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Solid-state fermentation for enhanced brewers‘ spent grain protein valorisation: A comprehensive review of conventional and emerging techniques

Growing demand for sustainable plant-based protein sources has increased in recent years. Brewers’ spent grain (BSG), the most abundant brewing by-product by volume, has attracted considerable attention due to its high protein content (18 to 30%) and its desirable physicochemical and nutritional properties. In this review, the advantages and limitations of conventional and novel BSG protein (BSGP) extraction techniques were discussed and compared. Solid-state fermentation (SSF) was highlighted as a sustainable and industrially adaptable biological process that enhances protein accessibility and recovery for waste valorisation. The commercial viability of SSF-regulated BSG bioconversion, key operational factors and future insights were also examined. Overall, this review provides an updated evaluation of BSG protein extraction methods, thereby contributing to the advancement of sustainable resource utilization and promoting circular bioeconomy.

Descriptors: Brewers’ spent grain; bioactive compounds; protein extraction techniques; waste management

1 Introduction

The global population is expected to reach 8.5 billion in 2030 and over 9 billion in 2050, resulting in a 70% increase in demand for food production [1]. Food security worldwide is facing unprecedented challenges due to the negative environmental impacts of unsustainable traditional intensified agriculture [2]. Global demand for protein, is expected to surge to 943.5 million metric tonnes by 2054 [3]. In such a context, the growing population and increasing consumer consciousness are driving the protein market and increasing the demand for alternative sustainable protein ingredients. Hence, there is an urgent need for alternative protein sources and innovative production methods to meet consumer demand and satisfy the anticipated global protein requirements.

Over the last five decades, global beer consumption has grown to more than 150 billion litres per year, making it the most dominant alcoholic beverage worldwide [4]. It is estimated that every 100 litres of beer produced generates around 15-20 kg of fresh BSG, which represents 85% of the solid brewing byproducts by wet weight from the brewing industry, resulting in 38-40 million tonnes of BSG being produced globally each year [5]. Despite its large production and easy accessibility, BSG remains underutilized, with approximately 70% being used to produce animal feed, 10% used for biogas and the remainder 20% being disposed of in non-intended landfills [6,7]. While BSG is a valuable source of nutritious functional proteins [8–10], its limited exploitation is mainly due to its complex composition and high moisture content, resulting in susceptibility to rapid microbial degradation and posing challenges for storage and transport [11]. Thus, there is an urgent need to explore suitable, efficient, scalable, and cost-effective technologies to ensure the effective recovery of protein from BSG. By doing so, it is expected to provide a sustainable option for plant-based proteins, reduce the environmental problems associated with waste accumulation, and significantly increase the profitability of the industry.

The bioactive compounds in BSG and its potential future applications have been thoroughly reviewed in several recently published articles [12–14]. Nevertheless, with the recent considerable advances in the techniques used for BSG protein extraction [15–18], there is a clear need for an updated review that compares these extraction techniques and evaluates their suitability for BSG protein recovery. The scope of this review covers conventional and emerging techniques for brewers’ spent grain protein valorisation, including solventbased extraction, acid and alkaline treatments, thermal and enzymatic processes, as well as novel approaches such as microwaveassisted, ultrasoundassisted, supercritical fluid extraction, and pulsed electric field technologies, with solidstate fermentation critically evaluated as a sustainable standalone strategy and as a pretreatment to enhance protein accessibility and recovery. For extraction methods not yet applied to BSG protein recovery,

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examples of other similar lignocellulosic materials were given with the aim of providing valuable insights into its valorisation.

2 Extraction strategies of bioactive proteins from brewers' spent grain

Brewers' spent grain has emerged as a noteworthy source of bioactive proteins due to its abundant and consistent production throughout the year. Although brewers' spent grain (BSG) is produced globally in large quantities, its effective utilisation is strongly constrained by geographic location, high moisture content, and dependence on proximity to end-users [19]. Research on the recovery of proteins from BSG was initiated in the mid-1940s [20]. However, the intricate and rigid lignocellulosic structure of BSG poses challenges to effective protein recovery, which traditionally limited its use mainly to animal feed [8]. To mitigate potential future food scarcity, there is a pressing need to convert this promising by-product into value-added commodities, thereby enhancing economic benefits and establishing a sustainable 'green' protein source. The protein extracted from BSG has drawn significant attention in the food manufacturing sector, owing to its chemical, functional, and nutritional capacities [21]. Addressing the demand to broaden protein sources and surpass constraints linked to traditional dairy proteins, a range of conventional and novel methodologies has been investigated to extract proteins from BSG, each method possessing advantages and limitations as detailed in Table 1.

2.1 Conventional extraction strategies

Conventional extraction methods refer to the widely employed techniques for the isolation of plant-derived proteins from complex cellular matrices. These methods normally involve the use of various solvents and different physical processes to break down cell structure, alter protein solubility, and consequently liberate protein from plant materials. Four of the most widely used conventional extraction methods are discussed below.

2.1.1 Solvent extraction

Two frequently employed solvent extraction methods are deep eutectic solvents and pressurised liquid extraction [6]. Deep eutectic solvents have found widespread application in the fractionation of lignocellulosic materials and the extraction of valuable components, while pressurized liquid extraction involves utilizing solvents under elevated temperatures and pressures, facilitating penetration of the solvent into the sample matrix and efficient transfer of solubilized compounds [30,31]. In a recent study by Hernández-Corroto et al. [32], it was demonstrated that a substantial amount of protein can be extracted from BSG using deep eutectic solvents composed of guanidinium chloride and urea at a (1:2) ratio, with an optimized procedure employing Box-Behnken Design involving the mixing of 20 mg of BSG in 80% high intensity focused ultrasound (HIFU) amplitude for 20 minutes at 37°C, resulting in final yields of 13 ± 2 g protein/100 g BSG (equivalent to a recovery of 41 ± 6% of BSG proteins). The authors further noted that the deep eutectic solvents can be effectively washed out by ethanol and the final product is phenol-free. However, the application's scalability in an industrial context may be hindered by the cost of these solvents and the complex procedure involved in the extraction.

Contrastingly, González-García et al. [30] observed that higher temperatures (155°C) enhance protein extraction from BSG, achieving a maximum protein yield of 69% under optimized conditions

Table 1 The advantages and disadvantages of protein extraction strategies from brewers' spent grain.

Extraction methods		Advantages	Disadvantages	Reference
Conventional	Solvent	Simple and cost effective	Potential co-extraction of undesirables, time consuming, large Volume of waste solvent	[22]
	Acid/base	Low cost, simple operations	Large amounts of solvents, protein might be denatured	[23]
	Thermal	High selectivity and no chemical addition	Time consuming	[24]
	Enzyme	High selectivity	High cost of enzymes, not suitable for industry-scale production, yields and quality vary greatly depending on the enzyme used	[25,26]
Novel	Microwave-assisted	High efficiency, less solvents, less energy	Equipment more expensive, low selectivity, high temperature may degrade the extracted compounds	[27]
	Ultrasonic assisted	Cost-effectiveness, faster energy transfer, high production, reduced heat gradient and extraction temperature	Prolonged sonication and power may stimulate protein aggregation, and reduce protein yield	[28]
	Supercritical fluid	Renewable, non-toxic, non-flammable, CO_2 is cheap and easily available	Not suitable for large scale production, low purity, and non-polarity of CO_2	[29]
	Pulsed electric field	Non-thermal, time-efficient, and high yields	High energy consumption, equipment cost, and scalability issues	[27]

(4.7% ethanol at 155 °C for 10 hours during a five-cycle extraction process). However, the obtained protein had a low purity of 20%. While the solvent extraction method offers relatively high protein yields and is viable in terms of equipment and cost, the use of solvents and high temperature/pressure during the extraction procedure can alter the structure and functionality of BSG proteins. This method was also noted to be time consuming and generate large quantities of solvent waste.

