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Brewers' spent grain protein: A promising functional ingredient for innovative food applications

The global food system is confronted with major challenges including food waste and food insecurity, driving the urgent need to find alternative sustainable food sources. At the same time, the brewing industry faces growing economic and environmental pressure to reduce both energy consumption and waste generation. Brewers' spent grain (BSG), the dominant by-product of the brewing industry, represents a promising resource due to its widespread availability and rich content of proteins and bioactive compounds. The incorporation of BSG-derived ingredients can enhance the nutritional profile of food products and may contribute to the reduction of diet-related disease risk and associated mortality. Nevertheless, further research and technological innovation are needed to broaden its applications in food manufacturing. This review provides a comprehensive overview of the physicochemical, functional and bioactive properties of BSG protein, as well as its digestibility and role in functional food development, thereby providing valuable insights for brewing by-product valorisation and identifying directions for future research.

Descriptors: Brewers' spent grain protein, functional properties, bioactive activities, functional food ingredient, waste valorisation

1 Introduction

Food insecurity remains one of the greatest challenges facing humanity, with approximately 733 million people undernourished due to inadequate access to safe, high-quality protein and nutrient-dense diets [1]. This issue is further exacerbated by limited natural resources, imbalances in food supply and demand, climate change, and environmental degradation, highlighting the need for sustainable alternative protein sources [2]. Insufficient availability of high-quality protein in adequate amounts can result in poor health and malnutrition, leading to a cellular imbalance between nutrient availability and energy demand [3]. According to the United Nations World population prospects 2022 revision, the world's population is expected to reach 9.7 billion in 2050 and could peak at nearly 10.4 billion in the 2080s [4]. This rapid growth of the world population together with increased urbanization and population aging, is expected to double the demand for food by 2050, raising serious concerns for both environmental sustainability and food security. However, conventional livestock-based food production is associated with multiple adverse impacts, including greenhouse gas emissions, intensive land use, wastewater generation, public health concerns, biodiversity loss, and ethical challenges [5]. Thus, there is an urgent need to identify more sustainable and environmentally friendly protein sources to ensure long-term food security and meet the United Nations Sustainable Development Goals (SDGs), particularly those related to ending hunger (SDG 2) [6].

Growing awareness of the importance of dietary protein, coupled with the rising popularity of plant-based and vegan lifestyles, has significantly increased interest among researchers and the food industry in identifying affordable, abundant, and sustainable plant protein sources. This momentum is further driven by global sustainability concerns, the need to reduce dependence on animal-based proteins, and the shift toward circular food systems that promote the valorisation of agro-industrial by-products. In parallel, there is an increased emphasis on developing cost-effective and scalable technologies for protein extraction, modification, and incorporation into food as functional ingredients. Additionally, evolving consumer preferences for sustainable, clean-label, and health-promoting foods have further accelerated the demand for innovative plant-derived proteins that can meet both nutritional and environmental requirements [7]. Agro-industrial residues, wastes, and by-products are increasingly recognized as valuable sources of bioactive proteins

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with significant health-promoting potential. Among these, brewers' spent grain (BSG) stands out due to its high protein content (15–30%) and widespread availability [8–11] Improved valorisation of such by-products can reduce reliance on conventional protein sources while aligning with circular economy principles by enhancing resource efficiency and minimizing waste as illustrated in figure 1. However, the effective utilization of BSG is often constrained by its high moisture content, which makes it highly perishable, susceptible to microbial spoilage, and challenging to store and transport without proper stabilization [12] Different drying techniques can have a significant impact on nutrient retention in BSG [13] Oven drying has been shown to be an effective method for preserving brewers' spent grain (BSG), by reducing its moisture content below 10%, it inhibits microbial growth, reduces its weight and volume and lowers associated transport and storage costs [14]. Although freeze drying provides better preservation of heat-sensitive nutrients and organoleptic qualities of BSG, oven drying is cost-effective, scalable, and easier to implement at industrial scale [13] Other preservation methods, including pressing, ensiling, membrane filter pressing, and the use of additives, have also shown potential for improving the stability and shelf life of BSG during storage and transport; however, further research is needed to evaluate their long-term effectiveness, economic feasibility, and scalability at industrial level [14–16]. Overall, the selection of a BSG preservation method depends on balancing product stability, nutrient retention, cost, and scalability, with oven drying currently representing the most practical option for large-scale industrial applications. After stabilization, extraction and fractionation processes can facilitate the recovery of valuable nutrients and bioactive compounds from BSG, with effective preservation playing a key role in maintaining the nutritional quality and functionality of the resulting fractions [12, 17, 18]. Despite these limitations, the development of cost-effective and scalable processing and preservation strategies that maintain the nutritional quality of BSG can enable the effective utilization of BSG as a sustainable and environmentally friendly protein source, contributing to long-term food security and nutritional sustainability [19, 20].

Although several recent reviews have examined brewers' spent grain protein (BSGP), their focus has primarily been limited to the influence of protein extraction techniques on its functional properties [11, 21, 22]. Consequently, a more comprehensive evaluation of BSGP is needed to support its application in food systems, particularly one that integrates recent advances in its techno-functional characteristics, bioactive properties, and potential food applications. Accordingly, this review examines the physicochemical and techno-functional properties of BSG proteins, including solubility, thermal stability, emulsifying and foaming properties, and water/oil holding capacity. It also critically evaluates the major bioactive activities reported for BSG-derived proteins and peptides, including antioxidant, antihypertensive, and antimicrobial effects, together with their behaviour during gastrointestinal digestion, bioavailability, and safety considerations. Finally, current and emerging applications of BSG-derived ingredients in functional foods are discussed to provide a clearer understanding of the opportunities and challenges associated with the commercial exploitation of BSGP. Where BSG-specific evidence is limited, relevant findings from other cereals and legumes are included to highlight existing knowledge gaps and future research needs.

2 Physicochemical and functional properties

As an abundant by-product of the brewing industry, BSG promising characteristics that extend beyond conventional applications, particularly in food development applications. Evaluating its techno-functional properties, which are influenced by its physicochemical characteristics, is essential for understanding how its proteins behave within food systems. [11]. A comprehensive study of the techno-functionality of BSG proteins is essential for their application in various industries and for advancing the broader discourse on sustainable waste valorisation.

2.1 Protein solubility

Protein solubility is a key thermodynamic property that describes the concentration of protein that can dissolve in an aqueous solution at equilibrium with its solid phase, while also reflecting the practical ability of proteins to remain dispersed in solution under specific conditions such as pH, temperature, ionic strength, and the presence of solvents or additives [23]. Its significance extends beyond simply dissolution, influencing functionalities such as emulsification, viscosity, foaming, and quality attributes including appearance, sedimentation, and flavour, while also defining the nature of the food matrix (liquid or solid), the phases stabilized (oil or air), processing requirements (thermal treatment, size reduction), and the time involved in these operations [24].

Generally, the proteins present in BSG exhibit notably low solubility within the pH range relevant to food applications (pH 2 to 9), primarily due to their chemical composition [25]. Soluble proteins are extracted into the liquid phase during the mashing process, leaving behind water-insoluble protein fractions, predominantly prolamins and glutelins, in the BSG residue [26]. Alkaline conditions, particularly pH 10, have proven most effective for solubilizing BSG proteins, although the partial solubilization of BSG lignin in alkali (pH 10), potentially poses fractionation challenges [27]. To enhance protein release from BSG, several attempts to fractionate protein have shown that enzymatic treatment on BSG can significantly improve the solubility, emulsifying, and foaming properties of extracted protein [28, 29]. Celus et al. [29] reported that enzymatic pretreatment, particularly with pepsin, was effective in increasing the solubility of proteins present in a BSG extract. At a 6% degree of hydrolysis, the solubility exceeded 90% across the pH range of 2-10, due to the enzymatic hydrolysis reducing protein size and increasing hydrophobicity. However, the duration of enzymatic hydrolysis poses a delicate balance, as prolonged periods may yield excessively small peptides, diminishing foaming and emulsifying properties, while shorter durations could lead to undesirable bitterness and odour [30, 31].

