could be convertible into useful products. Economically, the valorization of cereal by-products streams to obtain high added value compounds is primarily focused on pharmaceutical and cosmetic applications, followed by food and feed uses, elaboration of biomaterials, chemicals and combustibles, and energy production. This chapter provides an overview on the applications of high added value compounds that could be valorized from cereal by products by employing biotechnological approaches such as the production of microbial enzymes. By-products resulting from secondary processing of cereals as in the case of brewing are also presented and their possible applications to the food and the health care industries are discussed.

9.1. Introduction

Cereal grains are a primary human food source since thousands of years ago and a vital source of calories for a large sector of the world population (WHO, 2003). Typically, cereal grains used for human consumption are milled to remove the bran and germ in order to enhance the shelf-life and the sensory attributes and also to produce a wide variety of cereal-based products.

Typical primary processing approaches of cereal grains are aimed at producing a wide range of food commodities. In applications of such processes, it is inevitable that waste and by-product material are also produced. In the case of wheat grains, the bran, germ and endosperm are separated prior to the milling of the endosperm. This is usually done by a sequence of breaking and grinding processes and these components are separated from each other by a series of separating operations (Van Der Borght et al., 2005).

Barely can be either milled or malted. A pearling process is applied to separate surface layers of the grain after a conditioning process and pearl barley could be milled to produce barely flour. Alternatively, barely can also be malted through the germination process to produce a wide range of brewed alcoholic products. The kilning brittle rootlets by-product produced are utilized as a high protein feed.

In the case of rice, the removal of the hard protective husk surrounding the grain is a crucial step. This is done by passing the rice through the spinning rubber roles which results in the high-fibre brown rice. Further fine milling results in the separation of the bran layers from the grain resulting in what is typically known as white rice (Esa et al., 2013).

Corn processing requires the removal of the hull, germ and endosperm prior to milling. Corn can also be processed by wet-milling where the grains are soaked in dilute sulfur dioxide solution. The corn germ is subsequently removed to recover the oil, and the remaining corn germ meal is utilized in feed use.

Cereal by-products are one of the most contributing biodegradable wastes that are drained to landfills with biochemical oxygen demand (BOD) and chemical oxygen demand (COD) levels that can reach up to 5×10^4 and 1.5×10^5 mg/L, respectively, (Pant et al., 2007). Following processing, the energy values for the parent grains are higher than that of mill-feeds. In contrast, the protein contents for the parent grains are commonly lower than that of mill feeds.

The by-products resulting from primary processing have received considerable attention for re-utilization. In particular, the application of biotechnology for the production of high-value added products such as microbial enzymes, with a wide range of applications, provides tremendous opportunities for many industries including food, feed, pharmaceuticals and textile industries. Applications of by-products resulting from secondary processing concern basically products for the promotion of health. Most alcoholic drinks are made by fermentation of grains, fruits or vegetables. Variations in the fermentation process including pre-treatment approaches and further processes such as separation, alcohol concentration, addition of other ingredients and maturation are elements that allow the production of innumerable types of alcoholic beverages. Among them, drinks prepared by means of germinated grain wort fermentation are the most common and called beer. Barley is the preferred and main grain used for brewing, followed by wheat, rice, maize, sorghum, millet and corn. The use of alternative grains is very much influenced by local production of grain types and by any associated economic values. The FAOSTAT report (2014) showed that the quantity of barley processed worldwide to produce beer exceeded 180 million tons, 64 of which were produced in Asia and 42.5 in Europe. Barley used to produce spirits is significantly less than that used for beer. The production of beer world-wide reached 1.96 billion hectoliters in 2015 (Statista GmbH, 2015).

Distilling and brewing industries follow close processes to manufacture alcoholic drinks and generate similar waste streams. In recent years, many food and beverage related processes have been adapting to and adopting a biorefinery approach such as most of the previously called waste streams could be referred to as by-products. The bio-refinery concept refers to a comparison with the refinery industries of petroleum, in which the operational flowchart is

optimized to minimize the production of residues and all the streams generated are reused and valorized (Wu et al., 2008, Xiros et al., 2012, Musatto et al., 2013 and Lynch et al., 2016).

Brewers spent grain (BSG) is the main by-product of the brewing industry, since it constitutes 85% of the total by-products (Nigam, 2017). Annually, around 30 million tons of BSG are generated worldwide (del Río et al., 2013). BSG is the leftover of the processing of dried malted grains after mashing and filtration; mainly malted grains husks and the remaining parts of endosperm (Aura et al., 2013). The composition of BSG is variable and it depends mainly on the type of grains utilized, the brewing processes and any applied pre- or post-treatments of the exhausted grains (Blezinger, 2003). However, BSG is always rich in proteins (14.2-31%), and fibre (70%), particularly polysaccharides, making it a potential by-product for valorization, but also highly susceptible to deterioration induced by the growth of bacteria and yeasts (FAOSTAT, 2014).

9.2. Applications of by-products resulting from primary processing of cereals

The production of microbial enzymes requires a detailed knowledge of pre-treatment methods that should be applied to the by-products before the onset of fermentation to produce the added value microbial enzymes. There have been a number of reviews that discussed in details the different pre-treatments that could be applied and their benefits or shortcomings (Ravindran and Jaiswal (2016a).

9.2.1. Pre-treatment approaches

Most of the cereal by-products are lignocellulosic in nature and thus can be potential substrates for the production of high added value products. Upon hydrolysis, lignocellulosic material releases reducing sugars with applications in bioethanol, biogas, organic acids or enzymes. However, due to the high lignin composition and the crystalline nature of cellulose, pre-treatments are required to achieve the level of degradation necessary for further applications. The need for pre-treatment approaches is particularly important for the production of enzymes. While it might appear possible to recycle cheap carbohydrate sources to produce sugar for enzyme production, the heterogeneous nature of biomass carbohydrate sources is not an efficient source of nutrients for the enzyme producing microorganisms. Additionally, the fact that cereal by-products contain also other substances that may act as inhibitors for microbial growth, can compromise the fermentation process and the expected yield thus raising the production cost for the desired products and so defeating the initial concept of using cheap available resources.