2.1.2 Acid/base extraction

Alkaline solubilization is a well-known method to extract protein from a wide range of agro-industrial residues, including BSG. The mechanism of this method was well explained by Cavonius et al. [33]. In a strongly alkaline environment, proteins receive a net negative charge, leading to repulsion within and between protein molecules, which facilitates interactions with water, thus increasing the solubility of proteins in water. Upon reducing the pH to the isoelectric point (PI) of the protein, the negative charge associated with the protein diminishes, resulting in minimized interaction with water. Consequently, the protein becomes insoluble and precipitates out of the solution. According to Connolly et al. [34], protein extraction effectiveness using the alkaline method significantly depends on the type and concentration of alkali used, extraction temperature, solid-liquid ratio, and particularly the isoelectric point used. In their study, maximum protein yields of 59% were achieved from BSG by employing NaOH at a concentration of 110 mM, maintaining a weight/volume ratio of 1:20 over a 1-hour extraction at 50 °C. In the study conducted by Vieira et al. [35], a three-step sequential extraction approach was employed to extract proteins from brewers' spent grain. It examined the influence of various alkali solutions (KOH and NaOH) at different concentrations (0.1-4.0 M), along with other relevant parameters such as time, temperature, and solid: liquid ratio, on the protein recovery yields. The protein was subsequently precipitated in a strongly acidic environment (pH 3) using citric acid, resulting in protein extraction yields ranging from 79% to 83%. This study suggests that the alkaline extraction method presents a viable and effective strategy for extracting functional BSG protein.

In another study, Qin et al. [24] achieved a protein yield of 90% from defatted BSG.using a one-step dilute acid method (11,400 mg H_2SO_4/g BSG), followed by autoclaving at 121 °C for 1 h. The protein yield increased to 95% using a sequential 2 step alkaline and dilute acid method extraction (1 h alkaline extraction using 110 mM NaOH (1:20 w/v) at 50 °C and 200 rpm followed by a 1 h 1 M H_2SO_4 (16,150 mg H_2SO_4/g BSG) at 25 °C, 250 rpm, though reportedly a high amount of carbohydrates and lignin was also solubilized together with the protein highlighting a lack of selectivity for protein extraction. Although solvent extraction is generally favoured by researchers due to its simplicity, rapidity, and affordability, several drawbacks for the large-scale production of high-quality protein from BSG are also recognised, including the generation of significant amounts of sodium salts, low selectivity, potential loss of functionality, and the possible denaturation of extracted protein [23].

2.1.3 Thermal extraction method

Thermal extraction involves the application of heat to disrupt plant cell walls, and separate proteins from the polysaccharide structure [6]. The protein extraction yields obtained through thermal methods were found to be comparable to those achieved using alkaline extraction methods, highlighting the viability and advantages of thermal methods as chemical-free and environmentally friendly alternatives for recovering protein from brewers' spent grain [6]. In a study conducted by Qin et al. [24], BSG samples were subjected to solubilization in various water ratios (solid: liquid 6.67, 5, 4, and 2.5% w/v) followed by heating at different temperatures ranging from 35 to 135 °C for durations from 1 to 24 hours. The maximum protein yield reached was 66% with a purity of 53%, achieved by treating BSG at 60 °C for 24 hours. While the protein yields obtained through the thermal method were notably lower than those achieved in this study through the one-step dilute acid method (90%) and the sequential alkaline and dilute acid method (95%), they are still significant, especially given that they were obtained under non-optimized conditions [24]. Furthermore, the selectivity for protein extraction in the thermal method was higher than that observed in chemical processes. As a result, thermal extraction is recognized as an attractive procedure for BSG protein extraction, though further optimization studies are still needed to enhance protein yields and investigate the impact of heat on the functionalities and bioactivities of the extracted protein isolates.

2.1.4 Enzymatic extraction method

Enzymatic hydrolysis is another widely used method to solubilize protein from the polysaccharide matrix, breaking down complex structures into smaller hydrolysates and peptides, thereby enhancing the bioavailability and functional properties of the resulting protein extract [36]. Generally, the enzymatic extraction process is greatly regulated by factors such as the enzyme type, hydrolysis temperature, pH, duration, and the enzyme-to-substrate ratio. In this regard, enzymatic hydrolysis can be categorized into one-step and two-step processes based on the specific enzymes employed. He et al. [37] generated protein-rich hydrolysates with the optimal treatment of Alcalase (20 µl enzyme/g of dry BSG) achieving over 80% separation efficiency in a one step process. In contrast, Niemi and Aura, et al. [36] employed carbohydrase digestion on BSG at 50 °C for 5 hours, followed by proteolytic treatment with Alcalase 2.4L, Promod 144GL, and Acid Protease A. Alkaline protease (Alcalase) isolated 76% of BSG protein, significantly exceeding the yields obtained by neutral (21%) or acidic proteases (30%), consistent with findings by Treimo et al. [38], affirming Alcalase as the most effective proteinase for BSG protein solubilization, releasing up to 77% of total protein. Although appreciable protein extraction yields were obtained through these enzymatic solubilizations, no significant difference was noticed between one-step processes and two-step processes.

In another study conducted by Connolly et al. [39], the alkaline-extracted protein was compared with the two-step enzyme-solubilized protein. More specifically, BSG was sequentially hydrolysed by a mix of carbohydrases (Shearzyme® and Ultraflo®) and various proteinases (Prolyve®, Protease P, Alcalase®, Flavourzyme® and Corolase PP®) under optimal pH and temperature to generate bioactive hydrolysates. After all, the extraction of protein via enzymatic hydrolysis resulted in significantly higher yields when compared to those obtained from direct alkaline extraction (63.09 ± 0.27 and 58.90 ± 1.45%, respectively). The enhanced protein yields from enzymatic processes were theoretically attributed to the breakdown of complex cell walls by carbohydrate-degrading enzymes, facilitating access to internal cell structures [40]. Furthermore, studies indicate that high pH aids in protein recovery by decreasing cell wall integrity and increasing protein solubility. However, the low purity of extracts remains a limitation due to the solubilization of small molecular weight sugars by carbohydrases. Commercial enzymes, though effective, are expensive and sensitive to environmental factors such as temperature and pH, posing challenges for industrial-scale BSG protein extraction. As a result, numerous novel extraction strategies have been explored.

