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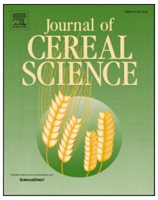

From Brew to Table: Unleashing the potential of spent grains protein

Vishal Ahuja [a,b,1], Shikha Chauhan [a,1], Yung-Hun Yang [c,d], Shashi Kant Bhatia [c,d,**], Vinod Kumar [e,*]

[a] University Institute of Biotechnology, Chandigarh University, Mohali, 140413, Punjab, India
[b] University Centre for Research & Development, Chandigarh University, Mohali, 140413, Punjab, India
[c] Department of Biological Engineering, College of Engineering, Konkuk University, Seoul, 05029, Republic of Korea
[d] Institute for Ubiquitous Information Technology and Applications, Seoul, 05029, Republic of Korea
[e] Centre for Climate and Environmental Protection, School of Water, Energy and Environment, Cranfield University, Cranfield, MK43 0AL, UK

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ABSTRACT

United Nations and Upcycled Food Association have suggested the possible use of food processing residues and waste materials to improve food quality as well as overcome hunger and malnutrition. Brewer's spent grains (BSG) account 85% of brewery waste having high nutritive value. Spent grains are initially exploited for animal feed only and restricted to the local area due to high moisture content that leads to spoilage. Spent grains are rich in fibre and proteins and were explored for the preparation of cookies, bread, pasta, and noodles which showed higher nutritive value and health benefits in comparison to conventional materials. The bioactive compounds in spent grains also provide additional advantages and can be used in drug formulation for commercial products. However, it needs in-depth research and technical support that aid in contributing to the circular economy.

1. Introduction

The increasing global population has raised the alarming situation due to low food availability and an increased hunger index. As per an estimate, the population that remains food insecure was 200 million in 2020 which is expected to cross 345.2 million marks by 2023. Unavailability of food and insufficient nutrition are reflected as the biggest barrier to food security as more than 828 million people were undernourished across the globe in 2021 (WHO 2022). Moreover, it is expected that with current performance, United Nations Sustainable Development Goal (SDG) 2 Zero Hunger can not be achieved by 2030 without major breakthroughs or changes. Reduced food waste is defined as one of the major changes that lead to the loss of one-third of food per year and cost UDS $936 billion (Nyhan et al., 2023).

Current lifestyle and food habits have raised the risk of diabetes and hypertension in society and currently around 20–25% population is at risk of premature death due to hypertension (including both men and women) (Mills et al., 2020; WHO 2023). Alongside this, more than 400 million people have been diagnosed with type 2 diabetes mellitus due to insulin resistance and its impaired secretion (Singh et al., 2021). Prolonged high blood glucose leads to neurological injuries, kidney failure, and cardiovascular illnesses. In addition, both conditions are associated and promote each other due to modulation in renin-angiotensin-aldosterone system, oxidative stress, mitochondrial dysfunction, sympathetic nervous system, fibrosis, and inflammation (Garzón et al., 2022). Malnutrition and unavailability of food further worsen the scenario that needs urgent attention. In order to provide food for all, it becomes much more necessary that food waste must be eliminated from society along with the inclusion of alternative nutrient sources.

In 2020, Upcycled Food Association defined upcycled food as "materials that otherwise would not have gone to human consumption, are procured and produced using verifiable supply chains, and have a positive impact on the environment" (Moshtaghian et al., 2021, 2024). Therefore, upcycling of food processing side streams and process byproducts with some positive food value gained attention in the last few years to reduce the waste and its transformation into sustainable foods (Nyhan et al., 2023). Brewer's spent grains (BSGs) are one of such byproducts available in ample amounts and have some nutritive value. It is insoluble solid waste residue, left after the malting of barley grains. It

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* Corresponding author.
** Corresponding author.Department of Biological Engineering, College of Engineering, Konkuk University, Seoul, 05029, Republic of Korea.
E-mail addresses: shashibiotechhpu@gmail.com (S.K. Bhatia), vinod.Kumar@cranfield.ac.uk (V. Kumar).

[1] the authors contributed equally.

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forms more than 85% of the total brewing waste. According to an estimate 20 kg/hL of beer resulted in the generation of 36.4 million tonnes of BSG. As mentioned, BSG is the major by-product of beer making left after wort extraction (Zeko-Pivač et al., 2022). On average, 0.2 kg and 2.5–3.0 kg of spent grain is obtained, respectively for each liter of beer and whisky produced (Chetrariu and Dabija 2023). Spent grains are mainly comprised of cellulose, hemicellulose, lignin, proteins, and lipids that make it 15%–28%, 19%–20%, 3–11%, 15–20% and 6–8% (on dry matter basis), respectively (Zeko-Pivač et al., 2022). The BSG proteins are mainly comprised of hordeins or barley prolamins followed by glutelins, albumins, and globulins. However, a major fraction of protein in BSG remains insoluble form as a soluble fraction has been utilized during brewing (Abeynayake et al., 2022). In addition, BSG is also rich in phenolics like hydroxybenzoic acids, hydroxycinnamic acids, ferulic and coumaric acid, vitamins including biotin, choline, folic acid, niacin, pantothenic acid, pyridoxine, riboflavin, and thiamine, etc. (Ikram et al., 2017).