The aim of pre-treatments is to facilitate or increase the efficacy of lignocellulose hydrolysis by the removal of lignin and hemicellulose which subsequently will lead to improvement in the accessibility to the cellulose-rich fractions mainly by microorganisms and ultimately leads to the production of new added value products.

The pre-treatment process is considered the most expensive step in the valorisation of lignocellulosic by products reaching up to 30% of the total cost (Yang et al., 2008).

Ravindran and Jaiswal (2016b) classified pre-treatments into physical, chemical, physio-chemical and biological methods.

Physical methods such as grinding are directed towards size reduction through the formation of ultra-fine powder resulting in the increase of the surface area accessible for the conversion of cellulose to glucose as reported by Silva et al., (2012). The application of ultrasound results in the breakdown of the complex network of polymerisation in biomass thus facilitating better enzymatic degradation. Centrifugal grinding is another physical pre-treatment approach which provides a superior grinding capacity in comparison with ordinary grinders by exerting multiple effects via impact and shear.

Chemical pre-treatments include alkaline or acid hydrolysis usually performed at temperatures reaching as high as 160°C. The primary objective is to achieve hydrolysis of the hemicellulose using acids such as sulfuric acid thus making cellulose more susceptible to enzymatic degradation (Martin et al., 2015). Alkaline potassium permanganate, generally considered as safe in comparison to ozone and ionic liquids, has the ability to break the ester and the ether bond between lignin and the carbohydrate fraction,

The application of hot water and steam explosion are some of the oldest and yet the most effective pre-treatments methods to break down the lignin-carbohydrate complex. Such approaches do not require the utilization of corrosion resistant reactors or chemicals and the formation of toxic compounds is almost absent (Jiang et al., 2015). The process requires the biomass to be exposed to high pressure and temperature up to 230°C.

Biological pre-treatments rely on biological agents to delignify the material. This includes a consortium of microbial cultures such as *Lactobacillaceae* which is the dominant species in biomass fermentation during ensilation (Chen et al., 2007). Fungal species, including white rot and brown rot fungi, are capable of breaking down and demineralizing lignin as reported by Jensen et al., 2001. Unlike physical and chemical pre-treatment methods, biological pre-treatments do not involve high temperature or pressures and do not require the utilization of acids, alkali or other reactive species thus requiring less capital cost with high environmental benefits. One of their drawbacks, and due to its biological nature, is that there is little limited control over the process in addition to being characterized as slow process and time consuming.

There is a need for integrated technologies for an efficient pre-treatment and the maximal recovery of energy from lignocellulosic by-products. On the other hand, the use of untreated lignocellulosic biomass as substrate can cause several processing issues such as agglutination and clogging in addition to microbial degradation resistance as highlighted by Fan et al., (2013).

9.2.2. Application of biotechnology to cereal by-products

According to Karl Ereky who used the term biotechnology in 1919 for the first time, biotechnology is a process by which raw material are converted to new products by the living microorganisms such as yeast, bacteria and fungi. Biotechnology offers many treatment methods to overcome environmental pollution and recovering value from waste.

Industrial enzymes:

The global enzyme industry is growing at a rapid rate. In 2013 it was worth $4.8 million and is expected to reach $7.1 billion by 2018 covering a wide range of products and processes (BCC, 2014). This significant growth stems from the basic characteristics of enzymes of being substrate and product specific, moderate reaction conditions, minimal by-product formation and high yield. However, enzyme manufacturing is a costly process, and up to 30% of the total production cost is attributed to raw material. Large-scale enzyme production is a capital intensive process, particularly purified enzymes which are finding extensive applications in a wide range of manufacturing processes covering food, beverages and textiles. The lignocellulosic material associated with cereal by-products can replace conventional carbon sources in media preparations for industrial microbial processes such as enzyme production (Ravindran and Jaiswal, 2016). Microorganisms produce enzymes that are specific to each polysaccharide component for the release of sugars that can be metabolized for growth energy and cell maintenance. Screening of microorganisms is critical for identifying new viable enzymes. For example, thermophilic microorganisms are of particular interest for enzyme production due to the reduction in contamination risk associated with bioprocessing operations conducted at higher temperatures. Additionally, as highlighted by Vandenberghe et al., (2016) high temperatures enhances the solubility of the substrates resulting in increased product yield. When evaluating the safety of an enzyme, the safety of the production strains is the primary consideration and microbial strains should be selected based on being non-pathogenic and non-toxigenic. A rather small group of bacterial and fungal strains have been used in the production of industrially important enzymes, primarily *Bacillus subtilis*, *Bacillus lichenifrmis, Aspergillus niger* and *Aspergillus oryzea*. This group of microorganisms can be genetically manipulated easily and are also known for their ability to overexpress proteins of interest in the fermentation media, thus making them very desirable as hosts for a variety of enzymes.

The microbial production of enzymes is mostly carried out both by submerged (SmF) and solid state fermentation (SSF). SmF is any aqueous fermentation process occurring in the presence of liquid substrate, whereas, SSF is any fermentation process occurring in the absence or near-absence of free water by employing a natural substrate as a solid support (Ng et al., 2010). Solid state fermentation (SSF) is currently being considered as a preferred method of enzyme production as it stimulates the natural growth of microorganisms on a moist insoluble solid support in the absence (or near absence) of free water. SSF compared to SmF is more simple, requires lower capital, has superior productivity, reduced energy requirement, simpler fermentation media and absence of rigorous control of fermentation parameters, uses less water and produces lower wastewater, has easier control of bacterial contamination and requires low cost for downstream processing. In the SSF process, the solid substrate not only supplies the nutrients to the culture, but also serves as an anchorage for the microbial cells. Agro-industrial

residues are generally considered the best substrates for the SSF processes. These characteristics offer several opportunities to be applied to cereal by-products.