2.2 Novel extraction strategies

Despite the fact that conventional extraction methods are well acknowledged for their simplicity, versatility, and economic sustainability, many drawbacks have been associated with these methods including low isolation protein yields, accelerated protein denaturation, generation of substantial wastewater causing environmental hazard, high addition of chemicals, and large time consumption, resulting in a major bottleneck for the applications of plant-based proteins [2]. Hence, increased attention has been placed on the development of non-thermal green technologies for promoting protein extraction while reducing protein degradation and maintaining protein functionalities [27].

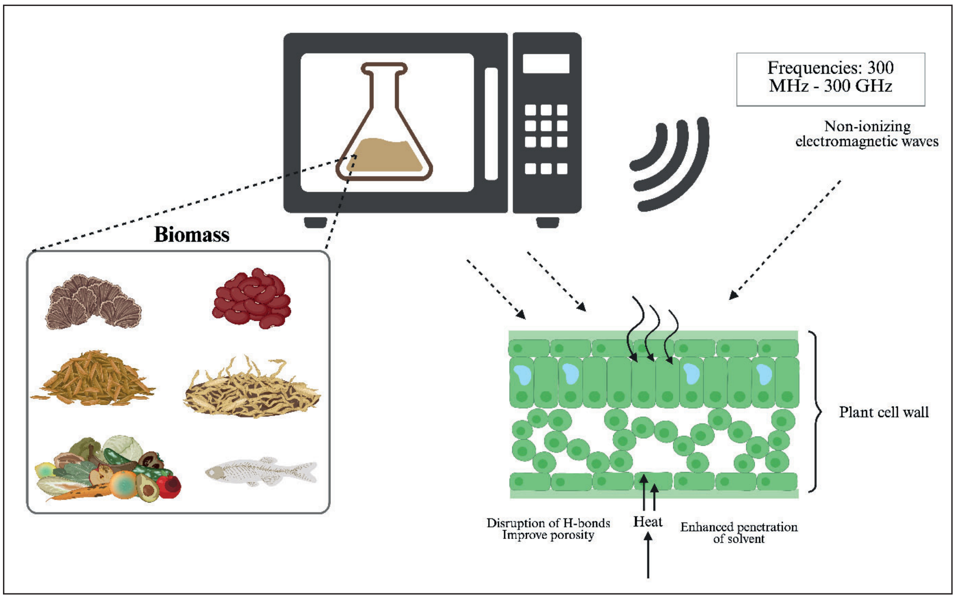

Fig. 1 **Representation of experimental operation of protein extraction from biomass using the microwave-assisted method (created on Biorender)**

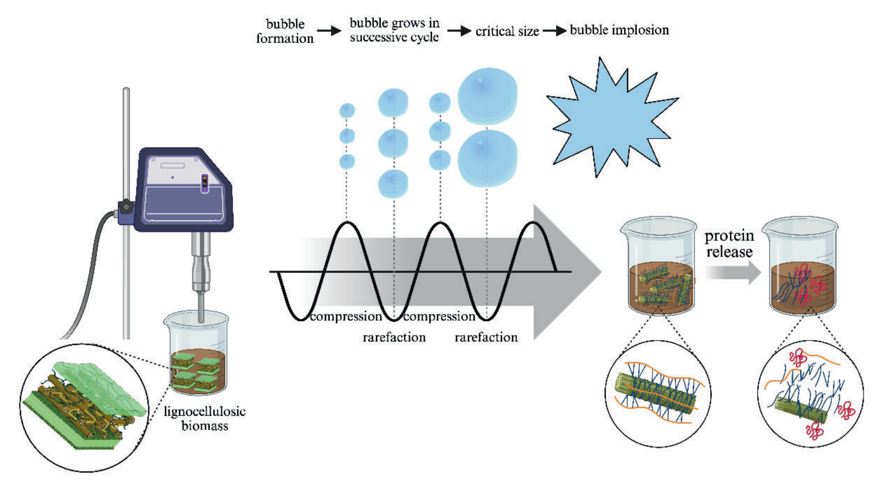

Fig. 2 **Illustration of the ultrasonic-assisted protein extraction method (created on Biorender)**

2.2.1 Microwave-assisted extraction (MAE)

MAE utilizes non-ionizing electromagnetic waves at frequencies ranging from 300 MHz to 300 GHz to disrupt H-bonds present in the cell walls of plant matrices. As demonstrated in Figure 1, these waves selectively interact with polar molecules and generate heat throughout the sample [27]. These electromagnetic reactions facilitate the porosity of plant cell walls, therefore improving the penetration of solvents into the sample which effectively assists the release of proteins into the solvent media [41].

It was observed that MAE can significantly reduce the extraction time compared to the conventional extraction methods, but its efficiency is greatly affected by the microwave energy, processing duration, temperatures and type and concentration of solvents used [41]. Barrios et al. [42] employed MAE in combination with sodium hydroxide to separate protein from BSG. The impact of variables including temperature, time, and alkali concentration on the recovery efficiency was also investigated, with the highest protein yield 93.7% achieved under optimal conditions of 90 °C, 6.25 min and 0.3 M NaOH. However, undesirable components such as sugars and phenolic compounds were also extracted along with the protein, thereby decreasing the protein purity. Moreover, microwaves convert energy to heat which causes the denaturation of heat-sensitive bioactive compounds and enhances the Maillard reaction, subsequently leading to darker BSG extracts [43]. Furthermore, the high temperatures generated during the MAE process have also been reported to promote depolymerization, debranching, and de-esterification of polysaccharides, indicating that extra caution should be taken for optimizing temperatures while using microwave-assisted extraction methods [44].

2.2.2 Ultrasound-assisted extraction (UAE)

UAE is another novel, sustainable, and non-thermal technology that provides an effective alternative to conventional strategies for the extraction of BSG protein, with advantages including economic viability, faster energy transfer, high efficiency, reduced thermal

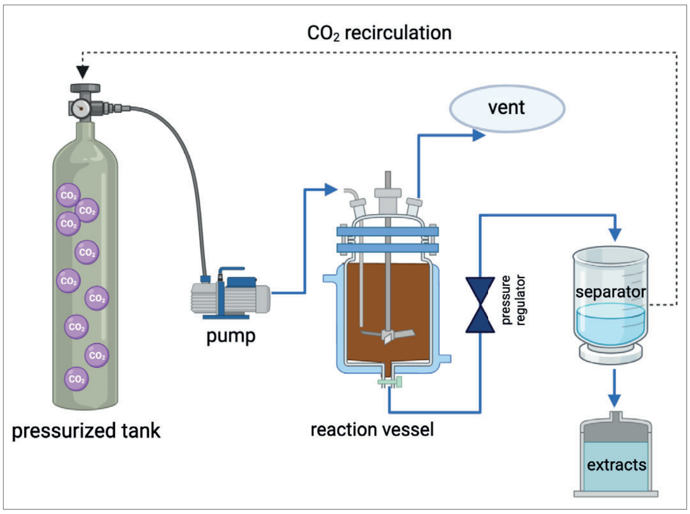

Fig. 3 **The illustration of the supercritical fluid-assisted extraction method (created on Biorender)**

gradients and extraction temperature, as outlined in Table 1. In UAE, ultrasonic radiation at frequencies higher than 20 kHz generates acoustic cavitations and produces hotspots of higher temperature and pressure, thereby liberating proteins from the plant cells [27]. A schematic diagram for the ultrasonic-assisted protein extraction method is presented in Figure 2. The UAE process includes the modulation of mechanical vibrations dependent on a physical medium for propagation, setting it apart intrinsically from MAE (microwave-assisted extraction), where electromagnetic waves can propagate through a vacuum [41]. The rapid generation and collapse of bubbles induced by sonication at the cell surface of plant material, coupled with micro-streaming and shockwaves, apply significant shear and mechanical forces, leading to the disruption of membranes and cell walls, reduction in particle size, and separation of cellular constituents [45].