The presence of nutrients makes it suitable for reutilization in industrial processes. The major issue with BSG valorization is high moisture content that makes it susceptible to microbial degradation (Bianco et al., 2020; Terefe 2022) and hence local consumption is preferred mostly as animal feed or discarded in landfill that releases approximately 513 kg CO_2 equivalent greenhouse gases (Kavalopoulos et al., 2021). Dried BSG will not only reduce pollution but also increased its shelf life followed by its use as a food ingredient or for the extraction of bioactive compounds (Wang et al., 2023; Nyhan et al., 2023).

The availability of nutrients like proteins, fibres, fatty acids, phenolics, etc., also increased its value as food additives as well as raw materials for pharmaceutical industries (Chetrariu and Dabija 2023; Patra et al., 2023) due to their anti-oxidant (Schettino et al., 2021a), anti-atherogenic (Verni et al., 2020), anti-inflammatory (Merten et al., 2022), and anti-cancer potential (Wang et al., 2023). In order to improve the food quality, protein, and bioactive peptides can be extracted or solubilize by grains pretreatment. Numerous methods have been developed for biomass hydrolysis and as a result output also varies *i.e.* acid hydrolysis targets saccharides and alkaline treatment targets lignin, however for protein recovery, alkaline treatment (Junttila 2022) and enzymatic hydrolysis is preferred along with solvent extraction (Alonso-Riaño et al., 2023). For extraction, operating conditions like pressure and temperature can be changed which also affects the outcome. The use of spent grains as animal feed is one of the most common applications due to their high fibre content as well as their conducive effect on gut microbiota (Zeko-Pivač et al., 2022). Now it has also been considered a food ingredient for humans and integrated into breadsticks, bread (Nyhan et al., 2023), biscuits (Wang et al., 2023), muffins (Nyhan et al., 2023), noodles (Shi et al., 2023), pizza dough (Nyhan et al., 2023), pasta (Schettino et al., 2021b), frankfurters, sausages and yogurt (Nyhan et al., 2023) etc. It has also been reported that BSG consumption has a direct impact on gut microbiota in humans as well and has an antioxidant effect.

Even with such high nutritive value and possible application, spent grains valorization suffers from some prominent challenges including microbial degradation due to high moisture (Mitri et al., 2022), microbial toxins such as mycotoxins (Nyhan et al., 2023), non-nutritive elements (Fărcas et al., 2022a) and allergens (Turck et al., 2023; Nye-Wood and Colgrave 2024). These toxins and unwanted chemicals compounds might have side effects upon consumption which lower their integration into food products (Fig. 1). In common consideration for such toxin's detection was poor detection and masking (Ahuja et al., 2023). This has created the need for advanced detection systems to ensure the safety of consumers. Some of the literature has summarized various aspects of BSG as animal feed as well as a food additive for human edibles however in the current review, the potential of BSG as a protein source has been elucidated with the exploration of its hidden potential.

2. Spent grain composition

Alcohol fermentation and beverage industries have used different types of grains as feedstock that vary in composition and hence spent grains' composition also varies with the source and type of grains and processes like fermentation and extraction applied (Table 1). Among different types of grains, barley is the most common grain used for beer making and hence dominates the waste residues. BSG has a high nutritional value composed of both macronutrients as well as micronutrients including fibers, sugars (primarily glucose, xylose, and arabinose), proteins, lipids, vitamins, and minerals. In spent grain, carbohydrate components make up approximately 50%–60% of the dry matter (Chetrariu and Dabija 2023). Spent grain contains predominantly hemicellulose (19–20% of dry matter), cellulose (15.2–28.7% of dry matter), and lignin (3.35–21% of dry matter) as dietary fibers, with hemicellulose being the most prevalent whose composition can reach up to 41.3% in some cases (Tišma et al., 2021; Zeko-Pivač et al., 2022). In addition to dietary fiber, BSG is rich in protein contributing to 15–30% of the biomass that can be utilized as a potential source for plant-based protein

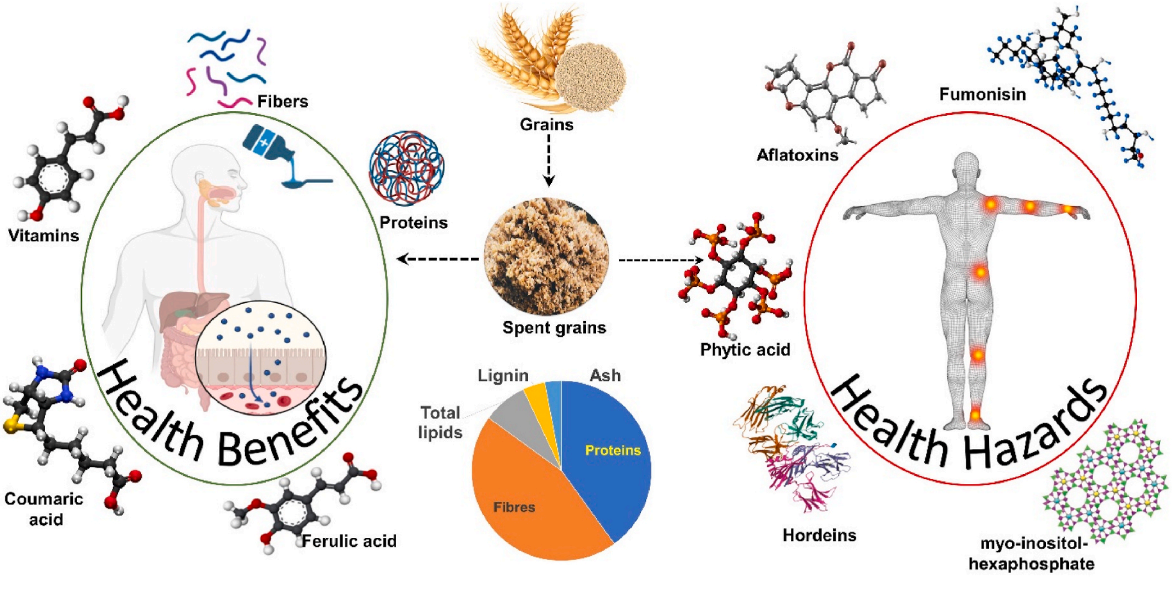

Fig. 1. Advantages and disadvantages of spent grains as food additive and supplement.