9.2.3. Amylases

Amylases (E.C. 3.2.1.1.) are hydrolytic enzymes responsible for the complete hydrolysis of starch. Currently, amylases have a great importance in biotechnology with a wide spectrum of applications, such as in textile, leather, detergents, food, brewery, starch processing and in various strategies in the pharmaceutical and chemical industries for the synthesis of optically pure drugs and agrochemicals (Kammoun et al., 2008). There is a high industrial demand for these enzymes which necessitate the need for efforts to explore various technologies to maximize yield at a low cost. Kunameni et al., (2005) investigated cereal by-products (including millet cereal, crushed wheat, corn cobs, wheat flakes, barely bran and rice bran) as potential substrates for amylase production upon the application of SSF. From all the residues investigated, wheat bran resulted in maximum enzyme production, showing an enzyme activity of 261 U/g followed by millet cereal. Combining cereal by-products as substrates seems to enhance enzyme yield as observed by Singh et al., (2012) whereby they have observed that a mixture of rice bran and wheat bran in the ratio of 1:2 gave maximum enzyme production of 549.11 U/mL using Streptomyces.

It is also important to investigate the role of the starter culture used on the enzyme yield production. In a study by Hussain (2014), six different cereal-by products (rice straw, rice bran, corn flakes, wheat bran, wheat flakes and grinded wheat kernel were individually submitted to a novel isolate *A. oryzae* IIB-6 for extracellular enzyme production of α-amylase. Among all the residues, wheat bran gave the maximum yield of 7800 U/g dried solid at 80% moisture content during a 72hour of solid state fermentation.

In a study carried out by Hashemi et al., (2011) brewer's spent grain (BSG) was used as a solid substrate for the production of α-amylase by *Bacillus* sp. KR-8104 in a submerged fermentation system. The utilization of up to 5% (w/v) of BSG in the growth media showed up to 5 fold enhancement in the enzyme production. Additionally this study showed that simultaneously adding BSG, omitting dextrin, and reducing the other ingredients concentration in the culture medium improved the production of α-amylase and made the production process more economical.

Gruel, which is wheat milling by-product, was also investigated by Kammoun et al., (2008) using submerged fermentation as the sole carbon source for the production of α-amylase from *Aspergillus oryzea* showing an enzyme production of 151.1 U/mL. Upon the application of solid state fermentation, Anto et al., (2006) showed that using paddy rice processing by-products a substantial increase in the α-amylase enzyme was observed reaching 211.5 U/g dried solid.

9.2.4. Proteases

Protease (EC.3.421.40) is a hydrolytic enzyme, which catalyzes the hydrolysis of the peptide bonds linking the amino acids in the polypeptide chain (Gupta et al., 2002). They account for nearly 60% of the total industrial enzyme market and microbial proteases account for approximately 40% of the total worldwide enzyme sales. There are four types of proteases (serine, aspartic, cysteine and metallo proteases). Alkaline proteases have a wide range of industrial applications as in detergent, leather, pharmaceutical and chemical industries. In the food industry, proteases are widely applied in meat tenderization, cheese production and in bakery products. While proteases can be found and extracted from a range of natural resources, the preferred production route relies on microbes, including bacteria, yeast and fungi (Mabrouk et al., 1999). In particular, *Bacillus* species possess remarkable biotechnological value due to the nonpathogenicity of its various species and the ability to produce extracellular protease in large amounts.

Bacteria generally synthesize proteolytic enzymes, when grown in protein medium, however, very few bacteria can also produce proteases on protein-free media. Although cereal by-products are generally characterised as having a lignocellulosic nature, with moderate amounts of protein, there has been reported studies indicating the feasibility of producing microbial proteases from such wastes.

Johnvesly et al., (2002) investigated the production of protease from the thermoalkaliophilic Bacillus JB-99 using a range of agro-industrial residues and found that maximum enzyme production was obtained from pigeon pea waste at 12,430U/mL. A range of agro-wastes were employed for thermoalkali-stable protease production from *Bacillus subtilis* K-1 under solid-state fermentation. Agricultural residues such as cotton seed cake supported maximum protease production (728 U/mL, which was followed by gram husk (714 U/ mL) mustard cake (680 U/ mL) and soybean meal (653 U/ mL).

9.2.5 Cellulases

Cellulases are a family of enzymes that hydrolyze the β-1,4 linkages of cellulose. To degrade cellulose to glucose, at least three classes of cellulolytic enzymes are required, including (1) endo-glucanase (EC 3.2.1.4) which randomly cuts cellulose chains to yield glucose and cello-oligosaccharides; (2) exo-glucanase (EC 3.2.1.91) which exolytically attacks the reducing or non-reducing end of celluloses to yield cellobiose; and (3) beta-glucosidase (EC 3.2.1.21) which hydrolyzes cellobiose and cello-oligosaccharides to yield glucose (Ng et al., 2010). Cellulases are used commercially in many applications, such as in detergents, pulping and textile industries, as animal feed additives for improving the nutritional quality and digestibility, and for clarification of fruit and vegetable juices (Rolle, 1998). Application of this enzyme in detergent, leather and paper industries demands identification of highly stable enzymes active at extreme pH and temperature. Cellulases have also been considered to play a critical role in the generation of potentially sustainable energy sources such as glucose, ethanol, hydrogen and methane (Bayer et al., 2007). A reduction in cellulase production cost, improvement in cellulase efficiency and an increase in sugar yields are all vital to reduce the

processing costs of biorefineries. The use of abundantly available lignocellulosic crop residues, such as rice straws, rice bran and wheat straws offers potential substrates to achieve this goal.

Bacteria and fungi have been exploited to produce a wide variety of cellulases. Fungi tend to be heavily utilized due to their ability to produce higher yields of cellulolytic enzymes that tend to be less complex than bacterial cellulases and further easily extracted and characterized. In the last decade, it has been noted that the isolation and characterization of novel cellulase from bacteria is gaining a lot of momentum. Miranda et al., (2009) highlighted that this could be attributed to:

i) bacteria often have a higher growth rate than fungi allowing for higher recombinant production of enzymes,
ii) ii) bacterial cellulases are often more complex and are in multi-enzyme complexes providing increased function and synergy
iii) iii) bacteria inhabit a wide variety of environmental and industrial niches like thermophilic or psychrophilic, alkaliphilic or acidiophilic and halophilic strains, which produce cellulolytic strains that are extremely resistant to environmental stresses.