Frequency, power, duration, temperature, pH, along with ultrasound intensity play crucial roles in the UAE processes. In a study conducted by Tang et al. [46], UAE was used for isolating protein from BSG while Central Composite Design was designated to assess the impact of extraction time, ultrasonic power, and solid-liquid ratio on the protein extraction yield. The three examined variables showed significant linear and quadratic effects on the protein yield, reaching a maximum of 104.2 mg from 1 g of dried BSG under optimized conditions (extraction time of 82.4 minutes, ultrasonic power of 88.2 W/100 ml of extractant, and a solid-liquid ratio of 2.0 g/100 ml). Li et al. [17] employed a similar process for protein extraction from brewers' spent grain, demonstrating that ultrasound-pre-treated BSG at 25 °C with 110 mM NaOH concentration, 1:15 (w/v) solid to liquid ratio under ultrasound treatment at 250 W, 20 min and 60% duty cycle. yielded a significantly higher crude protein yield (86.16%) compared to conventional alkaline extraction without ultrasound (45.71%) under the same conditions. This study also reported on the impact of ultrasonic pretreatment on the chemical structure and functional properties of BSG protein. Structural analysis revealed modifications in the BSG protein structure under UAE, leading to enhanced functionalities, including fat absorption capacity, emulsifying activities, and foaming properties, although water absorption capacity exhibited no improvement. While UAE has been widely acknowledged for favourable results, it presents two main drawbacks: non-uniform distribution of ultrasound energy and a decline in protein recovery with prolonged duration [47]. Nevertheless, the UAE method displays efficacy as an extraction technique with potential high-scale applications in the food and natural product industry, especially when considering the optimization of relevant parameters for different plant matrices.

2.2.3 Supercritical fluid extraction

A supercritical fluid refers to the state of a compound, mixture, or element above its critical temperature and pressure, but below the critical pressure required to condense it into a solid [48]. Carbon dioxide (CO_2) at its supercritical state (31.3 °C and 7.39 MPa) has high diffusivities as a gas and good solvation power as a liquid, making it the most used supercritical solvent [43]. The use of CO_2 as a supercritical solvent offers multiple advantages over conventional solvent extraction, such as its renewability, non-toxicity, non-flammability, cost-effectiveness, and general recognition as safe by the FDA and EFSA [49]. In this context, supercritical CO_2 extraction (scCO_2) is an innovative and promising strategy for the recovery of bioactive compounds from natural sources for the development of functional food, nutraceuticals, and pharmaceuticals [50,51]. During supercritical fluid extraction, temperature and pressure play crucial roles in controlling the scCO_2 process by influencing key properties such as density, diffusivity, viscosity, and dielectric constant, which in turn influence the solubility of solutes in scCO_2 and mass transfer, the two main mechanisms involved in scCO_2 processes [52]. A diagram of the supercritical fluid-assisted extraction method is presented in Figure 3.

Chee et al. [53] explored the use of supercritical fluid technology under varied operational conditions for protein extraction from defatted rice bran. Optimized results revealed that an extraction pressure of 450 bars, for 90 minutes at 60 °C with a flow rate of 17.5 g/min were optimal parameters, yielding a maximum protein content of 17.44%, however, while increased pressure and time led to small increases in protein levels, these effects were not statistically significant. These findings aligned with those reported by Knez et al. [54], affirming that supercritical fluid extraction is an effective technology for converting underutilized agro-industrial by-products into valuable proteins and amino acids. While the current applications of scCO_2 mainly focus on extracting polyphenols, and essential and non-essential oils from various plant materials, it represents a promising approach for recovering functional proteins intended for use in the food industry.

2.2.4 Pulsed electric field (PEF)-assisted extraction

PEF is a novel, non-thermal, and emerging protein extraction technology in which high electric current ranging from 10–80 kV/cm is subjected to biomasses (such as BSG) for a short time, ranging from a few microseconds to milliseconds [27]. The underlaying mechanism of this technique relies on the fact that the structure of the cell membrane permeability is altered or irreversibly damaged due to the action of high voltage (as shown in in Figure 4), resulting in rapid penetration of internal compounds [55]. This theory suggests that membrane permeabilization occurs through the formation of water pores induced by an external electric field on the cell membrane, a phenomenon known as electroporation

[56]. In a study performed by Kumari et al. [57], BSG samples mixed with distilled water (ratio of 1:6 w/w on a dry basis) were subjected to PEF pretreatment under the conditions of 2.8 kV/cm voltage with 3000 pulses of 20µs pulse-width and exposure duration of 60s, followed by a traditional solid-liquid protein extraction procedure at 55 °C, 220 rpm for 16 h. Results indicated that the PEF treatment substantially increased the yields of carbohydrates, starch, reducing sugars, and especially proteins, in comparison to untreated samples, and exhibited positive effects on antimicrobial and immunomodulatory capacities. Except for BSG, PEF has been effectively employed for the recovery of protein from a wide range of plant sources, such as soybeans, rapeseed residues, sesame cake, and microalgae, where PEF not only substantially improved the extraction rate but also enhanced the nutritional profiles and functional attributes of the extracted products [58].

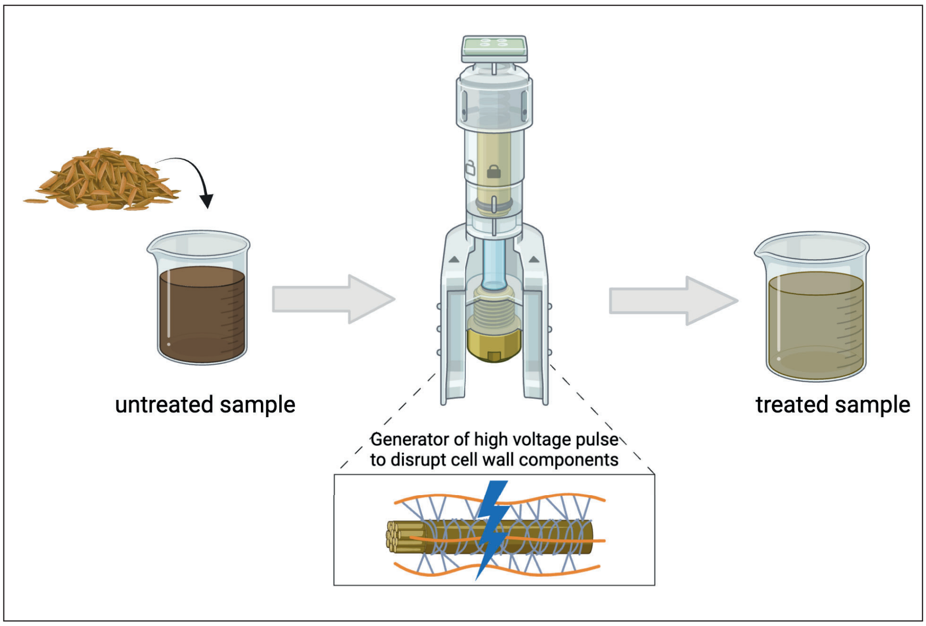

Fig. 4 **The schematic diagram of the pulsed-electric field-assisted protein extraction method (created on Biorender)**