Table 1
Composition of spent grains from different sources.

Spent grain source	Fibres %	Proteins %	Ash %	Carbohydrate %	Total lipid %	Miscellaneous	References
Red Sorghum spent grain	54	23.4 (Rich in aspartic, glutamic acid and leucine)	1.4	16.7	4.5 (Stearic acid 75.43% and palmitic acid 17.90 %)	Tannins 0.75 %; Ca 185 ppm, Cd 1.19 ppm, Zn 3.40 ppm	Adewusi and Ilori (1994)
White Sorghum spent grain	43	19.3 (Rich in aspartic, glutamic acid and leucine)	0.8	33.6	3.2 (Stearic acid 95.86%)	Tannins 0.1; Ca 140 ppm, Cd 1.12 ppm, Zn 1.30 ppm	Adewusi and Ilori (1994)
Corn distillers' grain	12–15	25.3	1.6	–	12	Moisture 4.6%	(Wall et al., 1984; Hernandez et al., 2016)
Barley spent grain (craft beer)	32–49	15.24	3–6.3	19–47.6 (starch)	3.10	Maltose 4.8; glucose 1.3; maltotriose 1.9	Jin et al. (2022)

formulations (Devnani et al., 2023). Proteins from BSG are known to have important amino acids (0.859 mg/g), predominantly proline (0.349 mg/g) and glutamic acid (0.340 mg/g), and trace amounts of serine, threonine, lysine, aspartic acid, tyrosine, and phenylalanine (Zeko-Pivač et al., 2022; Chetrariu and Dabija 2023). Barley proteins majorly (30–50%) consist of complex hordeins/prolamins (a rich source of proline and glutamine) followed by other proteins including glutelin, albumins, and globulins (Jaeger et al., 2021; Devnani et al., 2023). Additionally, BSG contains considerable amounts of lipids (3–10%), starch (1–12%), ash (2–5%), and traces of vitamins (B1, B2, B6, K), minerals, and phenolic compounds (Devnani et al., 2023) including ferulic acid (1219.40 μg/g) and *p*-coumaric acid (488.51 μg/g) being the most common phenolics (Zeko-Pivač et al., 2022; Chetrariu and Dabija 2023). Besides proteins and fibres, BSG is also a good source of lipids containing 67% triglycerides, 18% free fatty acids, 7.7% diglycerides, and 1.6% monoglycerides. Linoleic acid (18:2), linolenic acid (18:3), oleic acid (18:1), palmitic (16:0), and stearic acid (18:0) are the main fatty acids present in BSG (Chetrariu and Dabija 2023).

Waste disposal has become a major challenge in the agro-industrial sector. The circular economy has been established on the reduction of waste by reusing, regenerating, and recycling by-products (Chetrariu and Dabija 2023). Utilization of this massive amount of side stream is somehow limited to animal feed and for the production of compost and biogas. However, being nutritious, the application of BSG has been expanded to the food industry as well, particularly for the extraction of proteins to be utilized for human consumption (Devnani et al., 2023).

3. Spent grain protein extraction and processing techniques

In general, protein extraction involves two steps: solubilization and recovery. To facilitate the release of proteins, aqueous solutions containing different reagents are mixed with spent grain to disintegrate the matrix and reduce the protein interactions with other macromolecules followed by the recovery of these solubilized proteins through various techniques including precipitation, centrifugation, or membrane filtration (Devnani et al., 2023). In addition to classic potential methods of protein extraction such as alkaline and acid treatment, more advanced methods including ultrasonic and pulsed electric field treatment are also available (Jaeger et al., 2021). In order to aid in the release of proteins from the spent grain matrix, various pretreatments are often used before extraction. As spent grain procured after the brewing process has 70–85% of moisture content (Devnani et al., 2023), drying at 60–70 °C until the moisture content reaches <10% is the preliminary treatment followed by reduction of particle size through shearing or milling and sieving (Alonso-Riaño et al., 2021; Karlsen et al., 2022; Junttila 2022; Devnani et al., 2023). In addition to these treatments, defatting, enzymatic hydrolyzation, and ultrasound are the other usual pretreatment methods (Devnani et al., 2023). However, the effects of these pretreatments on the protein extraction yield are somewhat disparate. While several reports showed the fold increase in the extraction yield of protein after pretreatment (Li et al., 2021; Ampofo and Ngadi 2022), there were reports with no significant effect of these pretreatments (griding and defatting) prior to alkaline and acid extraction from spent grains (Junttila 2022). In fact, Karlsen et al. (2022) reported a decrease in the protein extraction yield from 45% to 38% after defatting, probably due to the protein solubilization in the methanol used for the pretreatment.