Despite the effectiveness of enzymatic processing, its high costs, along with the generation of wastewater and significant energy requirements during hydrolysis, have driven the exploration of alternative biomodification approaches such as fermentation [32]. Recent investigations have indicated that fermentation processes induce enzymatic activities, resulting in the modification of proteins, thus consequently enhancing protein bioactivity, nutritional value, and functional properties in plant materials [33]. A study conducted by Zadeike et al. [34] highlighted substantial improvements in

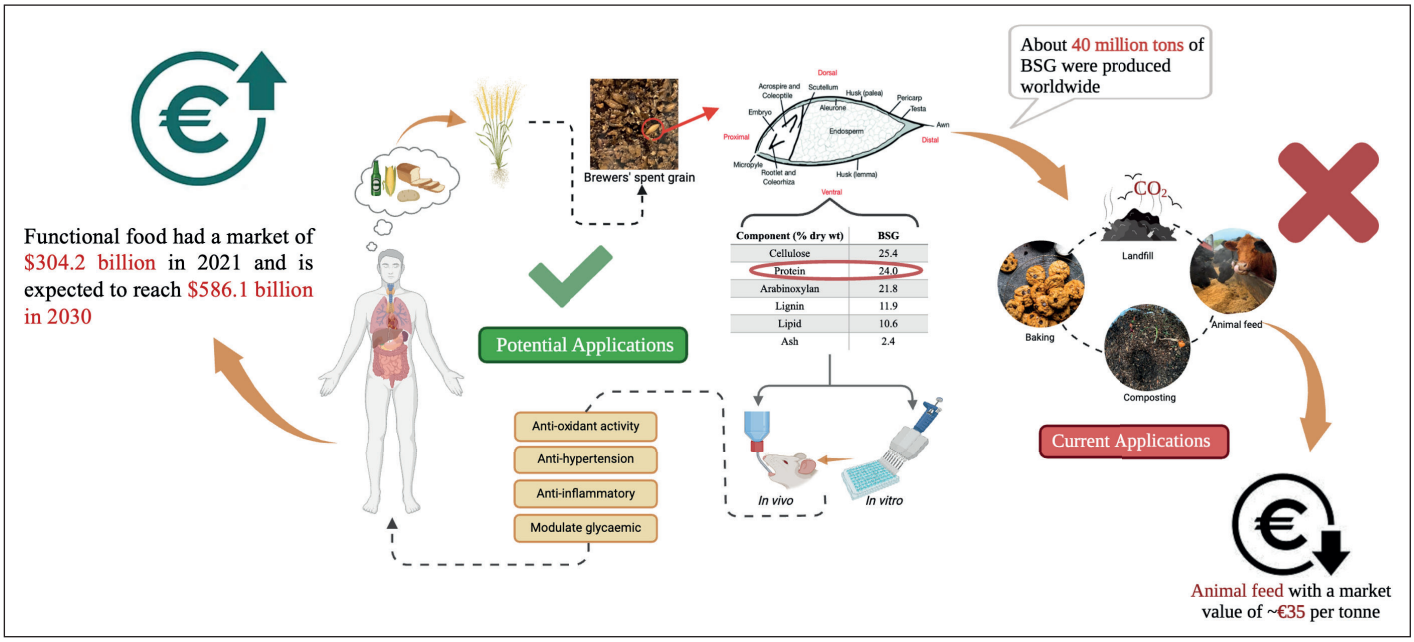

Fig. 1 **Current and potential applications of brewers' spent grain (created on Biorender)**

various aspects of solid-state fermented corn-milling by-products utilizing *Lactobacillus sakei* MI401 and *Pediococcus acidilactici* PA-2 strains, including nutritional quality, protein functionality, digestibility, and bioactivity, when compared to the untreated samples. Collectively, these findings demonstrate that although significant improvements in BSG protein solubility can be achieved through enzymatic and fermentation-based processes, the trade-offs between functionality, sensory quality, and processing costs highlight the need for more targeted and scalable approaches to fully exploit BSG proteins as food ingredients.

2.2 Thermal processing

Thermal treatments, such as pasteurization and sterilization, are fundamental in food processing for microbial control, shelf-life extension, inactivation of antinutritional factors, improvement of digestibility, and enhancement of flavour or texture [35]. At the molecular level, these treatments increase protein mobility, leading to the disruption of intra- and intermolecular interactions and consequent alterations in secondary and tertiary structures. As a result, hydrophobic amino acid residues become exposed, promoting protein denaturation, aggregation, or cross-linking with higher molecular weight components, which ultimately reduces solubility and may lead to precipitation [36]. In this context, understanding the denaturation temperature of proteins present in BSG is essential for predicting their behaviour during thermal processing. This can be assessed using techniques that measure protein thermal properties, such as differential scanning calorimetry, which directly determines denaturation temperature; however, limited data are available for BSG proteins [11]. Consequently, simpler experimental approaches are often employed, including immersion of protein samples in an oil bath at 140 °C. In this case, thermal stability is evaluated using heat coagulation time (HCT), defined as the time elapsed between placing the sample in the oil bath and the onset of protein coagulation, providing an indirect measure of protein stability under heat [28, 37].

Various factors, such as pH, ionic environment, hydrolysis, and protein concentration, influence the thermal behaviour of plant-based proteins [38]. Generally, BSG protein or hydrolysates exhibit optimal technological performance under alkaline conditions, with solubility gradually diminishing as pH shifts towards acidity [39]. In addition to pH changes, enzymatic hydrolysis of BSG protein, as observed by Connolly et al. [28] significantly increased heat stability at pH 6.0, with heat coagulation time at 140 °C rising from 40 ± 3 s to > 300 min for all hydrolysates. This differs from soy protein hydrolysates, where the thermal aggregation times depend on the proteolytic preparation used. Kim et al. [40], also found that increasing the concentration of soy protein isolates and their hydrolysates (from 1.8 to 3.6 to 7.2% w/v) led to a significant decrease in thermal stability, due to the resultant increase in protein-protein interactions, promoting aggregation in the presence of heat. Overall, while pH adjustment and enzymatic hydrolysis appear to markedly improve the thermal stability of BSG proteins, the limited availability of direct denaturation data and the variability of responses across different plant protein systems suggest that current understanding remains insufficient for reliably predicting BSG protein behaviour under diverse industrial processing conditions.

2.3 Emulsifying properties

Emulsions, defined as dispersions of two immiscible liquids with one liquid dispersed in the other as small droplets (0.1–100μm), play an important role in the food industry, with common examples being oil-in-water (O/W) mixtures, such as cream and milk, and water-in-oil (W/O) mixtures, such as margarine [41, 42]. Proteins isolated from various materials such as casein, ovalbumin, soy, wheat, and peas are favoured in the food industry for their emulsifying abilities, owing to their amphiphilic nature (i.e., having both hydrophobic and hydrophilic groups) and film-forming capacities [30, 43–46]. The amphiphilic nature of proteins allows them to migrate to the interface in both oil-in-water (O/W) and water-in-oil (W/O) systems, where hydrophobic regions drive adsorption by fa-

cilitating attachment to the interface [47] Once adsorbed, proteins undergo structural rearrangements, partially denaturing to align hydrophilic portions with the aqueous phase and hydrophobic portions with the oil phase, thus forming viscoelastic films that prevent the emulsion or foam from breaking apart when physical forces are applied. [48].