At present, the growing emphasis on sustainability and the efficient use of brewing by-products has led to increased attention toward the valorisation of BSG, given its status as the most abundant brewing by-product. However, BSG extraction presents some inherent challenges associated with its high moisture, polysaccharide, and protein content making BSG susceptible to microbial contamination shortly after production. This, in turn, underscores the need to find suitable, cost-effective, and environmentally friendly upcycling methods for its extraction [59]. While traditional extraction methods exhibit limitations particularly due to low extraction efficiency, limited purity and waste generation which restricts their overall effectiveness, innovative extraction techniques such as microwave-assisted methods, ultrasonic-assisted methods, supercritical liquid methods, and pulsed electric-assisted methods have demonstrated significant improvements in protein extraction rates and selectivity, accompanied by reduced extraction duration and the consumption of chemical reagents and energy, as indicated in Table 1. However, their relatively high installation costs pose challenges for large-scale industrial-level production, potentially increasing the cost of isolated BSG protein and limiting its commercial viability. Therefore, the absence of suitably effective, reliable, and environmentally friendly approaches for BSG valorisation underscores an urgent need to explore biotechnological procedures capable of achieving its efficient bioconversion. Microbial fermentation, particularly solid-state fermentation (SSF), appears to be a promising option for the valorisation of this high-volume by-product.

2.3 Solid-state fermentation

Fermentation is a biological process that involves various microorganisms such as fungi, yeasts, and bacteria in the transformation of an organic substrate into nutritional compounds that can be used in food production and preservation, energy generation, pharmaceutical industries, and material manufacturing [60]. This bioprocess can be primarily divided into two systems: submerged fermentation (SmF), a process where microorganisms grow in a liquid medium containing nutrients [61], and solid-state fermentation (SSF), in which microorganisms are placed on a moist, solid, natural, and non-soluble matrix that acts as support and/or nutrient source in the absence or near absence of free water [62]. In recent years, SSF has gained significant attention across scientific and industrial sectors worldwide, owing to its notable advantages over SmF, especially in terms of agro-industrial waste valorisation and environmental sustainability [63]. Solid-state fermentation has been shown to enhance protein accessibility, increase relative protein content and nutrient concentration while reducing total dry matter by selectively metabolizing carbohydrates during fermentation [64].

2.3.1 The role of solid-state fermentation in agro-industrial waste management

The excessive overproduction of agro-industrial waste has become a recurring problem worldwide, driven by rapid population growth, socio-economic developments, and accelerating urbanization [65]. Globally, 140 billion tonnes of lignocellulose-related organic agricultural waste are produced annually [66]. The management and disposal of agricultural and industrial processing waste represent a widespread and persistent global challenge. At present, the prevalent practices involve the dumping, burning, or burial of most of the agro-industrial waste, either at farm sites or in the backyards of food processing industries [67]. However, these methods have inherent limitations, notably poor efficiency, low selectivity, high cost, and potential environmental and health risks. Inappropriate dumping and burial of agro-industrial waste can generate leachate that contaminates surface and groundwater resources, lead to soil nutrient loss and degradation, result in inefficient land use, and promote the proliferation of pathogenic microorganisms and disease-spreading insects [62].

As land resources become more limited, incineration has emerged as a preferred alternative to landfills for waste BSG due to its ef-

Table 2 Comparisons between commonly used methods and solid-state fermentation for protein extraction from plant sources.

	Traditional and novel methods (solvent/thermal/microwave/supercritical)	SSF
Environmental impact	• High solvent usage and energy intensive (such as solvent and microwave techniques) [74].	• Minimum solvent usage and waste discharge [18].
Protein yields	• Supercritical: low polarity limits protein solubility and purity [29].	• Yield depends on microbial strain/ substrate [18,81].
	• Solvent/thermal: risk of denaturation and high resource usage [75].	
Scalability	• Solvent: cost-effective but increased disposal costs [82].	• Cheap and versatile substrates (e.g., agricultural waste) [81].
	• Supercritical/microwave/ultrasound: high installation cost and energy intensive [2].	• Scale-up challenges: inhomogeneous mixing, downstream processing [71].
Protein selectivity	• Thermal/microwave: Low selectivity (co-extraction of undesirables) and may induce undesirable structural modifications [83].	• High selectivity due to enzyme specificity (e.g. protease targeting proteins) [80].
Time consumption	• High-efficiency (minutes or hours) but may degrade heat-sensitive compounds [2].	• Slower (several days) but gentler; allows simultaneous biotransformation [84].

fectiveness in managing the growing volume of agro-industrial waste, alongside its ability to harness the heat generated as a viable alternative energy source [68]. Nevertheless, significant concerns accompany the incineration process, including the release of harmful organic pollutants, dust, and ashes, posing threats to both the environment and human health [68]. Moreover, waste collection and transportation processes usually consume substantial human, time, and financial resources, causing various adverse environmental and societal impacts such as air pollution, greenhouse gas emissions, acidification, and noise [69]. Improper and inefficient recycling systems further exacerbate environmental concerns including climate change, ecosystem destruction and adverse impacts on human health [70]. In addition, the destruction of valuable lignocellulosic biomass represents a missed opportunity for resource recovery and circular bioeconomy development.

Solid-state fermentation (SSF) emerges as an alternative technology to conventional and novel extraction methods, offering promising prospects and aligning closely with the waste-to-wealth concept through the valorisation of biomass and the reduction of downstream wastewater production [71]. Although SSF is not a novel concept in bioprocessing, its potential for utilizing diverse agro-industrial waste as substrates and for producing value-added compounds was only identified recently [63]. Amid growing interest in sustainable bioprocessing, SSF stands as an economically viable and practically acceptable technology for large-scale microbial biotransformation of agro-industrial waste and by-products including brewers' spent grain, fruit waste, and fish by-products into valuable resources, such as bioactive compounds, bioenergy, and biomaterials [72,73]. Its renewed attention in both scientific and industrial domains is largely attributed to its distinct advantages, including the utilisation of agro-industrial wastes as substrates, higher product yields, the absence of organic-solvent waste, cost-effective downstream processing, reduced capital and operational expenses, and an industry-friendly process configuration [71]. However, the successful development of bioprocesses in SSF relies on crucial factors, including the selection of suitable microorganism strains, substrate, and process parameters, such as pH, temperature, moisture, inoculums, particle size, aeration, and nutrients, among others [71].