Alkaline extraction is the most commonly utilized method for the extraction of proteins from spent grains (Jaeger et al., 2021; Silva et al., 2023) where the alkaline environment alters the charge and structure of SG proteins facilitating their solubilization (He et al., 2019; Junttila 2022; Devnani et al., 2023). Extraction yield is dependent on the alkali concentration, temperature of extraction, and the isoelectric point for precipitation. He et al. (2019) achieved an extraction yield of 82% with 37% protein purity by utilizing 5% w/w NaOH concentration for extraction from BSG. A 2-fold increase in the protein extraction yield was reported when alkali (NaOH) concentration was raised from 0.01 M to 0.1 M (Junttila 2022). Increased alkali concentrations facilitate the extraction yield, however, it lowers the purity of extracted proteins certainly due to increased carbohydrates solubilization as well, so must be optimized (Devnani et al., 2023). Recently, reduced pressure alkaline pretreatment was investigated resulting in an 80% of protein extraction yield with 24.7% of glutamic acid in the hydrolysate under optimal conditions of 180 mM NaOH; 455 mBar pressure; 70 °C temperature for 1 h treatment (Arantes da Fonseca et al., 2023). In extraction processes using chemicals such as sulfuric acid and sodium bisulfite (reducing agents), the hemicellulose in spent grains is hydrolyzed, and the disulfide bonds in proteins are broken, increasing their solubility and recovery (He et al., 2019). Compared to alkaline extraction, acid treatment resulted in higher extraction yields (63–90%), but lower protein purities (24–39%). Solubilization of spent grain protein can also be achieved by combining salt extraction with surfactants such as sodium dodecyl sulfate and disodium phosphate (Chetrariu and Dabija 2020; Devnani et al., 2023).

In order to isolate proteins from BSG, solvent extraction techniques include deep eutectic solvents and pressurized solvent extraction. Proteins are released more easily from spent grains if lignin and starch are fractionated and dissolved by deep eutectic solvents (DES). An extraction yield of 79% with 54% protein purity was achieved after extraction with sodium acetate-urea (1:2) DES at 80 °C for 4 h. When using pressurized solvent extraction, high temperature, and pressure facilitate penetration of solvent into the BSG matrix resulting in a higher yield of extraction with fewer chemicals utilization (Devnani et al., 2023). In hydrothermal extraction, proteins are released from spent grains through the use of water as a solvent at a mild to high temperature for a short or long duration whereas, in subcritical extraction, water is used at temperatures above 100 °C and its aqueous state is maintained through pressure arrangements. Alonso-Riaño et al. (2023) achieved a high extraction yield (78%) of proteins from the BSG by applying subcritical conditions at 185 °C and 5 MPa for 150 min. However, this method is not energy efficient, and such extreme conditions might result in the denaturation of extracted proteins.

The enzymatic extraction method utilizes enzymes such as carbohydrases and proteases that hydrolyze the spent grains which leads to the solubilization of proteins (He et al., 2019). In this typical two-stage process, firstly carbohydrases are added to disintegrate carbohydrates of BSG matrix (hemicellulose and cellulose) releasing proteins followed by the addition of proteases that partially hydrolyze the protein matrix resulting in the extraction and solubilization of proteins. Following enzyme extraction methods, protein extraction yields up to 86% with 40% protein purity have been reported in the literature (Devnani et al., 2023). Reaction parameters such as concentration of enzyme, pH of the medium, and reaction time are critical in attaining maximum extraction yields. An increase in the protein extraction yield from 77% to 84% has been obtained by increasing the concentration of enzyme (protease) from 5 to 35 μL/g of BSG (He et al., 2019). The major issue related to the enzyme extraction treatment is the low protein purity (~40%) that can be overcome by methods such as microfiltration of the hydrolysate after enzymatic treatment that could separate sugars from proteins therewith increasing protein purity (Devnani et al., 2023). A number of methods have been evaluated for protein extraction and each method has its own advantages and disadvantages (Table 2).

4. The potential of spent grain as a protein source

The protein and fiber content of BSG is the main attraction. BSG has much higher fibre content than conventional food grains like wheat, oat, and unmalted barley *i.e.* 9–20%. Even in legumes, fiber content was lower *i.e.* 14-21% in pea, 22–25% % in soybean, and 11–28% in faba bean. However, protein in BSG was higher than in normal food but lower than in soy (33–50%) and legumes (16–29%) but BSG has been considered a better alternative to legumes and cereals due to amino acid composition. Digestible indispensable amino acid score (DIAAS) for different grains is maximum for pea (98–103%) followed by faba beans (64–76%), wheat, barley–rice mixture (43–56%) while it is not well documented for BSG (Nyhan et al., 2023). The differential amino acid composition of BSG proteins *i.e.* richness in glutamine/glutamic acid, proline, and leucine (Shroti and Saini 2022) suggested its integration with conventional food.

Table 2
Advantages and disadvantages of protein extraction methods.

Method	Advantages	Disadvantages
Grinding and milling	•Particle size reduced •Increased surface area •Applicable at large scale	•Laborious and energy intensive •Need additional treatment for extraction •Generated heat may denature proteins
Microwave treatment	•Use microwave for energy transfer •High energy transfer rate in comparison to conventional methods •Less time consuming	•Scale up is challenge •High heat generated that denature proteins
Ultrasonic treatment	•High energy and mass transfer •Higher extraction efficiency	•Heat generated during operation •Energy intensive •Poor cell rupture and energy transfer at higher scale
Alkali treatment	•Cost-efficiency •Less time consuming •Can be scaled up	•Generate hazardous residues •Corrosion to vessel on regular operation •Not a eco-friendly method
Detergent treatment	•Dissolve cell wall and release soluble proteins	•Recovery becomes difficult •Detergent may also denature proteins
Solvents	•Short time of operation •Can be used at higher scale •Adaptable for various conditions •Solvents can be recycled	•Solvents can be toxic •High cost of solvents can be a major issue
Enzymatic hydrolysis	•Specific action •High yield •High chance for catalytic recycling	•High cost of enzyme production and purification •Long processing time •Complex treatment system and control