Turbidimetric techniques, conductivity, and droplet size measurements are commonly employed to assess emulsification properties, with parameters like emulsifying activity index (EAI) and emulsifying stability index (ESI) being key indicators of protein performance [49]. EAI represents the protein's adsorption ability and ESI measures the stability of the adsorbed layer over a defined duration [49]

Several studies have indicated that factors such as pH, protein concentration, and temperature significantly influence emulsion formation and stability. For instance, the emulsifying properties of BSG protein are least favourable around its isoelectric point, exhibiting a negative relationship between droplet size in stabilized emulsions and protein solubility, with increased solubility correlating with decreased droplet size [28]. The application of solid-state fermentation has exhibited positive effects on the functional properties of brewers' spent grain (BSG) protein. Chin et al. [50] conducted a study on BSG protein using *Rhizopus oligosporus* ATCC 64,063 through solid-state fermentation at 37 for three days, revealing enhanced EAI and ESI values compared to unfermented BSG protein. These observations are consistent with those reported by Park et al. [51], who demonstrated improved emulsifying properties in protein hydrolysates obtained from fermented soybean meal, characterized by increased amounts of shorter chain peptides and amino acids. Overall, their findings demonstrated the potential of the hydrolysates obtained from microbial fermentation to serve as emulsifiers in formulated food products such as mayonnaise, soups and sauces. However, current findings are largely based on laboratory-scale studies, limiting the ability to predict their effectiveness across diverse food matrices and commercial processing environments.

2.4 Foaming capacity

Foaming represents a significant aspect of food processing, characterized by the dispersion of gas (such as air, nitrogen, or carbon dioxide) within an aqueous solution or solid matrix [52]. In the context of protein foaming mechanisms, proteins, characterized by the presence of both polar and non-polar groups, adsorb at the gas-liquid interface, rapidly forming a protein film that reduces the surface tension of water to stabilize foam, with its characteristics mainly manifested by various molecular interactions, such as hydrophobic interaction, Van der Waals forces, hydrogen bond interaction, and disulphide bond interaction, among others [53]. The secondary and tertiary structure of the protein is unfolded under the action of high-speed shearing or whipping, exposing hydrophobic groups which promote polymer formation through cross-linking, thereby enhancing emulsifying and foaming properties [52]. Thus, partial denaturation or aggregation of proteins typically improves their foaming characteristics. Like emulsion stability, factors affecting foam stability include disjoining pressure, surfactant film viscoelasticity, and interfacial tension, with liquid drainage and gas disproportionation resulting in foam instability [54]. Drainage leads to the coalescence of bubbles due to thinning and rupture of the liquid film, while disproportionation, also known as Ostwald ripening, involves gas diffusion from smaller to larger bubbles.

In foam analysis, foaming capacity (FC) and foaming stability (FS) are commonly used to describe and quantify foam formation and characterization [55]. FC denotes the foam-generating ability, typically measured by foam/liquid height or maximum volume or weight of foam, while FS indicates foam volume stability, represented by the foam height after a specified duration [52, 56]. The Waring-Blender method, also referred to as the whipping method, is a commonly used method for evaluating foam systems' properties by determining the foaming volume obtained after whipping (FC) and the foam drainage volume overtime (FS) [56]. It is preferred in laboratory works over alternatives like the Bikerman and Ross-Miles method due to its straightforward and easily comprehensible operation process, as well as its capability to ensure uniform distribution of solution throughout the whipping process [52].

Chin et al. [50] investigated the influence of pH and solid-state fermentation treatment on the FC and FS of proteins derived from BSG. It was found that higher pH resulted in more stable foam, with fermented BSG protein exhibiting significantly higher FC and FS (16–30% of FC and 7–14% of FS) compared to unfermented BSG protein (7–14% of FC and 2–7% of FS) across all pH ranges. Connolly et al. [28] also reported similar observations that FC and FS

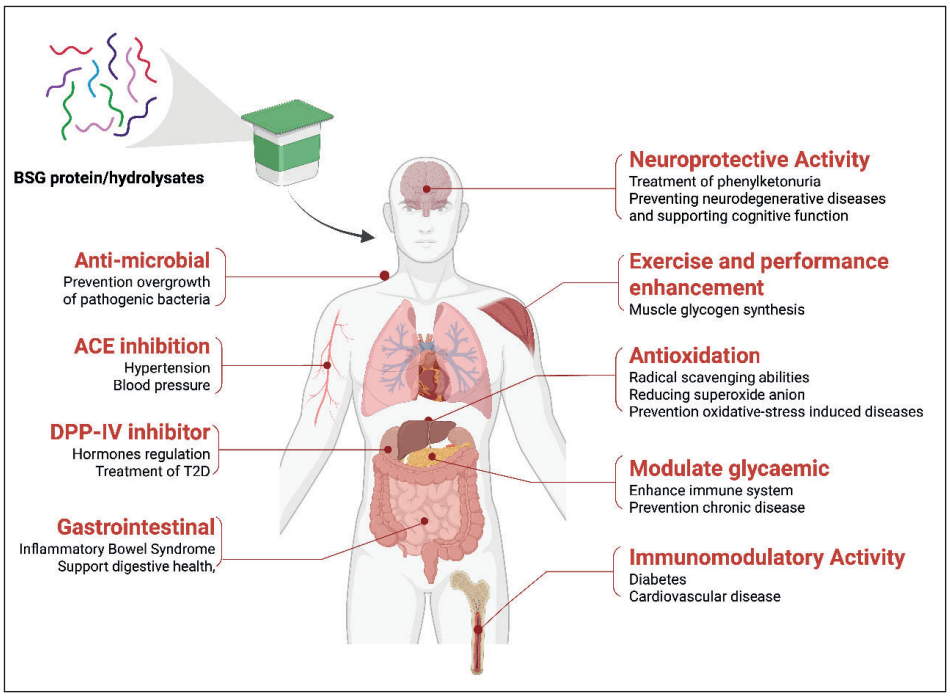

Fig. 2 **Potential health benefits of protein and bioactive peptides isolated from brewers' spent grain (created on Biorender)**

of BSG protein increase with increasing pH values. This is possibly attributed to enhanced net charge on the protein under alkaline conditions, which reduces attractive hydrophobic forces and facilitates rapid diffusion to the air-water interface. Apart from pH, microbial fermentation processes were also shown to positively impact protein foaming ability as demonstrated by Meinlschmidt et al. [57] who observed nearly doubled foaming activity in fermented soy protein compared to non-fermented samples. However, FC and FS are influenced by factors such as time, pH, and the microbial strains used during fermentation, with the potential to result in undesirable properties, including off-flavours, reduced nutritional value, or impaired texture.[58]. Although these preliminary studies indicate that fermentation may enhance the foaming functionality of BSG proteins; the available evidence remains limited and further research is required to confirm their suitability for diverse food applications.

2.5 Water/oil holding capacity (WHC/OHC)

Water-holding capacity (WHC) refers to the water-binding ability of protein by polar side chains, while oil-holding capacity (OHC), describes the binding of lipid by nonpolar side chains of proteins [59]. These properties are important in food applications as they influence textural characteristics and mouthfeel properties [60] Intrinsic factors affecting the water/oil holding capacity of food proteins include amino acid composition, protein conformation, and surface polarity/hydrophobicity [61]. However, protein extraction methods can significantly influence protein conformation and hydrophobicity, thereby altering WHC and OHC. This is illustrated by Silva et al.[62] who reported a significant increase in WHC of alkaline-extracted BSG protein concentrates with a rise in pH from 8 to 12 during extraction, while no substantial difference was observed in OHC values of BSG protein obtained using different preparation methods. It is noteworthy that both the WHC (3.2-5 g/g) and OHC (3.2-5.1 g/g) values of BSG protein were superior to those reported for other plant-derived protein concentrates such as pea, faba bean, lentil, chickpea, wheat, oat, and soy, which varied between 0.6 to 4.9 g/g, and 1.0 to 3.96 g/g, respectively. High WHC values are desirable in gel-like foods such as sausages, custards, or mayonnaises, as they contribute to a desirable texture, while OHC is crucial for enhancing mouthfeel and retaining flavour [63].