These strains can survive and produce cellulolytic enzymes in the harsh conditions which are found to be stable under extreme conditions and which may be used in the bioconversion process. Upon surveying the literature both fungi and bacteria are equally utilized in the production of cellulases and in many situations this can be dictated by available strains and resources.

A high yield of β-glucosidase (EC 3.2.1.21) of 159.1 U/g-solid activity on 4-nitrophenyl β-d-glucopyranoside (pNPG) was achieved by rice bran-based solid-state fermentation (SSF) upon using the fungus *Penicillium citrinum* YS40-5. The enzyme was both thermophilic and acidophilic at the optimized temperature and pH of 70°C and 5.0, respectively. Over 95% of the original β-glucosidase activity was maintained after a prolonged storage at ambient temperature for 4 weeks and showed comparable activity to that of commercial β-glucosidase (Ng et al., 2010).

Cellulolytic enzymes from fungi have been extensively studied but their cellulase system is, in general, deficient in β-glucosidases, causing accumulation of cellobiose, which results in repression and end product inhibition of the enzymes (Adsul et al., 2006). A lot of efforts have been devoted to preparing mutagenesis and genetic modifications and obtaining improved strains capable of producing high levels of cellulases. This is an observed trend currently in the production of microbial enzymes in order to maximize yield production. Liu et al., (2011) used *Penicillium decumbens* strain L-06 to prepare mutants with ethyl methane sulfonate (EMS) and UV-irradiation in order to produce cellulolytic enzyme efficiently. A mutant strain ML-017 exhibited an increased cellulase activity upon solid state fermentation using rice bran as a substrate. The activity observed increased by 44.12% in comparison to the original un-mutated strain.

Gomathi et al., (2012) studied submerged fermentation of wheat bran by *Aspergillus flavus* for the production of carboxy methyl cellulose. Wheat bran showed a high cellulase yield which could be attributed to its high nutritional content of: proteins (1.32%), carbohydrates (69%), fats (1.9%) and fiber (2.6%).

9.2.6. Xylanase

Xylan is a biopolymer comprising of D-xylose monomers which are linked through *β*-1,4-glycosyl bond, and is found abundantly in lignocellulosic biomass. Due to the complex structure of xylan, many different enzymes are needed for its complete degradation, but xylanase (EC 3.2.1.8) is sufficient to break down the xylan backbone. Xylanases are produced by different species of microorganisms and have been studied mostly from bacteria, actinomycetes, and fungi (Bajaj and Singh, 2010; Kumar et al., 2009 and Sanghi et al., 2008).

The xylanolytic enzymes of microbial origin have significant applications such as in animal feed preparation, food processing, textiles, pharmacy, paper, and pulp industries (Dhiman et al., 2008). Additionally, xylanases are utilized efficiently in the production of several valuable products such as xylitol and ethanol (Beg et al., 2001).

The utilization of agro-industrial by-products have been examined as possible carbon sources substrates for the production of xylanases. For example, wheat bran, rice bran and soy meal, have been found to be suitable substrates for xylanase production (Kumar et al., 2009 and Raj et al., 2013). Xylanase production of about 249.308 IU/mL was achieved at pH 8 and 37°C, within 48 h submerged fermentation in enzyme production medium supplemented with 2% (w/v) oat bran as an optimum carbon source (Sepahy et al., 2011). In a study conducted by Sanghi et al. 2008, Alkalophilic *Bacillus subtilis* ASH was reported to produce high levels of xylanase using easily available inexpensive agricultural waste residues such as wheat bran, wheat straw, rice husk, sawdust, gram bran, groundnut and maize bran in solid-state fermentation (SSF). Among these, wheat bran was found to produce the highest levels of xylanase as was also indicated by Kapoor et al., (2008). This could be due to the fact that it contains 54 % carbohydrates (pentoses and hexoses), 14 % protein, minerals, amino acids, and vitamins which supports the growth of the bacterium and hence xylanase production

9.2.7. Vanilin

Vanillin (4-hydroxy-3-methoxybenzaldehyde) is one of the most important and widely used flavours in the food industry and it is the main component of vanilla. *Vanilla planifolia* is the main source for natural vanillin and it can only supply less than 1% of the annual market demand. Lignin and guaiacol provide the majority of the commercial vanillin which is obtained through microbial conversion of substrates such as eugenol or ferulic acid (Ramachandra et al., 2000). Cereal bran represents one of the most widely produced agricultural processing by-products and are considered as a rich source of ferulic acid.

The cell walls of wheat, maize and rice, contain high amounts of ferulic acid which is linked to arabinoxylans through ester links. In order to utilize cereal bran in vanillin production, it is important to break the ester bond either chemically or enzymatically. Enzymatic release of ferulic acid is preferred as the vanillin produced could be labelled as natural. The utilization of enzymes such as cellulases and hemicellulases (as presented in the previous section) will degrade the polysaccharide fraction of the plant thus providing access of feruloyl esterases to their substrate, allowing a nearly complete recovery of ferulic acid from wheat bran and corn bran as reported by Faulds et al., (2004) and Shin et al., (2006). Di Gioia et al., (2007) explored the possibility of obtaining vanillin from the bioconversion of ferulic acid by the enzymatic

hydrolysis of wheat bran through the application of an engineered *E.coli* strain and achieving bioconversion yields up to 70%.

9.3. Secondary processing by-products

BSG has been generally under-utilized in low value applications or either considered as a waste. First uses reported were as landfill or fertilizer (Gupta et al., 2010). It also shows suitability as ingredient for animal nutrition formulation because of its protein and fibre content, especially for cattle (Gupta et al., 2010, Mussatto et al., 2006 and Crawshaw, 2004). Other applications include the valorization of BSG as a composting material for gardening and the horticultural sector, for energy generation by means of production of biogas or direct BSG incineration, as a substrate to grow mushrooms for food utilization and biochemical application, or to produce chemical products by fermentation such as ethanol (Nigam et al., 2017 and Mussatto et al., 2006).