A comparative study of the protein profile between EverPro (barley-rice protein) with pea and soy (the current gold standard for plant proteins) revealed that EverPro satisfies all the requirements and base composition of essential amino acids except the lysine content. Similarly, pea and soy lack methionine and cysteine. In terms of protein solubility, EverPro has a protein solubility of 100% which is 22% for peas and 52% for soy (Jaeger et al., 2023). The deficiency of food grains in terms of composition and nutritional efficiency, spent grains can be exploited as ingredients for the formulation of edibles like cookies, pasta, noodles, bread, etc. Cappa and Cappa (2017) evaluated the effect of the addition of BSG in egg pasta by central composite design and post quality was evaluated. The addition of BSG, lowered the average break strain in comparison to the control (conventional past) from 54 ± 4% to 26 ± 10% (for raw material) and from 54 ± 1% to 25 ± 8% (for cooked sheets). In comparison to BSG, egg white powder improved mechanical strength mainly due to dense protein network ovalbumin, The break load and strain in cooked pasta *i.e.* 6.5 ± 0.4 N and 33 ± 4% were higher than with egg white 1.4 ± 0.1 N and 18 ± 1%, respectively (Cappa and Cappa 2017). In terms of desirability function, both BSG and egg white have positive outcomes and thus can be included as ingredients. In another effort, Schettino et al. (2021) exploited BSG as an alternate ingredient for semolina in pasta. Native (nBSG) and fermented BSG (fSG; treatment with xylanase followed by fermentation with *Lactiplantibacillus plantarum* PU1) were used separately to evaluate the effect on the nutritional value of food. The addition of BSG has a minor change in protein and fat as it increased from 14.11% to 2.18% (conventional pasta; control) to 15.16% and 3.49% (nBSG) respectively but carbohydrate reduced from 79.44% (control) to 69.69% (fSG) while total dietary fiber content increased tremendously from 2.91% (CP) to 11.96% (fSG) and 11.88% (nBSG) (Schettino et al., 2021b). It has been seen that in comparison to nBSG, fSG has higher fiber, high protein digestibility, essential amino acid index, protein efficiency ratio, and nutritional index) along with a low glycemic index which makes it suitable for low carbohydrate diet. High starch content is a major obstruction to its consumption by diabetics. Shi et al. (2023) used BSG based protein and fibers as an ingredient for starchless noodles. The inclusion of BSG in noodles lowers the glycemic response by 93.16 ± 8.07% in comparison to conventional noodles (Shi et al., 2023).

Pasta and noodles are not the sole products that have been prepared with BSG but this list also includes edible films, bread, biscuits, etc (Fig. 2, Table 3). The inclusion of BSG in any edibles will not only change nutritional quality but also influence food texture, fragrance, and taste. Wang et al. (2023) fermented BSG (solid-state fermentation) with *Rhizopus oligosporus* followed by inclusion in biscuits. Three sets were prepared for evaluation *i.e.* biscuit without substitution (control), with autoclaved BSG (ABSG), and with fermented BSG (fBSG). fBSG and ABSG both make biscuits fracturable but 30% fBSG has a better impact on the nutrition level and anti-oxidant capacity of biscuits. The inclusion of fBSG, increased the antioxidant power by > 3 times (gallic acid equivalent), 2.7 times (Trolox equivalent), and more than 5 times (ferric reducing/antioxidant power). On the other side, fBSG also has an inhibitory effect on in vitro starch digestibility due to the inhibition of α-glucosidase (Wang et al., 2023). Microbial activity during fermentation increases the nutritional value. In another work, the protein was extracted from BSG for the preparation of edible film with the help of glycerol as a plasticizer. It has been observed that concentration of BSG has a direct influence on the physical properties of prepared films as protein film's solubility increased at higher pH along with puncture strength and elongation at break. The tensile strength of films increased from 0.71 to 1.44 MPa but water activity, swelling capacity, and water