3 Bioactivities of BSG protein and hydrolysates

Continuous exposure to physiological imbalances and external toxic substances can disrupt normal bodily functions, leading to the development of various health conditions [64]. In this context, alongside growing consumer awareness of the importance of protein intake, bioactive proteins and peptides have attracted considerable interest due to their ability to modulate, enhance, or inhibit a range of physiological pathways [65]. Recently, BSG has emerged as a promising source of natural bioactive peptides due to its economic viability and its high protein content and abundance of essential amino acids compared to other cereal products [66]. Bioactive peptides, initially inert within the parent protein sequence of BSG, can be released through enzymatic hydrolysis by digestive enzymes, fermentation of protein substrates with proteolytic starter cultures, or proteolysis by enzymes sourced from microorganisms or plants [67]. Once released, these bioactive proteins and peptides have the potential to contribute to the management and prevention of a range of health disorders. Consequently, the incorporation of BSG-derived proteins as functional ingredients in the food industry is of considerable interest, given their potential health-promoting properties, including antioxidant, antihypertensive, antimicrobial, glycaemic-regulating, and immunomodulatory [9, 68–71]. A summary of the main bio functional properties reported for BSG-derived protein and its relevant hydrolysates is displayed in figure 2.

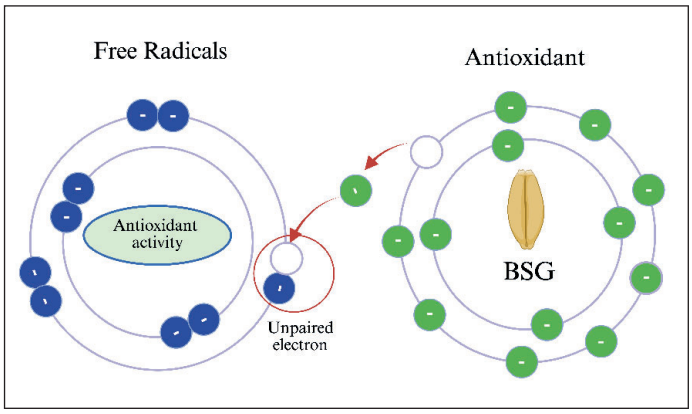

Fig. 3 Antioxidant bioactive molecules can neutralize free radicals by releasing electrons (created on Biorender)

3.1 Antioxidant activity

The overproduction of free radicals within the human body can induce oxidative stress, leading to damage to lipids, proteins, and DNA, and subsequently contributing to the onset of chronic diseases such as diabetes, cancer, inflammation, and cardiovascular diseases [72]. These free radicals, collectively referred to as oxidants, include reactive oxygen species (ROS) and reactive nitrogen species (RNS), such as superoxide (O_2^-), peroxynitrite ($ONOO^-$), and hydroxyl radicals (•OH), and are generated from endogenous metabolic processes, exposure to environmental or physicochemical stressors, or pathological conditions [73]. Oxidative stress occurs when the body's capacity to regulate free radicals is overwhelmed, resulting in an imbalance between oxidants and antioxidants and leading to cellular damage [74]. Oxidation is also recognised as a major contributor to food deterioration, leading to rancidity, undesirable flavour and texture changes, and ultimately reduced shelf life [65]. On the other hand, synthetic antioxidants like butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) have raised several concerns due to their instability at high temperatures and potential health risks, prompting a shift in consumer preference toward natural antioxidants [75]

Antioxidants, molecules capable of neutralising free radicals by donating electrons or hydrogen atoms, play a crucial role in mitigating cellular damage by inhibiting lipid peroxidation, scavenging reactive oxygen species, and chelating metal ions (Fig. 3) [76]. In addition to these direct mechanisms, antioxidant peptides and protein hydrolysates can enhance the body's endogenous defence systems by activating signalling pathways such as Nrf2/Keap1-ARE and NF-κB signalling pathways, thereby contributing to antioxidant protection [77].

In recent years, there has been growing interest in the generation of antioxidant peptides from brewer's spent grain (BSG) proteins. Given the large volumes produced, the associated environmental challenges, and the increasing global demand for sustainable protein sources, the valorisation of agro-industrial by-products such as BSG as natural antioxidants in food systems has gained significant attention [78]. For instance, Vieira et al. [79] conducted a study where alkaline extracted BSG protein was hydrolysed using brewer's spent yeast (BSY) proteases, Neutrase and Alcalase, at the same proteolytic activity (1 U/mL) with an enzyme/substrate ratio of 10:100 (v/v) at 50 °C for 4 h, resulting in fractions with enhanced *in vitro* antioxidant potential and protective effects against H_2O_2 induced oxidative damage in Caco-2 and HepG2 cell lines. Similar findings were also reported by Vieira et al. [80] that the use of the enzyme Alcalase for the hydrolysis of the protein concentrate obtained by alkaline solubilization and acid precipitation of BSG was effective for generating hydrolysate with antioxidant activity, where inhibition of the ABTS (2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic) acid) radical above 90% was achieved. Recently, Chai and Chen [81] isolated protein from solid-state fermented BSG with *Rhizopus oligosporus* ATCC 64063 using microwave assisted three-phase solvent partitioning, demonstrating significant ferric reducing power (Fe^{3+} to Fe^{2+}) and efficient $ABTS^{\cdot+}$ radical scavenging ability. These findings align with previous studies highlighting the efficacy and scalability of various techniques, such as solid-state fermentation, in enhancing the antioxidant properties of BSG [50, 82]. Overall, the available evidence suggests that BSG-derived protein hydrolysates may represent a sustainable alternative to synthetic antioxidants in food and health-related applications. However, most studies to date are *in vitro* which may not fully reflect antioxidant efficacy under physiological conditions or within complex food matrices. Variations in protein extraction, hydrolysis, and fermentation procedures make direct comparison between studies difficult and highlight the lack of standardized methodologies. Further research is required to identify the specific peptides responsible for antioxidant activity, evaluate their bioavailability and stability *in vivo*, and establish the scalability and economic feasibility of their production.

3.2 Antihypertensive activity

Normal blood pressure typically ranges from 100–140 mmHg (systolic) and 60–90 mmHg (diastolic), whereas values exceeding 140 mmHg systolic and 90 mmHg diastolic typically indicate hypertension, or high blood pressure [83]. Hypertension is a major risk factor for cardiovascular disease and stroke and remains one of the most pressing global public health challenges [84]. According to the Global Burden of Disease (GBD) report, an estimated 1.28 billion adults aged 30-79 years globally have hypertension, with a majority (two-thirds) situated in low- and middle-income countries [85]. Blood pressure regulation is intricately controlled by the renin-angiotensin system (RAS) and the kinin-nitric oxide (NO) system. Within the RAS pathway, angiotensinogen, a polypeptide derived from the liver, is activated by renin, producing angiotensin-I, which is subsequently cleaved to the potent vasoconstrictor angiotensin-II by the angiotensin I-converting enzyme (ACE) [64]. Angiotensin-II binds to receptors throughout the body, inducing vasoconstriction and retention of Na^+ and water to maintain normal blood pressure [86]. However, under pathological conditions, elevated levels of angiotensin II promote excessive vasoconstriction, leading to hypertension and increasing the risk of diseases such as myocardial infarction, as well as cardiac and renal failure [87]. On the other hand, the kinin-NO system produces bradykinin, a potent vasodilator, which exhibits hypotensive effects by increasing intracellular Ca^{2+} and activating nitric oxide synthases (NOS) to generate NO. Nevertheless, the ACE can also degrade bradykinin into inactive peptide fragments, such as bradykinin (1–7) and bradykinin (1–5), therefore preventing vasodilation and promoting vasoconstriction, resulting in hypertension [88].