Buffington (2014) reported a pyramid of prime applications for BSG, from high to low economic value as follows: pharma and cosmetics, food and feed, bioplastics and biopolymers, bulk chemicals and fuels, and energy and heat production. The increased interest in the valorization of BSG to recover high-added value compounds with a variety of applications saw an increase of about 5 times increment in the number of scientific publications since 2006 as seen in Figure 1.

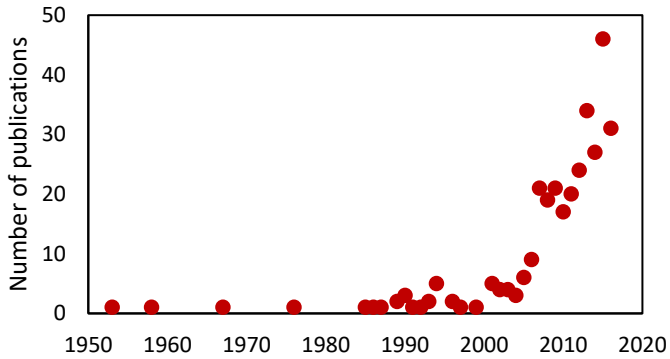

Fig 1 Number of papers retrieved in Scopus with the keyword 'brewers spent grain' and 'novel applications', 'nutraceutical', 'cosmetic', 'cosmeceutical', 'pharmaceutical', or 'food'.

The phenolic compounds, fibre, protein and β-glucan rich fractions of BSG have been reported to be the most valuable components due to their potential health-promoting and techno-functional properties (Lynch et al., 2016), which could be utilized as ingredients for functional food, fortified food products and pharmaceutical and cosmetic formulations (Gupta et al., 2010). The inclusion of BSG fibre fraction as an ingredient in bakery products and extruded snacks helps to enhance not only the total fibre content but also the protein and fat content (Stojceska et al., 2008a and Nigam et al., 2017). Fibre-enriched food products could aid in the prevention of diverse disorders such as cardiovascular diseases, diabetes, cancer and gastrointestinal ailments (Nigam et al., 2017). Some fibre fractions of BSG also show a prebiotic capacity (Bamba et al., 2002). Phenolic acids exhibit antioxidant activity, anti-cancer,

anti-apoptotic in immune cells, anti-inflammatory, anti-atherogenic functions and immunomodulatory effects (McCarty et al., 2013 and Lynch et al., 2016).

9.3.1 Extraction techniques and derived fractions

In order to use the fractions and components present in BSG, they need to be released and isolated, or at least concentrated. The hydrolysis of BSG results in the breakdown of the cellulose yielding easily fermentatable carbohydrates such as glucose and non-cellulosic polysaccharides such as xylose, mannose, galactose and arabinose, as well as arabinooligoxylosides, acetic and hydroxycinnamic acids, proteins, and phenolic compounds (Mussatto et al., 2006 and Lynch et al., 2016). The main conventional extraction techniques utilized including physical and chemical processes have been covered in chapter 7.

Novel extraction techniques can be classified as physical, chemical, biological and have been recently reviewed by Guido & Moreira (2017): rapid microwave assisted derivatization and extraction as reported to obtain phenolics (McCarthy et al., 2013b and Moreira et al., 2012); ultrasound-assisted extraction that allowed reduced times and lower energy consumption to produce arabinoxylan-rich extracts when compared to the conventional alkaline extraction (Reis et al., 2015); autohydrolysis as part of the purification process of arabinoxylo-oligosaccharides from BSG as reported by Gómez et al., (2015); pressurized fluid extraction and enzyme-assisted extraction as in the production of hydroxycinnamic acids by means of the action of the enzyme ferulic acid esterase from *Lactobaccillus acidophilus* K1 (Lynch et al., 2016); and fermentation applications. In particular, the biological approaches tend to be of interest as they allow the production of new compounds not present initially in the raw biomass.

9.3.2. Fractionation, refining and purification techniques of BSG fractions and derived products

Conventional sieving or screening can be simple and efficient techniques to separate fractions based on their gravimetry or particle size have been widely applied. Fonseca et al., (2014) reported this step as a pretreatment of the sample to be more susceptible to ultrasounds breakage and further acid hydrolysis of BSG to obtain pentoses. Ishiwaki et al., (2000) produced three fractions of different particle size with diverse bioactive or functional activities. Liquid to liquid extraction with ethylacetate was reported to refine an alkaline extract to produce a phenolic-rich fraction (Reis & Abu-Ghannam, 2013).

Membrane separation and resins are commonly used for refining fractions, as Gómez et al., (2015) used for the production of arabino-xylooligosaccharides. Liquid to liquid extraction with solvents of different polarity is a common practice, especially for phenolic recovery from brewing streams (McCarthy et al., 2013b). Supercritical CO_2 technology is useful and specific for fat fractions refining, as reported by Kitrytė et al., (2015) for collecting lipidic fractions with higher phenolic content, antioxidant and antiradical activities than similar fractions of malted barley.

Table 1. Main extraction and refining technologies for valorization of brewers' and distilling spent grain.

Extraction / Refining technology	Spent grain or fraction	Fractions / Compounds	Bio- and functional activities	References
Extraction				
Solid to liquid extraction	BSG	60% acetone extract: 9.90 mg GAE/g BSG	Antioxidant	Meneses et al., 2013
Acid hydrolysis	Dried distilling grains	Residual fibre Pentoses-rich fraction	Suitable for feed formulation Base of production of bioproducts	Fonseca et al., 2014
Saponification or alkaline hydrolysis	BSG	Phenolic fraction: 145.3 mg/L ferulic acid; 138.8 mg/L p-coumaric acid	Antioxidant	Mussatto et al., 2007
Microwave assisted derivatisation and extraction	BSG	Phenolic-rich fractions	Antioxidant	McCarthy et al., 2013b and Moreira et al., 2012
Ultrasound-assisted extraction	BSG	Arabinoxylan-rich extracts		Reis et al., 2015
Autohydrolysis	BSG	Purified arabinoxylo-oligosaccharides		Gómez et al., 2015
Enzyme-assisted extraction (enzyme ferulic acid esterase from *Lactobaccillus acidophilus* K1)	BSG	Hydroxycinnamic acids		Lynch et al., 2016
Refining				
Liquid to liquid extraction	BSG	Phenolic fraction	Antioxidant	Reis & Abu-Ghannam, 2013
Ultrafiltration with membranes	Pale BSG Black BSG	Low molecular weight phenolic fraction High molecular weight phenolic fraction	Antioxidant	Piggot et al., 2014