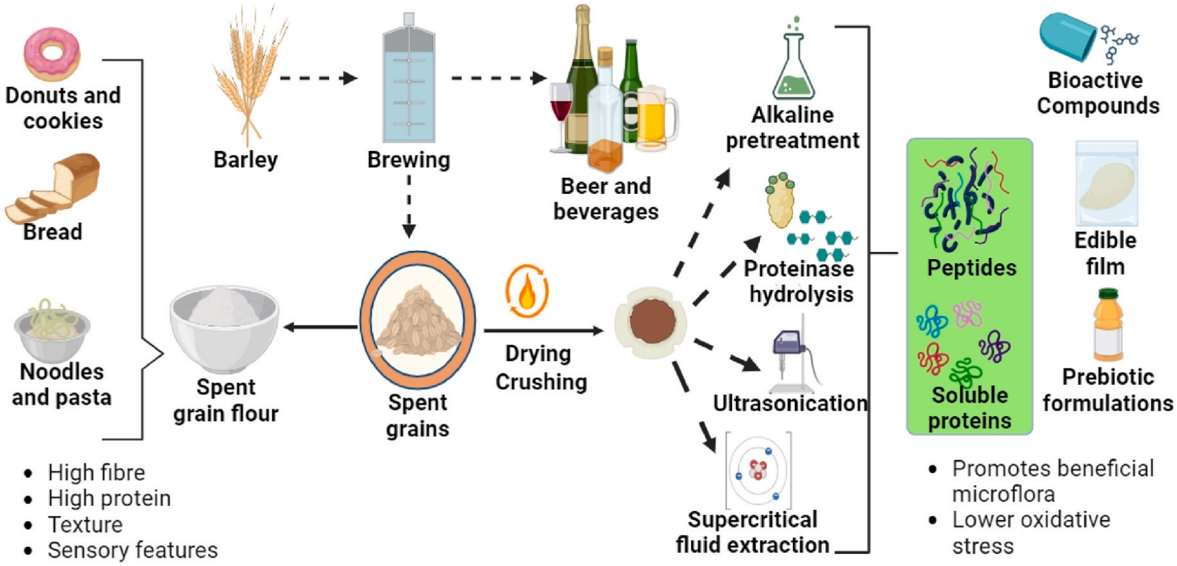

Fig. 2. Extraction methods of proteins from spent grains and use as flour alternative.

Table: 3
Commercial application for brewery spent grains in protein extraction and edibles formulation.

Target product	Method of processing	Operating conditions	Major findings	Outcome	Reference
Bioactive peptide	Alkaline hydrolysis + enzymatic hydrolysis	Time 2.12 h; temp 79.61 °C; H_2O_2 67.52%	Flavourzyme hydrolysate has a maximum α-glucosidase inhibitory 9.25 mg/mL (IC 50)	Alcalase hydrolysate ABTS (IC50) = 1.7 ± 0.08 mg/mL and DPPH radicals 2.47 ± 0.07 mg/mL	Bazsefidpar et al. (2023)
Protein	Alkaline hydrolysis under subcritical conditions	NaOH concentration 0.05 N; time 60 min; temp 40 °C	Treatment with H_2SO_4 before alkaline extraction increased protein yield; no drying and grinding needed for extraction	Total proteins extraction 69.7%	Junttila (2022)
Proteins	Reduced-pressure alkaline pretreatment	Temp 70 °C; pressure 455 mBr; time 1 h; NaOH concentration 180.00 mM	Extract comprised of glutamic acid 24.7%; leucine 8.4%; proline 11.8%; aspartic acid 8.1%	80% protein extraction from BSG	Arantes da Fonseca et al. (2023)
Proteins/ peptide	Subcritical water treatment	Temp 170 °C; time 22 min	Free amino acids/g protein 2.17%; total phenolic content 17.84 mg GAE/g dry BSG	Peptide 6.5 g/L, protein yield 64%	Alonso-Riaño et al. (2023)
Antioxidant and prebiotic activity and food products					
Prebiotics	Solvent extraction	80 % ethanol: water (v/v);	2% extract increased the growth of *Bifidobacterium animalis* spp. lactis BB12	Bioaccessibility index 99.77 % (ferulic acid); 72.68 % (4-hydroxybenzoic acid), 65.37 % (vanillin); 28.99 % (p-coumaric); 22.54 % (catechin)	Bonifácio-Lopes et al. (2023)
Antioxidant activity	Enzyme hydrolysis	0.5% bromelain or 1% neutrase or 1% trypsin at 55 °C for up to 67 h	Maximum protein recovery from neutrase 63.24% (4 h); degree of hydrolysis 22.70%	Antioxidant activity 50.06 ± 0.39 μM TE/ g dw	(Dumitrascu et al., 2023)
Ćupter candy	Ingredients in conventional food	22.5 g flour +13.5 g industrial BSG +180 mL white grape must	Nutritive value improved with BSG but processing must be improved for consumer acceptance	Protein from BSG 13.03%, water activity 0.86	Lalić et al. (2023)
Noodles	Ingredients mixing	5–20% BSG flour + wheat flour	Fibre 12.79% and gluten 4.54%; DPPH 31.08%; free phenolics 132.87%	Total protein 11.89% (15% BSG)	Anisha et al. (2023)

vapor permeability reduced. On the other hand mechanical properties and moisture content raised with protein concentration but transparency is reduced (Shroti and Saini 2022). The edible films can be used for drug coating as well as packaging material for edibles if permissible stability is observed.

5. Examining its digestibility and bioavailability

In the current scenario, oxidative stress is one of the biggest challenges that is increasing day by day due to associated health issues, and hence the demand for food with antioxidant potential is also on the rise (Pizzino et al., 2017; Zehiroglu and Ozturk Sarikaya 2019; Forman and Zhang 2021). Apart from proteins, fibre, and lipids, some of the researchers also emphasized spent grains as a rich source of bioactive compounds including phenolics, and polyphenols (Fărcas et al., 2022b). Abeynayake et al. (2022) showed the presence of bioactive peptides with antioxidant potential. In BSGs, the major fraction of protein is in insoluble form which also reduces the availability. Protease hydrolysis of BSGs increased the solubility of protein to 94.4% even at neutral pH. Different combinations of enzymes i.e. alcalase alone and in combination with neutrase, flavourzyme, or everlase followed by assessment of antioxidant potential. DPPH scavenging activity of hydrolysate ranged from 72.6 to 74.9% but maximum superoxide radical scavenging activity (19.3%) was reported from the combination of alcalase and flavourzyme while ferrous chelation was maximum in the case of everlase and foodPro PHT as a catalyst (Abeynayake et al., 2022). Naibaho et al. (2022) also revealed that enzymatic hydrolsysis of BSG with 0.5% protamex alone as well as a combination of protamex and flavourzyme have antioxidant activity in comparison to control (without hydrolysis) due to higher phenolic compounds. Ferric-reducing antioxidant power of extract was due to polyphenolic compounds while oxygen radical absorbance capacity (5.9–7.0 mmol Trolox/100 g) and ABTS (4.15–5.55 mmol Trolox/100 g) was due to proteins in hydrolysate (Naibaho et al., 2022).