Traditionally, chemically synthesised antihypertensive drugs such as enalapril, lisinopril, ramipril, and captopril have been widely used to manage hypertension; however, their prolonged use is often associated with adverse effects, including taste disturbances, dry cough, and skin rashes [89]. These angiotensin-converting enzyme (ACE) inhibitors exert their antihypertensive effects by blocking the conversion of angiotensin I to the potent vasoconstrictor angiotensin II, thereby reducing blood pressure. In contrast to synthetic ACE inhibitors, natural hypotensive peptides derived from a wide range of plant-based resources have emerged as promising alternatives due to their high bioavailability and reduced risk of adverse side effects [84]. At present, many hypotensive peptides have been isolated from plant sources such as cereals, beans, nuts, vegetables, fruits, mushrooms, and particularly agro-industrial by-products, several of which have demonstrated significant efficacy *in vivo* [89]. As illustrated in figure 4, bioactive peptides may exert antihypertensive effects through the direct

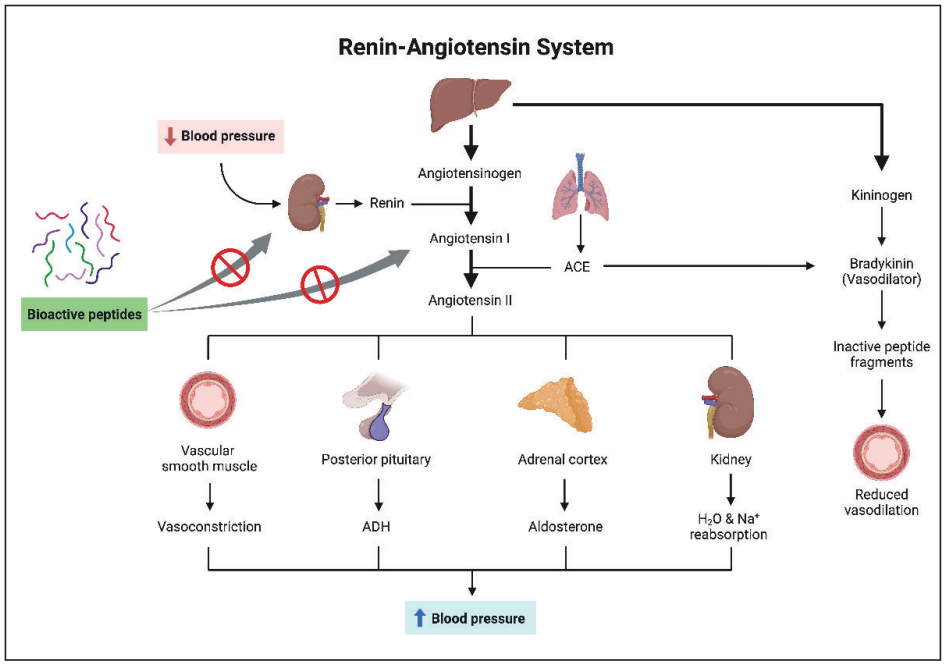

Fig. 4 **Antihypertensive mechanism of plant-derived peptides (created on Biorender)**

inhibition of renin, the enzyme that cleaves Angiotensinogen into Angiotensin I, or by suppressing ACE-mediated formation of angiotensin II.

Grain protein has been extensively researched as a source of peptides with valuable hypotensive activity through numerous *in vitro* and *vivo* studies [84, 86, 90]. Loponen et al. [90] through an *in silico* approach identified several hypotensive peptides within the sequence of barley proteins. This approach indicated the most potent C-terminal amino acids for dipeptides binding to ACE were tryptophan, proline, tyrosine, and phenylalanine, while valine and isoleucine are the most potent ones at the N-terminal. The ACE inhibitory activity of these peptides is quantified in terms of the micromolar concentration required to inhibit 50% of ACE activity (IC50), with lower IC50 values indicating higher inhibitory potency [86]. Notably, the most potent ACE inhibitory peptide identified was Ile-Val-Tyr, found in the B-hordein sequence, with an IC50 of 0.48 µM. Other peptides, such as Leu-Glu-Pro and Ile-Tyr found in B-, C-, and D-hordeins, as well as Leu-Arg-Pro and Val-Pro-Pro found in γ-hordein, also demonstrated significant ACE inhibitory activity [90].

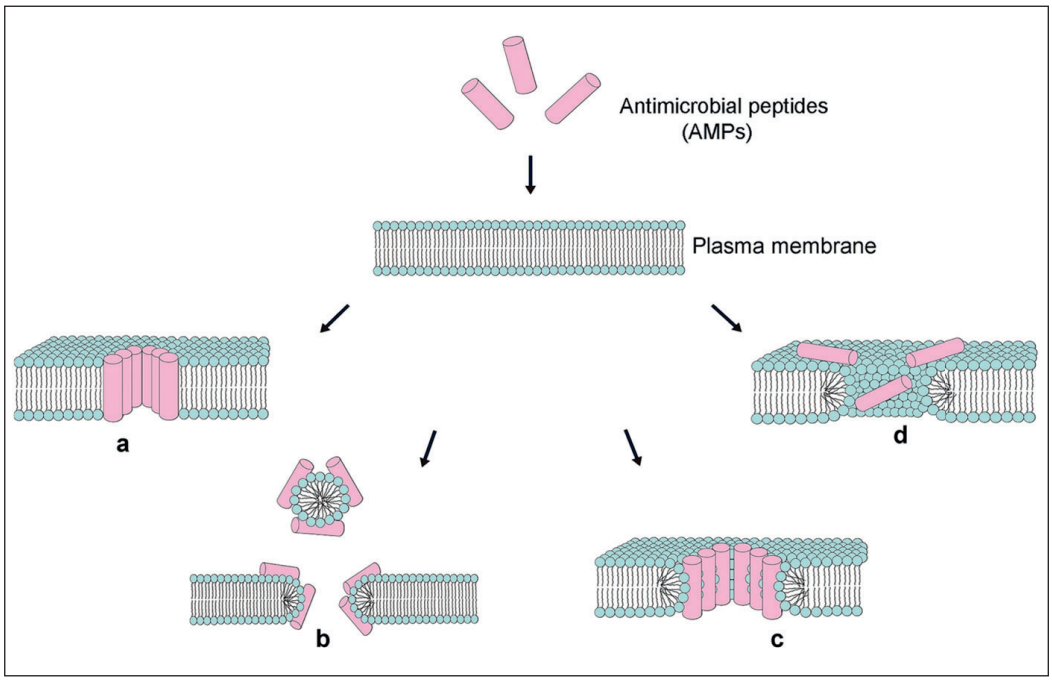

Fig. 5 **Four major mechanisms of antimicrobial peptides (AMPs). (a) barrel-stave pore: AMPs insert vertically in the bacterial cell membrane to form transmembrane pores. (b) carpet mechanism: Peptides are adsorbed parallel to the lipid bilayer to cover the cell surface, causing membrane destabilisation and disintegration. (c) toroidal pore: AMPs induce bending of the lipid bilayer, forming pores lined by both peptide molecules and lipid head groups. (d) disordered or transient pore formation: irregular and less structured pores may form with fewer peptides, requiring additional peptide interactions to stabilise the membrane disruption. (Adapted from Li et al., 2021)**