2.3.2 Can SSF overcome the limitations of conventional/ novel extraction methods?

Conventional and novel methods of BSG protein extraction including solvent, thermal, enzyme-assisted, microwave, and ultrasonic-assisted methods, among others, exhibit significant drawbacks. As shown in

Table 2, the traditional solvent extraction method, while easy and low-cost, consumes huge quantities of reagents and generates large wastewater, which can consequently lead to adverse environmental impact [74]. The thermal method, on the other hand, may result in protein denaturation or irreversible structural changes due to the high processing temperature [75]. Novel techniques, like the microwave-assisted approach, which has been widely demonstrated to improve protein recovery from a wide range of plant materials [76–79] suffers from high set up costs and can cause the thermal degradation of unstable compounds [27], while Supercritical CO_2, although environmentally friendly, is limited by polarity and scalability [29]. In contrast, solid-state fermentation (SSF) overcomes many of these constraints by eliminating the need for intensive solvent input, operating under mild environmental conditions, and utilizing enzyme specificity to retain protein bioactivity [18,80]. However, SSF presents different challenges, including longer operation times, issues associated with substrate homogeneity, microbial consistency, and product purification.

2.3.3 Solid-state fermentation: important variables

The SSF process offers a wide range of benefits, largely owing to its operational simplicity and its close resemblance to the natural habitats of many microorganisms. Given the special requirements for growth and metabolism among microorganism strains, the selection of solid substrate and bioreactor systems also significantly influences the efficacy of the SSF process. Solid-state fermentation typically requires several days to two weeks, with most processes reaching optimal performance within approximately 3–10 days, depending on the microorganism, substrate, and process conditions [85]. As illustrated in Figure 5, the productivity of this process is regulated by various parameters which can be categorized into biological (strains, inoculum, substrate), physicochemical (moisture content, pH, temperature, aeration, particle size), and mechanical (agitation and design of bioreactor) [63].

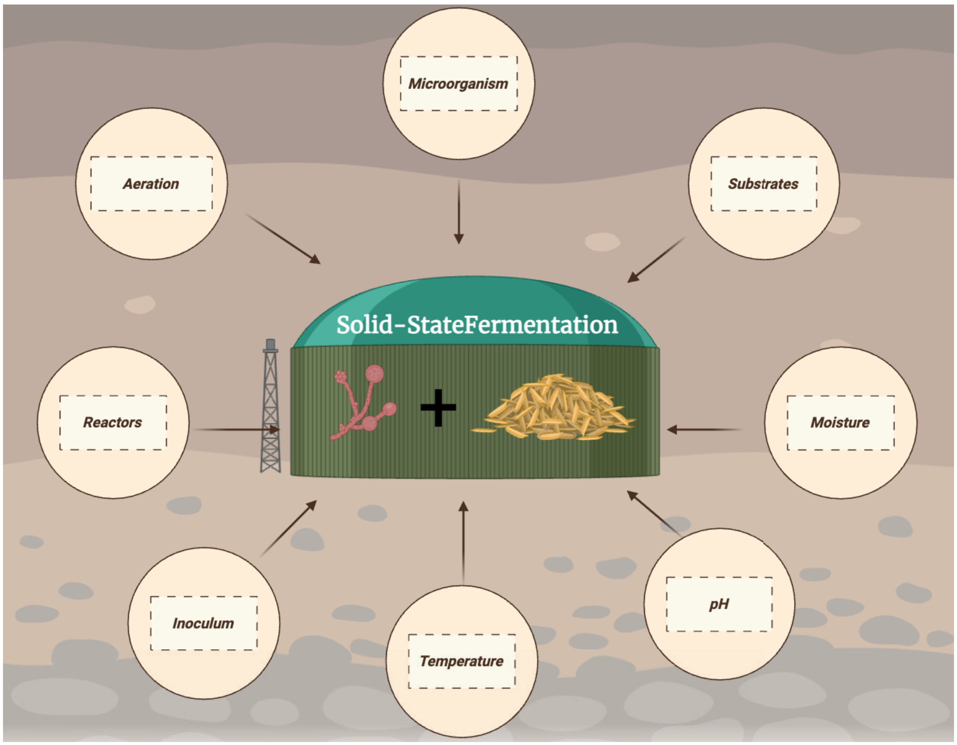

Fig. 5 **The important variables that regulate the solid-state fermentation process (created on Biorender)**

2.3.4 Parameters regulating SSF productivity

Microorganism

The selection of appropriate microorganisms serves as a critical determinant for the success of the SSF process, influencing the quality of the desired products, microbial growth behaviour, substrate degradation capabilities, pH and temperature tolerance, genetic manipulability, and the safety of the final fermented product for human and animal consumption [62]. Various microorganisms can be used in SSF, with fungi and yeast being particularly prevalent due to their compatibility with the fermentation media and the fermentation conditions along with their capacity to secrete thermostable enzymes with high commercial value [86]. The most used filamentous fungi in SSF include genera such as Aspergillus, Fusarium, Penicillium, Rhizopus, and Trichoderma. Yeast genera frequently utilised include Saccharomyces, Candida, and Kluyveromyces, while other relevant microorganisms include Actinomycetota and Aureobasidium [87,88]. Species such as Aspergillus niger and Thermoascus aurantiacus have been shown to produce lignocellulolytic enzymes that catalyse the breakdown of hemicellulose in BSG increasing the release of fermentable sugars [89]. Other fungi, including Rhizopus oligosporus, Rhizopus oryzae and Neurospora intermedia have also demonstrated the ability to improve the nutritional quality of BSG [90,91]. Although SSF typically supports the growth of fungi and yeast, there is increasing evidence to suggest that bacteria can also be successfully utilized in bio-production within SSF. Bacteria, notably Bacillus sp. and Streptomyces sp., are widely utilized in SSF, due to their ability to produce beneficial thermostable and hydrolytic enzymes while tolerating harsh environmental conditions [92,93].

Research on SSF has revealed that microorganisms can be employed in various combinations, including mono-culture, co-cultures (combinations of two strains), or consortia of mixed cultures (combination of three or more known pure cultures) [67]. AboSiada et al. [94] demonstrated that the protein content and nutritional value of five different substrates from agro-industrial wastes can be significantly improved with mono-culture Trichoderma reesei. Similarly, Zepf and Jin et al. [95] employed co-cultures of Aspergillus oryzae and Trichoderma reesei for the bioconversion of grape marc into protein rich animal feed, which resulted in an approximately 280% increase in protein content. Collectively, these examples highlight the versatility of utilizing diverse microorganisms either individually or in combination to valorise agro-industrial by-products, transforming them into valuable bioproducts.

Inoculum

As summarized by Manan and Webb [63], an inoculum can be defined as a preparation containing a high concentration of viable microorganisms, typically introduced to induce favourable modifications in the solid substrate. In SSF bioprocessing, inoculation of the culture medium, often in the form of spores or yeast cell suspensions, is a critical factor influencing productivity. The size of the inoculum directly affects microbial growth in the fermentation process; insufficient inoculum concentrations may inhibit microbe growth, while excessive inoculum levels can prevent mass transfer and potentially hinder metabolite synthesis due to rapid nutrient depletion after germination [71]. The age and physiological state of spores are also critical considerations in the fermentation process, that can lead to suboptimal fermentation rates and SSF performance [96].