Enzymatic extraction with alcalase	BSG	Protein-rich fraction	Anti-inflammatory (reduce IL-6 production when added to milk and subjected to simulated digestion)	Crowley et al., 2015
Membrane fractionation	Protein rich enzymatic fraction	5-kDa retentate, and 5-kDa and 3-kDa permeates		
Supercritical CO_2 extraction	BSG	Lipidic fractions	Antioxidant and antiradical	Kitrytė et al., 2015

BSG, Brewers' spent grain; GAE, gallic acid equivalents as phenolic standard; IL, interleukin.

9.3.3. Food applications

Enriched food products

Due to its nutritional composition, especially for its protein, fibre and mineral content, BSG and its derivatives are potential ingredients for incorporation in food preparations as they are considered as safe ingredients for food utilization. Proteins, phenolic compounds and fibre have been reported to retain their functional and biological activities after fermentation.

The incorporation of BSG as an ingredient in the bakery industry and in particular in bread making has been significantly studied in recent years. The BSG, mostly incorporated as flour, seemed to work well with other bakery ingredients and the equipment typically available in the bakery industry. Typical applications included breads, mixed grain cereals, seeds, fruit and vegetable loaves, cakes, muffins, cookies, waffles, pancakes, tortillas, snacks, doughnuts, and brownies (Gupta et al., 2010). BSG incorporation enhanced the fibre, protein and fatty acid content of the developed baked products. Protein and fibre-rich snacks and cookies prepared by adding BSG in a percentage of 40% to the dough maintained the physical properties as the controls with no BSG (McCarthy et al., 2013b). An addition of 40% of BSG to cookies increased the fibre content ten folds (Nigam et al., 2017). Stojceska & Ainsworth (2008b) found an increase of fibre from 2.3% to 11.5%, and from 3.4% to 4.4% in fat content in bread enriched with 30% of BSG. The compositional analysis of breadsticks containing 15% of BSG enhanced the fibre content from 6% to 15%, and an addition of 35% of BSG showed an increased protein content from 14.3% to 18.4% (Ktenioudaki et al., 2012). BSG was also reported as an ingredient in meat derived products. For example, the manufacturing of Frankfurters with 5% of BSG (coarse particle size 425-850 μm) increased the total fibre content by 4.6% and the moisture by 3.5%, while decreasing the fat content by 3% (Özvural et al., 2009).

Additionally, the sensory aspects of BSG incorporation in baked products were also investigated because BSG can confer brown colour and strong flavour that could affect the consumer acceptance of the products. For example, the use of BSG as an ingredient in baked snacks in a concentration of 20-30% notably affected the appearance (colour, volume, structure) and texture (crispiness, moisture) (McCarthy et al., 2013b). Ktenioudaki et al., (2013) reported that the incorporation of BSG in a wheat dough resulted in the production of

low volume and non-fluffy baked products, and some of the measured rheological properties that were negatively affected include: biaxial extension, peak and final viscosity, uniaxial extensibility, holding strength, and breakdown and setback. In an attempt to explain these somehow unacceptable textural attributes, Roth et al., (2016) proposed that the addition of dried BSG to the dough might inhibit the activity of *Saccharomyces cerevisiae* (responsible for dough fermentation and increasing the volume and modifying the texture) due to containing furfural generated during the drying process. Nevertheless, previous studies reported successful results with respect to consumer acceptance of baked goods with BSG additions of 5-15% to the flour mix (Gupta et al., 2010 and Prentice et al., 1978). The organoleptic parameters are of significant importance to consumer acceptance of the products, even though they could present enhanced nutritional value such as enriched protein and fibre content and some stable physical properties during storage.

BSG has also been incorporated in the development of extruded snack products. It seems that an addition of less than 30% of BSG to the dough in combination with rice flour was suitable to produce snacks with comparable appearance to those prepared with rice flour only (Nascimento et al., 2017). Stojceska et al., (2008a) added 30 % BSG in the mixture to prepare extruded ready-to-eat snacks at different processing conditions. The fibre content in the final products increased at least a 10% compared to the snacks with no BSG added.

Rice distilling waste (lees) is also used in the food industry. It was reported as an ingredient to produce seasonings and flavours, as part of diverse types of foods and beverages, and as a source of amino acids (Shao et al., 2011). It showed suitability as a source to extract vegetal protein for human consumption or as an ingredient in developing functional foods (Hua, 2009). Manaois et al., (2012) produced a flour from rice wine lees by washing all the soluble material from the residue, followed by drying and milling the retentate. A high percentage of crude protein (42-54%) and dietary fiber (11.20-12.15%), parameters which vary depending on the drying process, make this flour a nutritious ingredient. Rice cookies and polvoron prepared with up to 50% of flour from rice wine lees were considered as acceptable by a sensory panel (Manaois et al., 2012).

The polyphenolic fraction mainly p-coumaric, ferulic, sinapic and caffeic acids can be used as antioxidant ingredients in the preparation of food products and functional foods to extend their shelf-life and to confer free-radical protection in humans (Moreira et al., 2013). The antioxidant compounds delay the lipid oxidation of fatty acids in food products. Vieira et al., (2017) reported polyphenols as functional ingredients from BSG protein hydrolysates obtained by alcalase extraction, with an *in vitro* free-radical scavenging activity of 0.083 mg GAE/mg db for total phenolic content or 0.101 mg trolox equivalents/mg db.

The xylitol obtained by microbial fermentation of the xylose present in BSG acid hydrolysates is an artificial alternative sweetener which can be used in food and health care applications, such in sugar-reduced food products and snacks for diabetics (Mussatto et al., 2006 and Nigam et al., 2017). Niemi et al., (2012) reported BSG to have a fatty acid content of 11%, composed mainly of linoleic (18:2), palmitic (16:0), and oleic acids (18:1), which could confer a potential added value to BSG as a food ingredient.