It is well established that the protein content and amino acid composition of barley derived BSG are comparable to those of barley grain, with BSG proteins exhibiting a similar amino acid profile, notably rich in glutamine, valine, and leucine [65]. While this compositional similarity does not directly confirm bioactivity, it supports the potential of brewers' spent grain as a promising source of bioactive peptides with hypotensive effects. In support of this, Connolly et al. [91] revealed that enzymatic hydrolysis of BSG resulted in significantly superior antihypertensive effects compared to intact protein isolates, with Alcalase hydrolysates displaying the highest ACE inhibition (averaging 84.6 ± 2.89%). More importantly, the bioactivity of these hydrolysates remained high even after undergoing *in-vitro* simulated gastrointestinal digestion. These findings were further validated by Garzón et al. [92] in an in-vivo rat model with induced hypertension and insulin resistance by a post-weaning sucrose-rich diet (SRD), where administration of BSG protein hydrolysate microcapsules at 700 mg kg bw^{-1} day^{-1} for 100 days led to decreased enzyme activities and significantly reduced blood pressure in the tested rats. These studies highlight the potential of the BSG protein and its derived hydrolysates as functional ingredients in the management of hypertension. Collectively, these findings suggest that BSG-derived protein hydrolysates possess considerable antihypertensive potential, particularly through ACE inhibition, and may offer a sustainable alternative to conventional synthetic antihypertensive agents. However, much of the current evidence is derived from *in vitro* assays, *in silico* predictions, and a limited number of animal studies, which may not fully reflect efficacy in humans. Furthermore, differences in protein extraction methods, hydrolysis conditions, and peptide identification approaches complicate comparisons between studies. Therefore, further research is required to identify the specific bioactive peptides responsible for the observed effects, assess their bioavailability and stability following digestion, and validate their long-term safety and efficacy.

3.3 Antimicrobial activity

Antimicrobial activity is highly valued in the food industry for its ability to preserve food quality and prevent spoilage caused by fungal and bacterial microorganisms [89]. Recently, antimicrobial peptides have gained significant attention owing to their effectiveness, specificity, low toxicity, minimal drug interactions, and their lower propensity to induce microbial resistance compared to conventional antibiotics [93]. As illustrated in figure 5, antimicrobial peptides exhibit diverse mechanisms of action, including membrane disruption and, in some cases, the inhibition of intracellular processes such as protein and nucleic acid synthesis [94]. It is widely recognised that the net charge and hydrophobicity of antimicrobial peptides are critical structural features that determine their activity, particularly due to the presence of anionic lipids, such as phosphatidylglycerol and cardiolipin, in bacterial membranes [95]. Positively charged antimicrobial peptides are attracted to microbial cell membranes through electrostatic interactions with negatively charged phospholipids, leading to structural rearrangements of the peptides [94]. Upon binding, antimicrobial peptides interact with the membrane and can induce disruption through various

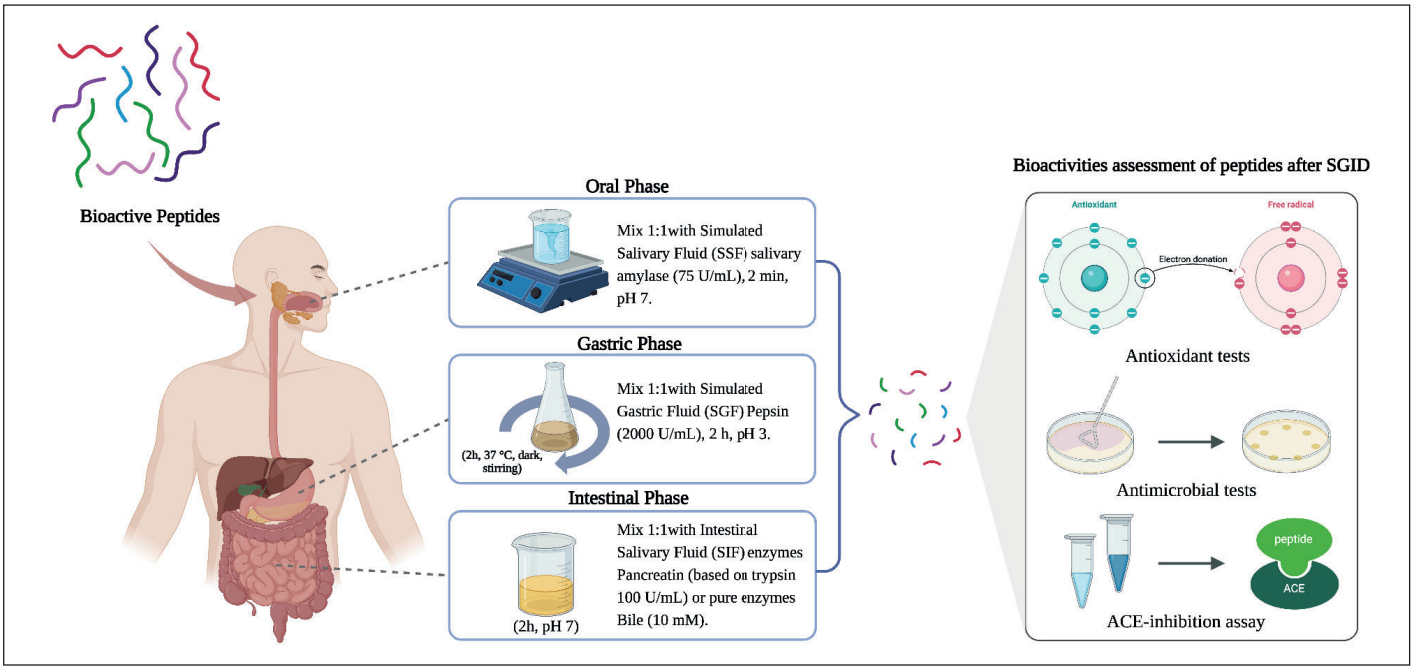

Fig. 6 ***In vitro*** **simulated gastrointestinal digestion (SGID) of bioactive peptides after oral administration, and the evaluation of bioactivities of final digests (created on Biorender)**

mechanisms, including barrel-stave and toroidal pore formation, as well as carpet-like membrane destabilisation. These interactions ultimately compromise membrane integrity, resulting in leakage of cellular contents and cell death (Fig. 5) [95].

Antimicrobial peptides are typically categorized based on their net charge, with cationic peptides enriched in arginine or lysine, and anionic peptides enriched in aspartic acid or glutamic acid [95]. Numerous studies have reported the isolation and identification of antimicrobial peptides from various plant sources. For example, in a study conducted by Tan et al. [96], hydrolysates generated from palm kernel cake, a by-product of palm kernel oil production, showed potent antimicrobial activity, particularly against *Bacillus* species. Similarly, antimicrobial peptides isolated from industrially processed soybean meal and soybean grains have shown significant inhibitory effects on Gram-positive and Gram-negative foodborne bacteria [97]. Hydrolysates of brewers' spent grain (BSG) protein, particularly those generated with Flavourzyme, exhibited strong antibacterial activity against *Staphylococcus aureus*, a common foodborne pathogen [98]. In the same year, significant growth inhibition against *E. coli* was observed for BSG protein hydrolysates produced by an enzyme derived from a *B. cereus* strain [70]. Comparable findings were reported by Taha et al.[99] regarding sunflower protein hydrolysates produced using enzyme mixtures (Alcalase and Flavourzyme), which displayed effective antimicrobial properties against various bacterial strains, including *E. coli*, *S. aureus*, *B. cereus*, *L. monocytogenes*, and *S. typhimurium*. Overall, smaller peptides generally exhibit greater antimicrobial efficacy compared to intact proteins, highlighting the importance of enzymatic cleavage. However, the selection of enzymes and reaction conditions significantly influences the antimicrobial activity of the resulting hydrolysates by modulating properties such as net charge, hydrophobicity (or lipophilicity), and amino acid composition, which in turn affect their interactions with microbial membranes. While BSG protein hydrolysates exhibit promising antimicrobial activity, further research is needed to clarify their mechanisms of action, standardise production processes and confirm their effectiveness in practical food applications.