Comparative evaluation of BSG and extruded BSG for their effect on human oral-gastro-intestinal digestion and colonic fermentation showed no change in the bioaccessibility of gluten and glucose after extrusion but an increase in amino acids and phenolics. The extrusion also reduced the formation of short-chain fatty acids formed in colonic fermentation. Extruded BSG lowered the formation of intracellular ROS in IEC-6 cells and had anti-inflammatory activity in RAW264.7 cells. In addition, it also reduces glucose absorption and has anti-carbohydrases activity that is required for the anti-diabetic potential of BSG (Gutierrez-Barrutia et al., 2022). The consumption of BSG extract has a conductive effect on gut microbiota and has higher prebiotic potential as well as antioxidant activity that increases with total phenolic compounds (Table 1). Vanillic, ferulic acids, vanillin, and catechin were the main phenolic compounds in the extract (Bonifácio-Lopes et al., 2022). The potential of BSG has been tested in the lab as well as in animal models successfully. The bioactivity of BSG and its extract was mainly due to the presence of peptides and phenolics (Fig. 3). In hypertensive and insulin resistant rat models, microencapsulated BSG peptides have shown antihypertensive and antidiabetic effects (Garzón et al., 2022). In obesity drives nonalcoholic fatty liver disease mice model as well, BSG reduced body weight, retroperitoneal white adipose tissue and liver weights, and lower plasma total cholesterol. The effect was reflected in reduced hepatic steatosis and higher fecal fat (Pei et al., 2022).

6. Future prospects and challenges

BSG waste is commonly used as animal feed or discarded in landfills. The availability of nutritive and non-nutritive components in BSG suggested its highly valued industrial applications but some technical constraints might need attention to overcome the challenge.

6.1. 6.1. shelf-life, transportation and degradation

The major issue with BSG is its short shelf life which is mainly due to high nutritive material for microorganisms and high moisture content. Both factors are collectively responsible for the high possibility of microbial activity and degradation (Wang et al., 2023; Nyhan et al., 2023). Azevedo et al. (2024) suggested that raw BSG discarded after malting has more than 80% moisture along with total lignin content of around 29.25%, 6–7% proteins, 4–5% ash, and fermentable sugar and residual polymer of 45–50%. The overall pH of BSG was in the acidic range (6.06) which also promoted fungal growth. As per the available literature, *Alternaria* spp., *Aspergillus* spp., *Fusarium* spp., and *Penicillium* spp., are prominent fungal pathogens that deteriorate the spent grains (Sarmast et al., 2021). In order to prevent microbial degradation, local consumption by the animal as a constituent of their feed is preferred but due to its high amplitude, it is insufficient to handle alone (Zeko-Pivač et al., 2022). Drying of biomass becomes necessary to lower the moisture content ultimately increasing the shelflife of biomass and easing transportation so that it can be used as an ingredient in various products like bread, cookies, muffins, pasta, cereal bars, and yogurt (Nyhan et al., 2023).

6.2. 6.2. microbial toxins and detection

The major issue with BSG valorization as edibles is contamination with mycotoxin phytoestrogens, pesticides like Zearalenone, T-2, HT-2, ametoctradin, mandipropamid and ergot alkaloids in varying concentrations that would be lethal to the consumer even at low concentrations (Penagos-Tabares et al., 2022). The malting and preparatory process increased the moisture content (around 80%) and make it prone for fungal and other microbial contamination (Mitri et al., 2022). Under favorable conditions, *Fusarium, Penicillium, Alternaria*, and *Rhizopus* are the prominent contaminating agents in barley grains. Drying of barley grains after malting and kilning reduced the fungal growth except production of mycotoxin and hence led to the production of aflatoxins, fumonisins, ochratoxin A, trichothecenes, and zearalenone in spent grains (Nyhan et al., 2023). The presence of mycotoxins can be very lethal and harmful to vital organs and tissues, and cause neuropathy, immunomodulatory, and carcinogenic effects. It may also have a negative influence on reproductive health in animals and humans (Mastanjević et al., 2019).