Substrates

In fermentation processes, the careful selection of a suitable substrate is essential for achieving efficient and cost-effective production. In SSF systems, the substrate must be accessible, economically feasible and able to provide the necessary nutritional components and physical structure to support optimal microbial growth and metabolic activity [73]. Heterogeneous agricultural products or agro-industry by-products are particularly suitable substrates due to their abundance and low or no cost, coupled with their favourable chemical composition. Agro-industrial residues, including BSG, rice bran, wheat straw, soybean hulks, and corn cobs, among others, present high levels of cellulose or lignocellulose along with other essential nutrients such as proteins, minerals, and amino acids, making them ideal to produce high-value compounds through SSF bioprocesses [63]. Moreover, the use of these agro-industrial by-products as carbon sources during SSF not only reduces the fermentation cost but also mitigates environmental pollution associated with their disposal, offering an attractive and feasible solution, particularly in regions where they are readily available [61]. Appropriate preparation and pretreatment of substrates is essential to ensure optimal quality and yield in SSF. These pretreatments generally fall into three categories: mechanical treatment methods such as grinding, rasping, or cutting reduce particle size and enhance substrate porosity and homogeneity, whereas chemical treatments such as pH regulation through the addition of acids/bases and supplementary nutrients facilitate microbial sporulation, and thermal treatments like heating or sterilization ensure the purity of fermentation cultures [71].

Moisture content

Moisture content plays an important role in SSF by regulating nutrient and gas diffusion, impacting the growth kinetics and physicochemical properties of solids, and ultimately affecting productivity [71,97]. Substrates typically exhibit a moisture content ranging from 30% to 85%, with fungi thriving in a lower range of 40%-60%, while bacteria prefer a higher range of 60%-85% [98]. Higher humidity levels contribute to particle agglomeration, inhibit gas transmission, and increase the risk of bacterial contamination. Conversely, lower moisture levels hinder nutrient diffusion, microorganism growth, enzyme stability, and swollen substrates [71]. Hence, it is vital to maintain and monitor the moisture to ensure it falls within the optimal range to achieve a successful fermentation.

pH

Maintaining appropriate pH is essential for fostering microorganism growth, facilitating biomass synthesis, and degrading nutrient substrates. However, measuring and monitoring pH variations during the fermentation process poses challenges due to the solid substrate's low water content, heterogeneity, and the absence of accurate pH measurement methods [63,99]. Additionally, the secretion of organic acids or specific metabolic reactions may induce pH changes, thereby affecting the growth and productivity of microorganisms [71]. Microorganisms exhibit specific pH ranges with optimum values for activity and growth, favouring slightly acidic environments for fungi and yeast, while bacteria thrive in at a neutral pH range [100]. To address pH variability during SSF, substrate formulations with buffer systems are often employed to maintain pH levels conducive to microbial activity without adversely affecting biological processes [101].

Temperature

The overall temperature of the medium varies considerably during the fermentation process due to the heat generated from the metabolic activities of microorganisms and accumulated in the system [98]. Suboptimal temperatures during fermentation can hinder the growth of microorganisms, reduce product consistency, promote moisture loss, and create heterogeneity within the solid substrate due to condensation [102]. To mitigate these issues, strategies such as aeration and/or agitation of the substrate medium, addition of water by direct injection, and addition of humidified air during the fermentation process coupled with ventilation have been reported to be the most effective approaches [103].

2.3.5 From lab to industry: is SSF-mediated BSG biotransformation commercially viable?

Solid-state fermentation (SSF) has historically found applications across various sectors including food, pharmaceuticals, cosmetics, and materials, to produce a diverse range of metabolites such as enzymes, antibiotics, organic acids, biosurfactants, biocontrol agents, as well as flavour and aroma compounds [104]. Currently, SSF is experiencing renewed interest owing to its versatile applications in bioprocess development, including bioremediation and biodegradation of hazardous compounds, biological detoxification of agro-industrial residues, biotransformation of crops and crop-residues for nutritional enrichment, bio-pulping, and the production of value-added product [105]. Given the growing global environmental concerns, SSF has attracted significant attention due to its sustainability, particularly through the valorisation of underutilised agro-industrial by-products, notably brewers' spent grain (BSG), to generate valuable products [106].

Lignocellulose, a heterogeneous polymeric material, forms a complex three-dimensional network through the interactions of its main components, cellulose (40–50%), hemicellulose (25–30%), and lignin (15–20%) [107]. During SSF, microorganisms initially face challenges in accessing cellulose, hemicellulose, and other constituents present in the plant cell walls of BSG due to its complex structure [81]. Over time, as part of their natural metabolic activity, microorganisms introduced to the BSG substrate generate substantial amounts of diverse enzymes including ligninases and cellulases, facilitating the direct degradation of the 3D lignocellulosic structure on the cell surface, leading to the subsequent liberation of phytochemical substances bound to the solid matrix, and ultimately enhancing their extractability [108]. Therefore, SSF can be applied directly to the biosynthesis of nutraceutical compounds or used as a pretreatment step to prepare bioactive-rich materials to be more readily accessible for extraction.

Multiple studies have demonstrated that SSF is a viable and effective strategy to significantly enhance the protein and amino acid content of BSG under optimized fermentation conditions. For example, Canedo et al. [109] conducted a study investigating the fermentation of BSG with R. oligosporus with different moisture levels and nitrogen sources for 18 days. Results indicated that an initial moisture level of 70%, coupled with the addition of nitrogen sources (ammonium sulfate, urea, and sodium nitrate), led to an almost two-fold increase in average protein content compared to unfermented BSG. This highlighted the potential use of solid-state fermented BSG as an additive in animal feed. Chin et al. [110] investigated the upcycling of brewers' spent grain via solid-state fermentation using Rhizopus oligosporus and showed that SSF increased the protein content of the solid residue from 25% to

34% (dry basis), while also improving protein extractability, with subsequent ethanolic-alkali extraction at 60 °C, pH 9 for 2h producing protein hydrolysates with a purity of 61–66% and enhanced emulsifying capabilities, demonstrating SSF as an effective, albeit time consuming pretreatment to increase protein content. Similarly, Ogunjobi et al. [111] also observed a nearly two-fold increase in protein content (from 18.22% in unfermented to 28.33%) during SSF initiated by A. oryzae after 35 days, accompanied by an overall decline in crude fibre content. The ability of A. oryzae to degrade crude fibre, which contributes to biomass accumulation, is most likely responsible for the changes in nutritional composition, particularly the increased in protein concentration. In another study, Tan et al. [112] highlighted SSF with Bacillus subtilis WX-17as an effective technology for converting complex macronutrients in BSG into valuable components. Their findings showed a two-fold increase in the total amino acid content (0.859 ± 0.05 to 1.894 ± 0.1 mg per g of BSG after fermentation) and notable enhancements in unsaturated fatty acids and total antioxidants quantity. These findings align with those reported by Cooray and Chen [113], where R. oligosporus fermented BSG exhibited increased levels of amino acids, citric acid, vitamins, and antioxidants compared to unfermented BSG.