4 *In vitro* digestion and bioactivity BSG protein and its associated hydrolysates

Numerous studies have demonstrated the health benefits of plant-derived peptides, with oral administration being the preferred delivery route as it reflects their natural dietary intake and enables absorption through the gastrointestinal tract [100] However, the human gastrointestinal (GI) environment presents significant challenges to the absorption of orally administered peptides, primarily due to physical barriers, such as the selectively permeable intestinal epithelium, and biochemical barriers, including digestive enzymes such as pepsin, pancreatic proteases, brush-border peptidases, and cytosolic peptidases [101]. Consequently, bioactive peptides may either reach the small intestine intact or undergo degradation by GI proteases, with pepsin hydrolysis typically resulting in the denaturation of food-borne proteins and the production of peptides with molecular weights exceeding 10kDa [100]. Following gastric digestion, the resulting products serve as substrates for further hydrolysis by pancreatic enzymes (pancreatin), leading to the formation of intermediate and small peptides, as illustrated in figure 6. Therefore, to overcome these challenges and ensure the bioavailability of peptide bioactivity, *in vitro* models that simulate the mechanical, chemical, and enzymatic processes of the human gastrointestinal tract are essential during food development.

One of the most widely adopted *in vitro* digestion models in the food industry at present is the INFOGEST method, a static model

developed collaboratively by an international consortium of scientists who worked to identify and then replicate the conditions required to adequately simulate the human mouth, stomach, and small intestine, including parameters such as temperatures, incubation times, pH values, ionic compositions, enzyme activities, bile levels, and mucin levels [102]. Currently, the digestion of bioactive proteins and peptides *in vitro* is primarily conducted through adaptations of the INFOGEST model. This approach holds numerous significant benefits, particularly providing insights into the potential functionality of peptides *in vivo*.

Agroindustrial byproducts are widely acknowledged as important sources of bioactive compounds with the potential to deliver multiple health benefits (Fig. 2). In this context, considerable attention has been given to understanding how *in vitro* gastrointestinal digestion affects the stability of these proteins and, consequently, their bioactive properties over the past decade. For instance, Silva do Nascimento et al. [103] revealed that hydrolysates derived from yam protein exhibited significantly enhanced antioxidant, ACE-inhibitory, and antibacterial properties following *in vitro* digestion, compared to intact protein. Similarly, Sánchez-Velázquez et al. [104] found that simulated gastrointestinal digestion (SGID) positively influenced the nutritional and bioactive attributes of oat peptides, with a remarkable 400% increase in DPPH scavenging activity post-digestion. Similarly, Nongonierma and FitzGerald [105] demonstrated a decrease in the DPP-IV IC50 from 1.33 to 1.02 mg/mL of a whey protein hydrolysate indicating an increase in inhibitory activity after *in vitro* SGID. However, it is important to note that the functional and bioactive properties of peptides are not always improved after SGID. Mune et al. [106]. reported a reduction in the ACE inhibitory activity of Bambara bean protein hydrolysates following *in vitro* gastrointestinal digestion, indicating a decrease in potency, likely due to further peptide degradation and the loss of bioactive sequences.

Several studies have demonstrated the antihypertensive potential and preliminary safety of bioactive peptides derived from brewers' spent grain (BSG), in animal models. Cermeño et al.[107] reported that a single oral dose induced a measurable hypotensive effect within hours in spontaneously hypertensive rats, while Garzón et al. [92] demonstrated that longer-term administration (up to 100 days) of microencapsulated BSG peptides resulted in sustained reductions in systolic blood pressure and improvements in metabolic parameters without reported adverse effects. Additional studies have further shown that these peptides can mitigate diet-induced oxidative stress and pro-thrombotic states *in vivo,* with microencapsulated BSG peptide supplementation restoring antioxidant balance and improving systemic biomarkers in rat models, thereby supporting their broader cardioprotective potential [108]. Blood pressure regulation is primarily attributed to angiotensin-converting enzyme (ACE) inhibition, while complementary mechanisms such as antioxidant and antithrombotic activities contribute to the overall cardiometabolic protective effects of BSG-derived peptides [91, 108, 109]. Taken together, these findings suggest that gastrointestinal digestion can substantially influence the bioactivity of food-derived peptides, either enhancing or reducing their functional properties depending on peptide structure and digestion conditions. While BSG-derived peptides have demonstrated promising antihypertensive and cardioprotective effects in animal studies, limited information is available regarding their stability, bioavailability, and efficacy in humans.

4.1 Safety of BSG protein and hydrolysates

Dietary proteins and protein hydrolysates are widely used in food products and are generally permitted in the European Union as conventional food ingredients. However, where novel sources or specific health claims are involved, safety and efficacy may require evaluation in accordance with relevant regulatory frameworks, including assessment by the European Food Safety Authority (EFSA). In the United States, many proteins and protein hydrolysates have been designated as "generally recognized as safe" (GRAS) by the Food and Drug Administration (FDA) on a case-by-case basis for specific applications [110]. Nevertheless, there is no dedicated regulatory framework specifically addressing amino acids and bioactive peptides as functional ingredients; instead, their use is typically governed within broader food, novel food, or dietary supplement legislation. Despite this, food protein-derived bioactive peptides are generally considered safe, as they are naturally generated during gastrointestinal digestion and are commonly produced using food-grade enzymes and processes [64]. Nevertheless, their safety may depend on factors such as peptide sequence, concentration, and source. Although systematic assessments of the safety of brewers' spent grain (BSG)-derived proteins and hydrolysates for human consumption remain limited, available evidence suggests that they are safe for use in food systems.

Brewers' spent grain (BSG) protein is derived from barley, a nutrient-rich cereal that has been safely consumed worldwide for centuries. The extraction of BSG protein typically involves mild mechanical and food-grade processing methods, yielding a product that remains compositionally similar to native barley. Although GRAS status is not based solely on historical use, the long-standing consumption of barley supports the safety of BSG-derived proteins, further reinforced by studies demonstrating their safe use in both animal and human models [11, 21, 111]. In a study by Teixeira et al. [112], Wistar rats were fed various plant-based diets, with one containing 15% brewers' spent grain (BSG) for twelve days. No adverse effects were observed in any treatment group, and rats with BSG diets exhibited increased gut microbiota diversity. Similar observations were reported by Kanauchi and Agata [113] with no side effects noted after feeding rats 6% BSG for 14 days. Additionally, Garzón et al. [92] administered microcapsules containing bioactive peptides from BSG to a rat model with induced hypertension and insulin resistance. This study confirmed *in vivo* antihypertensive and antidiabetogenic effects through the inhibition of enzymes related to blood pressure regulation and glucose metabolism, while no negative effects on the rats were observed. Furthermore, in a study conducted by Zhang et al. [114], ulcerative colitis patients with previous ileostomies were administered 62 g of BSG per day for a duration of 7 days. These patients experienced significantly decreased bowel transit times, and no adverse effects. In addition to animal and human studies, studies have demonstrated the absence of toxicity of isolated bioactive peptides in cell culture models [110]. Collectively, these findings suggest that BSG-derived proteins are appropriate for human consumption. Nevertheless, although bioactive peptides are generally regarded as physiologically safe, additional studies

are needed to further support their safety and potential benefits in dietary applications.