Techno-functional properties

Gupta et al., (2010) compiled a series of properties that define BSG flour as a potential ingredient suitable for food formulations and classified them as: easy to mill, up to 50% less calorie content when compared with the majority of whole cereal flours, high water activity leading to low fat absorption, a source of protein, fibre and minerals such as Ca, P, Fe, Cu, Zn and Mg, among others, and a roasted aroma and tan colour.

Some extracts obtained from BSG showed functional properties of potential interest for food applications. For example, BSG has been reported to have anti-foaming properties when reused as additive during the brewing process, and an increase in fermentation yield was reported when it was added to the wort during beer production (Mussatto et al., 2006). Kotlar et al., (2013) referred to a protein-rich hydrolysate, obtained by enzymatic extraction with an enzyme from *Bacillus cereus,* which could have food applications such as delaying the oxidation of fat and protein enriched food products. A protein-enriched hydrolysate, produced by enzymatic extraction with Alcalase, Corolase PP, Flavourzyme and Promod 144MG of an alkaline extract of BSG was proposed as a techno-functional ingredient in food formulations. Its addition enhanced the foaming properties up to 1177% in food products above pH of 8.0 (Connolly et al., 2014). High quality protein hydrolysates were obtained with the utilization of an extracellular peptidase from *Bacillus cereus* sp. from BSG. The resulting products showed improved water/oil holding capacity, emulsifying properties and foaming expansion capacities when compared to non-hydrolyzed BSG fractions. Additionally, the produced hydrolysates presented beneficial rheological properties that make them competing ingredients for food preparation formulas (Kotlar et al., 2013). Negi & Naik (2017) reported the use of a non-prolamin protein fraction isolated from wheat spent grain as an emulsifier ingredient in food applications to produce stable emulsions. The emulsification activity index and the emulsion stability of the preparations were pH dependent, with values of 245.6 m^2 g^{-1} and 9.8% at pH 4, and 560 m^2 g^{-1} and 90% at pH 9, respectively. Similarly, the addition of BSG as ingredient to produce fiber-enriched fresh egg pasta sheets made the dough more elastic, and showed lowered average break strain with respect to the control samples (from 26% to 54% for raw sheets and from 25% to 54%, for cooked sheets) (Cappa & Alamprese, 2017).

Protein-rich extracts and isolated proteins from distillers' dried grains were reported to enhance the flexibility in the production of protein-based biodegradable films for food purposes when added in a concentration up to 40% to the mixture. Nevertheless, the mechanical properties and the scaling process need to be improved to make them real alternatives to the conventional polymers made from petroleum (Chatzifragkou et al., 2015).

Health care applications

The incorporation of bioactive fractions from BSG in food products could provide potential for producing functional foods with possible health promoting properties (Fărcaş et al., 2014). Additionally, these bioactive fractions were reported to have biological activities such as immunomodulatory, anti-inflammatory and antioxidant properties when tested *in vitro* and in

cell culture experiments. Similarly, their capacity in preventing cardiovascular disorders, diabetes, cancer and gastrointestinal disorders were reported (McCarthy, 2013 and Nigam et al., 2017), in addition to their prebiotic and intestinal epithelial cell protection properties (Bamba et al., 2002 and Wilhelmson et al., 2009).

Connolly et al., (2014, 2017) reported the dipeptidyl peptidase-IV (DPP-IV) and angiotensin-converting enzyme (ACE) inhibitory activities of a protein-enriched fraction from BSG obtained by enzymatic hydrolysis with alcalase. This fraction was proposed as ingredient to produce functional foods with capacity to control hypertension and type 2 diabetes. McCarthy et al., (2013a) reported that some proteic fractions of BSG showed immunomodulatory activity by reducing IFN-γ production in Jurkat T cells. Higher molecular weight (>5 kDa) and unfractionated hydrolysates of BSG demonstrated greatest anti-inflammatory effects, while fractionated hydrolysates showed antioxidant activity by the SOD activity assay. These activities were also observed when the fractions were added to food formulations, such as yogurt, chocolate drinks and snacks, and further tested by simulated gastrointestinal *in vitro* digestion (McCarthy et al., 2013). In fact, the fortified yogurt protected against H_2O_2-induced DNA damage in Caco-2 cells (McCarthy, 2013 and McCarthy et al., 2015). Similarly, the addition of a BSG protein-rich fraction into milk, obtained by alcalase extraction and refined by membrane technology, provided anti-inflammatory activity by reducing the production of interleukin-6 (IL-6) in Jurkat T cells when subjected to simulated gastrointestinal digestion (Crowley et al., 2015). Vieira et al., (2017) reported that a protein hydrolysate obtained by alcalase extraction of BSG exhibit a total phenolic content of 0.083 mg GAE/mg db and an *in vitro* free-radical scavenging activity of 0.101 mg trolox equivalents/mg. Enzymatic fractionation of BSG with enzymes isolated from brewer's spent yeast (BSY) produced protein rich fractions with particle size <10 KDa, and showed a protective effect against free-radical induced cytotoxicity in Caco-2 and HepG2 cell lines (Vieira et al., 2017).

Some polyphenols, mainly hydroxycinnamic acids such as ferulic acid, p-coumaric acid, caffeic acid extracted from BSG were reported as potential functional food ingredients because of their antioxidant capacity (McCarthy et al., 2013). They are able to neutralize free radicals, reduce the oxidative stress and its damaging effects in humans, and inhibit the generation of new free radicals by controlling their production. Phenolic acids have anti-inflammatory, anti-atherogenic and anti-cancer activities, and show DNA protective effects against the genotoxic effects of the oxidants hydrogen peroxide (H_2O_2) and 3-morpholinosydnonimine hydrochloride (SIN-1), possibly by means of Fe chelation (MacCarthy et al., 2012). Furthermore, McCarthy et al., (2013a) found that phenolic compounds from BSG might have immunomodulatory effects because they can reduce the production of pro-inflammatory cytokine IFN- γ.