These studies demonstrate the effectiveness of SSF in the valorisation of BSG, highlighting its relevance to the feed industry but also its promising potential for the food and nutraceutical sectors. The use of BSG presents a valuable opportunity to generate a wide range of value-added products, encouraging industrial stakeholders to integrate this approach into broader waste management strategies within food, feed and agro-industrial systems. Mussatto et al. [64] showed that brewers' spent grain can be valorised via solid-state fermentation to produce high-value industrial enzymes, particularly xylanases and cellulases, as well as phenolic compounds such as ferulic and p-coumaric acids. Embracing environmentally friendly practices and harnessing BSG to produce functional protein, bioactive peptides, and other valuable secondary metabolites can effectively address environmental concerns linked to the disposal of BSG into landfills [7,114]. Despite the appealing benefits of SSF, future work should consider systems that combine the sustainability of SSF with the efficiency of solvent-based or mechanical-assisted methods (e.g. microwave-assisted extraction). For example, in the recent work of Zhang et al. [18], the combination of SSF with the pH-shifting method for the isolation of protein from brewers' spent grain was investigated. Under optimal conditions, the combined method significantly improved protein recovery from 0.352 ± 0.015 µg/µL to 0.678 ± 0.0047 µg/µL. In another study, a two-step process of dry fractionation, followed by SSF of chickpeas yielded 28.4% protein-enriched fractions and 47.7% enriched starch fractions [115]. Furthermore, despite the progress in feed applications, BSG bioconversion for human consumption remains limited to lab-scale production [81]. Therefore, further work is essential to advance the development of functional foods that incorporate proteins obtained through SSF.

In recent years, there is a growing trend for the use of artificial intelligence (AI) to improve the efficiency and quality of functional compound recovery from plant waste. AI-driven tools can be used to determine the optimal experimental parameters, including solvent type, concentration, and temperature for specific plant waste and target metabolites, ultimately resulting in a more efficient extraction process [116]. In addition, AI models and machine learning methods can also be used in the process of predictive maintenance of equipment, limiting downtime and ensuring continuous output in large-scale extraction/production plants [117]. Consequently, future studies should incorporate AI to optimise the SSF process to isolate bioactive metabolites from agro-industrial waste, with the goal to improve both economic returns and operating performance.

While a high level of protein can be recovered from BSG, substantial difficulties persist in maximizing the commercial competitiveness of BSG through SSF bioprocessing. Integrated approaches must be developed to facilitate the biotransformation of BSG from laboratory bench to industrial scale that address technological feasibility as well as environmental performance, economic viability, regulatory compliance and logistical coherence [118]. Techno-Economic Feasibility for SSF protein extraction from BSG traditionally has an intermediate to high Technology readiness level (TRL) [118]. However, key challenges remain in tailoring bioreactor configurations for brewers' spent grain, scaling up the use of SSF-fermented BSG to industrial levels, and optimizing fermentation and extraction conditions to achieve high protein yield [7]. The environmental performance of BSG valorization should be assessed through life cycle assessment (LCA) as it largely depends on the energy required for stabilization and drying, transport distances, and the ability of the resulting products to replace conventional materials [119]. To evaluate the economic viability of SSF for breweries, factors such as moisture content, stabilization requirements, transport distance, logistics infrastructure, local demand, brewery size and material classification of BSG within the European context should be taken into consideration [118–120]. Whether BSG is classified as waste or as byproducts under European waste legislation strongly influences regulatory requirements, logistics, costs, and ultimately the feasibility of their valorization, particularly for high-value food and feed applications, with materials classified as waste, typically being subjected to stricter requirements [121].

The handling and processing of large volumes of freshly often daily generated, highmoisture BSG require rapid stabilization or immediate utilization to prevent spoilage, as logistical constraints pose challenges for storage and transport, process control, uniform fermentation, microbial stability, and contamination management [122,123]. Transport distance, storage time, and collection frequency also affect environmental performance and economic viability of SSF particularly for smaller or decentralized breweries [124]. Drying, pressing, or thermal treatments, used individually or in combination to reduce the BSG moisture content below 10%, are scalable and effective in lowering product volume, reducing transport and storage costs, and inhibiting microbial growth. However, these treatments can significantly increase energy consumption and may diminish environmental and economic benefits if fossil-based energy is used or if they are not integrated with waste heat recovery or other energy systems within the brewery. Preservation of brewers' spent grain by oven drying, freezedrying, or solar drying has been shown to reduce product volume and associated transport and storage costs while largely maintaining its original composition, with oven drying preferred over freeze drying due to cost, practicality and scalability [122]. Alternative preservation methods including freezing, ensiling, membrane filter pressing and additive addition have also been shown potential to inhibit microbial contamination and reduce nutrient deterioration facilitating storage and transport [122,125,126].

Overall, the techno-economic feasibility of BSG valorization through SSF varies significantly and implementation should be evaluated on a case-by-case basis; however, for small and medium-sized breweries, on-site SSF protein extraction may generally be a more practical option than advanced biochemical extraction pathways that rely on costly stabilization and transport. However, the high initial capital expenditure required for specialized equipment, along with the technical expertise needed to operate and maintain SSF systems, may limit feasibility [120]. For large breweries, in areas with well-developed food and biotechnology industries, biochemical and material-based uses of BSG can offer a higher value if logistical challenges can be minimized [118,127].

3 Conclusion

BSG-derived protein has been extensively recognised as a valuable source of plant protein with desirable functionalities and bioactivities. Nonetheless, its application and commercialisation remain limited due to the complex composition of BSG, which makes efficient protein extraction difficult. Although conventional techniques, including solvent extraction, thermal extraction, and direct enzyme solubilisation, have been widely utilized due to their simplicity and cost-effectiveness, they are associated with various environmental concerns. To overcome these issues and follow sustainable principles, several novel extraction techniques have been developed as alternatives to conventional extraction methods, offering advantages in terms of extraction time, solvent consumption, protein extraction yield and reproducibility. However, most of these methods require high equipment investment and are only performed on a laboratory scale, so commercial applications are still at an early stage of development. Therefore, it is essential to develop bioprocesses that are economically feasible, environmentally sustainable, and capable of being scaled to industrial levels for the efficient recovery of proteins from BSG. In this context, solid state fermentation (SSF) has emerged as a promising approach due to its sustainability, high substrate-based productivity, and potential cost effectiveness. Although SSF is operationally simple its practical implementation is challenged by difficulties in controlling moisture, heat and mass transfer, and achieving uniform conditions during scale up. Moreover, despite its operational simplicity and potential scalability, SSF remains limited by factors such as dependence on the microbial strain used, relatively long fermentation times, and the complexity of downstream processing. To overcome these issues, future research should focus on the combined application of emerging, sustainable, and high-performance protein extraction technologies. In addition, the underlying mechanisms and potential modifications of these newly established extraction approaches must be further explored, as they can significantly influence the physicochemical and functional properties of the extracted proteins. Furthermore, due to the vast global production of BSG annually, a considerable amount of residual biomass remains after protein extraction. Consequently, there is a pressing need to further develop sustainable circular protein extraction systems to fully valorise brewing by-products and contribute to the advancement of the circular bioeconomy.

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Conflict of interest:

The authors declare no conflict of interest.

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