6.3. 6.3. allergens and antinutritive factors

Spent grains are a rich source of proteins and minerals however, the

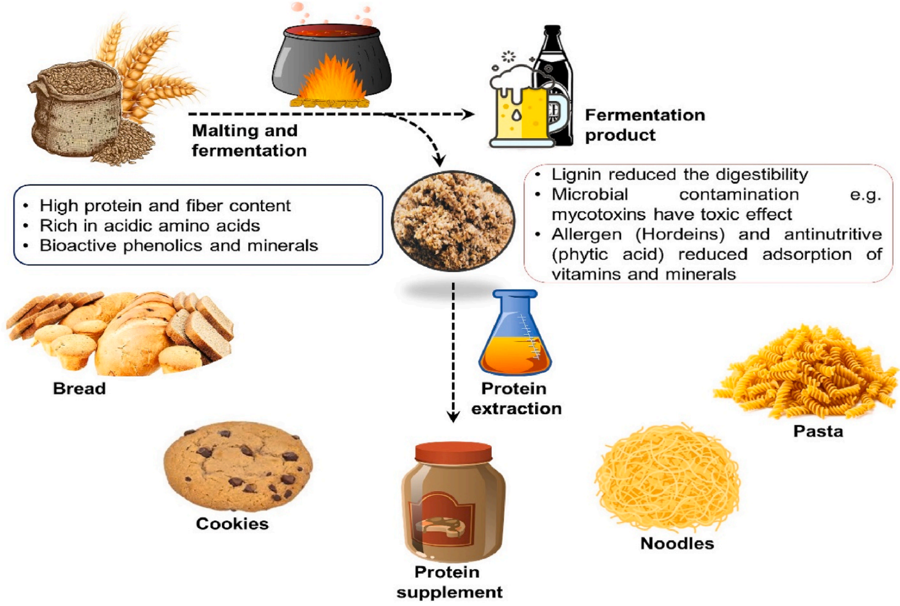

Fig. 3. Composition of residual grains after malting and possible outcomes.

presence of allergens like hordeins (Nye-Wood and Colgrave 2024) and antinutritive factors including lignin fibres, phytic acid, and myo-inositol-hexaphosphate (Fărcas et al., 2022a) are also reported which may lead to a serious allergic reaction. As per the European Union regulation (EU 1169/2011) in annexure II due to the presence of gamma-3 hordein and non-specific lipid transfer protein (Turck et al., 2023). The consumption of barley without removing such products formulated with spent grains might have a negative effect on consumers. Nye-Wood and Colgrave (2024) reported that malting selectively extracts non-protein fractions and makes spent grains protein-rich hence spent grains have a high level of hordein.

Presence of high levels of ANFs in animal feed or food reduced protein digestibility and bioavailability (Karlsen and Skov 2022) while high concentration of phytic acid reduced the solubility of vitamins and minerals (Lynch et al., 2016) and myo-inositol phosphates can form complex with divalent cations like Ca^{+2}, Cu^{+2}, Mg^{+2}, Mn^{+2}, and Zn^{+2} and hinder the absorption of minerals (Silva et al., 2020). The major concern with microbial toxins and antinutritive factors is poor detection and a tedious preparatory-detection process. The available methods for mycotoxin detection include thin-layer chromatography (Akinola et al., 2019; Awan et al., 2022), enzyme-linked immunosorbent assay (Wang et al., 2020), and gas chromatography (Cirio et al., 2019). The conventional methods have issues with low detection, poor reproducibility, accuracy, and high cost of operation (Wu et al., 2023). Some of the modifications in conventional models like integration of mass spectrometry (MS), Gas chromatography–mass spectrometry (Mahmoud et al., 2018), high-performance liquid chromatography (Moez et al., 2020; Li et al., 2020), and HPLC–tandem mass spectrometry (González-Jartín et al., 2019; Rausch et al., 2021) have high sensitivity and can be used for new compounds identification as well. GC and GC–MS techniques can only be used for the detection of heat-stable and volatile mycotoxins (trichothecene) while HPLC relies on ultraviolet or fluorescence detectors and hence needs related moieties for detection (Wu et al., 2023). In addition, chemical masking and modified forms of the toxins can become serious concerns for food safety (Ahuja et al., 2023).

6.4. 6.4. spent grain pretreatment and fermentation inhibitors

Biopolymers like cellulose and hemicellulose have complex structures that slow down valorization and biotransformation. BSG also possesses high amounts of phenolics, fibres, and fatty acids that may have a negative effect on sensory features, and the technofunction of food and other edibles (Nyhan et al., 2023). Pretreatment strategies can be implanted to increase the availability of monomers like fermentable sugars and amino acids that can be used for microbial biotransformation. Numerous methods including acid and alkaline hydrolysis, enzymatic saccharification, and the use of supercritical fluids and solvents, have been reported for pretreatment and facilitate protein extraction. Chemicals like acids and alkali are commonly preferred due to lower reaction time however both are corrosive and generate hydrolysis byproducts such as furfurals and hydroxymethyl furfurals that further affect the microbial activity. The selection of a suitable method not only depends upon hydrolysis efficiency but also includes environmental feasibility and cost-effectiveness. The hydrolysate or extract can be treated with adsorbents like activated charcoal and char or processed with membrane filtration to remove inhibitors and to ensure health and food safety. Furthermore, process integration has shown the path to valorize the waste from one process in another process as raw material. This approach not only improved the environmental feasibility but also reduced the net cost of investment.

7. Conclusion

Brewery and beverages are an exponentially growing industry across the globe. The process also generates a significant amount of waste mainly dominated by spent grains (more than 85%). The major fraction of nutrients have been taken up during malting but spent grains are also rich in proteins, vitamins, minerals, and fibres. Direct utilization of spent grain and its microbial transformation is hindered by the complexity. Grain processing and pretreatment might solubilize the nutrients and improve their availability. Considering the potential source of protein, spent grains have been integrated into edibles and food for human consumption which have shown a conducive effect on gut microbiota. The bioactivity of peptides and phenolics present in spent grains can also be used for the extraction of drug compounds however, researchers need to evaluate the process and lower the process cost.

CRediT authorship contribution statement

Vishal Ahuja: Conceptualization, Writing – original draft. **Shikha Chauhan:** Writing – original draft. **Yung-Hun Yang:** Writing – review & editing. **Shashi Kant Bhatia:** Conceptualization, Supervision, Writing – review & editing. **Vinod Kumar:** Conceptualization, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no competing interests.

Data availability

No data was used for the research described in the article.

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