Due to their non-toxic character, lipids obtained from BSG are already widely used for oral drug delivery applications as carriers and excipients. In particular, fatty acids, triglycerides and phytosterols showed important nutraceutical, pharmaceutical and cosmetic properties (del Río et al., 2013). Phytosterols, tocopherol, and unsaturated PUFAs extracted from sorghum brewer's grains showed protection against the development of cancer, diabetes and coronary diseases (Singh et al., 2003). Some polysaccharides could have a role in preventing and alleviating diabetes. For example, the arabynoxylans and the (1-3, 1-4)-β-D-glucans isolated

from spent wheat endosperm were reported to reduce postprandial glycaemic responses (Steiner et al., 2015).

In addition, Kim et al., (2011) showed that the consumption of brown rice lees from rice wine during a 12-week period reduced the waist circumference in patients with type II diabetes.

Apart from the direct use as ingredients in health care and food formulas, BSG and some of its derivatives obtained by hydrolysis and other pretreatments could be utilized as substrates for biotechnological processes. They have been reported as precursors for complex processes, mainly as carbon sources, such as microbial fermentation for the production of high added value compounds (i.e. lactic acid, ethanol, xylitol, arabitol, pullulan) (Mussatto et al., 2006 and Aliyu & Bala, 2011), and as a cheap alternative substrate for the production of enzymes, microorganisms and single cell protein at high production yields and low cost (Xiros and Christakopoulos, 2012 and Nigam, 2017). For instance, ferulic acid was reported to be utilized as a substrate in the microbial process to produce the aromatic compound bio-vanillin, which has food and health care applications, such as a flavoring agent in foods, drinks and pharmaceuticals (Chatzifragkou et al., 2015). Knob et al., (2013) reported the production of a *Penicillium glabrum* xylanase using BSG, which showed an optimum activity at pH 3.0 to be useful in biotechnological processes including clarification and maceration of juices and wines.

Pre- and probiotics

A prebiotic product is a carbohydrate-based fraction characterized as being non-digestible by humans, but can be fermented by the gastrointestinal bacteria leading to the increased growth in health beneficial bacteria such as bifidobacteria and lactobacilli.

BSG is a good source of arabinoxylooligosaccharides that are typically isolated by an autohydrolysis process and further refined by enzymatic hydrolysis and ion exchange technology. Gomes et al., (2015) reported that arabinoxylooligosaccharides isolated from BSG performed slightly better as prebiotics than fructooligosaccharides when administrated to elderly people. The fermentation of arabinoxylooligosaccharides produced short chain fatty acids and increased the population of bifidobacteria and lactobacilli.

The incorporation of fibre in the diet improves the speed of stool formation and increased fecal weight because of its high water-holding capacity (Bamba et al., 2002). It also provokes a faster elimination of undesirable products and fat and cholesterol excess from the colon (Gupta et al., 2010).

Germinated barley, containing hemicellulose-rich fibre fraction, as obtained from BSG through milling and sieving had an effect in maintaining the epithelial cell condition and renewing the intestinal mucosa in patients with colonic injury such as ulcerative colitis (Kanauchi et al., 2001, Bamba et al., 2002 and Wilhelmson et al., 2009). This fraction enhanced the *in vivo* production of fatty acid chains, such as butyrate by *Bifidobacterium* and *Eubacterium* spp., which can suppress the epithelial nuclear factor kB-DNA binding activity and therefore prevent or alleviate the condition of colonic injury in human patients.

BSG can be used as a low cost nutrient-rich substratum for the growth of microorganisms to be used as probiotics. Novik et al., (2007) obtained high yields and viability of probiotic bacteria using hydrolysable proteins and polysaccharidic fractions as substratum. Apart of being used as probiotics, these bacteria are producers of prebiotics and immunostimulant components, such as polysaccharides and glycolipids for vaccine production.

9.4. Conclusion

Valorising of cereal by-products through its transformation into added value biomolecules presents a significant opportunity given the global magnitude of the cereal industry. With the diversity of cereal crops and the multitude of processing stages for the production of a wide range of cereal-based commodities, it is expected that there will be a variety of by-products with potential applications. This chapter provided some insights into the role of biotechnology in utilizing primary processing by products for the production of microbial enzymes for a wide range of applications in food, feed, health and textile industries. The application of enzymes as processing agents in the food industry is gaining a lot of momentum currently and is growing at a very fast rate. Enzymes are characterised as capable of producing superior products with improved yield, in addition to reducing carbon footprint, energy consumption and environmental pollution. Additionally, novel enzymes can be further engineered and improved using knowledge of enzyme structure and random mutagenesis techniques of the microorganism that produce them. Secondary-processing of cereal crops as in the production of alcoholic beverages also produce by-products, such as BSG, with wide potential applications in the food and health industries. However, *in vivo* human clinical trials of product intake, digestibility and absorption need to be performed in order to validate the reported effects of the utilization of BSG. Studies on the safety, sensory properties, rheological and suitability of many ingredients and prepared products for specific uses in humans need to be investigated before implementation in real products and in new applications. The increasing concern for a healthy lifestyle and diet will slowly allow accepted modifications in some sensory attributes such as colour and flavour in favour of an enhanced nutritional content and health care benefits.

However, the recovery of high added value compounds is not always possible. High-tech equipment and facilities are not always within an accessible range from breweries, and as transportation and drying costs are high, there is a tendency to use no or low-processed brewers' spent grain in applications such as feed and agricultural use. The increase growth in the number of small breweries and distilleries provides an impetus to develop an infrastructure that allows pooling of the by-products and hence a better approach and strategy to harness possible benefits. The brewing processes applied in artisan breweries seem to leave by-products with high bioactive compounds thus reinforcing the need for further research in this aspect. A multidisciplinary approach need to be put in place to harness the value of the by-products produced from the alcoholic beverage industry encompassing in addition to technology, aspects such as environmental responsibility, economy and social ethics. Finally, a cost-analysis process is required to evaluate the financial rewards of scaling up from laboratory to industrial scale.

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