In this context, anti-nutritional factors remain an important consideration for plant-derived proteins and bioactive peptides. Cereals such as barley contain compounds including phytic acid, tannins, and protease inhibitors, which can limit nutrient and mineral bioavailability. However, as BSG is derived from malted and brewed barley, these processing steps, particularly germination, are likely to significantly reduce antinutrient levels, for example through phytase-mediated degradation of phytic acid. Moreover, additional processing steps such as thermal treatment can further reduce heat-sensitive antinutrients, including protease inhibitors. Consequently, BSG-derived protein is unlikely to retain antinutritional factors at levels that would pose significant safety concerns [115–117].

Additionally, the allergenicity of proteins and peptides remains a common public concern. However, barley is not classified as a major allergen requiring mandatory declaration under the U.S. Food Allergen Labelling and Consumer Protection Act (FALCPA) and the Annex II of Regulation (EU) No 1169/2011, however, it must be declared under Regulation (EU) No 1169/2011 as part of the category "cereals containing gluten," due to its relevance for coeliac disease and other gluten-related disorders [118, 119]. While true IgE-mediated allergy to barley is considered relatively rare, with only infrequent reports of adverse reactions despite its widespread global consumption [120], barley contains gluten proteins (hordeins), and studies have shown that these proteins can persist and even become enriched in brewers' spent grain (BSG) following the brewing process, meaning that BSG-derived ingredients are not suitable for individuals with celiac disease or gluten-related disorders [121]. Accordingly, formulated value-added food products should clearly declare the presence of barley-derived proteins (e.g., BSG protein) in the ingredient list, enabling consumers to make informed dietary choices. Overall, while individuals sensitive to barley or its protein fractions may experience adverse reactions, such occurrences are expected to be exceedingly rare in the general population and thus represent a minimal public health risk when appropriate labelling is applied.

In general, conventional foods are typically regarded as safe without defined intake limits, whereas functional foods and nutraceuticals containing bioactive proteins or peptides are specifically formulated to confer health benefits and support disease prevention. Consequently, establishing an appropriate dosage is essential to ensure their efficacy while avoiding potential adverse effects [122, 123]. Overall, the available evidence suggests that BSG-derived peptides are promising natural antihypertensive agents with no observed toxicity in preclinical studies; however, the safety and functionality of BSG protein hydrolysates are strongly influenced by processing conditions, which determine peptide composition and potential bioactivities and human clinical data remains lacking, highlighting the need for further investigation into their efficacy, dose–response relationships, and long-term safety in humans.

4.2 Functional food

The term 'functional food' originated in 1984 in Japan from a study exploring the correlation between nutrition, sensory satisfaction, fortification, and modulation of physiological systems, defining these as food products fortified with special constituents having beneficial physiological effects [124]. Despite numerous attempts at defining 'functional food', a universally accepted definition remains unclear [125]. European legislation considered functional food not as a concept but rather as a specific food category [126]. Recently, the Functional Food Centre (FFC) provided a definitive description of functional food, as "it must not only deliver basic nutritional value but also enhance well-being, health, and/or reduce the risk of disease, thereby improving the overall quality of life including physical, psychological, and behavioural aspects, supported by scientifically validated claims, while being consumed in amounts and forms typical for dietary purposes and not in the form of pills or capsules" [127]. This characterization distinguishes functional foods from nutraceuticals, which are food-derived products often sold in non-food forms such as pills, powders, or other medicinal formulations [128].

4.3 The role of functional food in human health

Functional foods have emerged as a cornerstone of modern dietary habits, offering a pathway towards optimizing human health and overall well-being [129]. In recent decades, poor adherence to balanced dietary patterns has contributed to a rising prevalence of metabolic disturbances, thereby increasing the risk of chronic conditions such as obesity, type 2 diabetes, hypertension, food allergies and intolerances, as well as gastrointestinal and inflammatory disorders [130]. Consequently, there has been a notable shift in focus from basic sustenance to actively promoting health and enhancing performance through dietary choices that extend beyond traditional nutritional requirements [131]. This shift has led to an increased global interest in and acceptance of functional foods among consumers.

Numerous studies have demonstrated the health-promoting properties of ingredients derived from diverse plants and animals, leading to the successful commercialization of many products [132]. Within the category of functional foods, dietary probiotics represent a significant market segment, exerting their effects by enhancing the growth and/or activity of specific bacteria in the colon, thereby improving overall host health [133]. Another significant and rapidly expanding product category within the functional food segment comprises non-alcoholic beverages fortified with vitamins A, C, and E, or other functional ingredients such as omega-3, lutein, minerals, amino acids, and antioxidants [134]. In addition, functional cereals, bakery products, spreads, meat products, and eggs have gained significant consumer interest, largely due to their capacity to deliver bioactive components at physiologically effective levels while maintaining desirable sensory attributes such as appearance, taste, and texture [132]. Incorporating consumer preferences into the development of functional foods is essential to enhance their market acceptance and successful commercialization.

Recently, BSG has been recognised as a valuable ingredient in the human diet [135] and has demonstrated considerable success in bakery and cereal based applications, including bread [66], biscuits [136], noodles [137], and pasta [138]. In these cases, BSG inclusion was able to enhance the nutritional profile of these products through increased dietary fibre, protein, and bioactive compounds

while maintaining acceptable technological performance and sensory properties when incorporated at optimised levels. Pioneering companies in this area include Regrained® (San Francisco, CA, USA) that began commercialising BSG-based food products in 2013, and Evergrain (Wilmington, Delaware, USA) that released its BSG-derived ingredients, EverPro and EverVita, in January 2020 [11].

However, despite its high nutritional value, the application of BSG protein and peptide ingredients in functional foods remains largely at the laboratory and pilot scale. This is primarily due to challenges related to raw material stability, including susceptibility to microbial spoilage during storage, the presence of certain anti-nutritional components typical of cereal matrices, and dietary restrictions associated with gluten-containing proteins [66, 121, 139]. In addition, variability in composition and the need for optimised processing conditions can further limit large-scale incorporation. Therefore, advanced processing technologies, effective stabilisation strategies, and comprehensive bioactivity and safety evaluations are required to support the wider utilisation of BSG-derived ingredients in food systems.

5 Conclusion

At present, functional foods represent a significant area of innovation within the brewing industry, holding substantial potential for enhancing health and aiding in the prevention of certain diseases when incorporated into a balanced diet and healthy lifestyle. However, the comprehensive assessment of the long-term benefits and risks associated with functional foods remains a critical issue that requires further investigation [140]. Although accumulating evidence indicates that BSG-derived proteins may offer potential benefits for disease prevention and health promotion, it is essential to simultaneously consider their safety profile [110]. Despite substantial evidence supporting the beneficial physiological effects of BSG-derived proteins and peptides, most of these studies have been conducted *in vitro*, primarily focusing on the activity of isolated compounds against specific health-related biomarkers [141]. However, the specific mechanisms underlying the activity of some of these bioactive compounds remain insufficiently understood, highlighting the need for further research to elucidate their full physiological roles following consumption. Moreover, the functional effects of BSG-derived proteins are strongly influenced by the food matrix and inter-individual variability among consumers, which may contribute to inconsistencies observed in *in vivo* outcomes. Therefore, advancing the development and market potential of functional foods requires a deeper understanding of the interactions between functional ingredients and the food matrix during processing, alongside well-designed clinical studies to more comprehensively evaluate their health effects. While these aspects are important for substantiating functional claims, foods that do not fully meet such criteria may still provide nutritional value, even if their functional benefits are not yet conclusively established [142].

Furthermore, the functional food sector requires substantial investment in terms of time and financial resources to support fundamental research, technological development, product approval processes, and effective commercialization strategies, and is often sensitive to broader economic conditions [143]. In this context, optimisation of extraction techniques and processing parameters is essential to enhance the yield, chemical stability, and functional performance of BSG-derived proteins and their hydrolysates [142]. Additional research is also needed on the application of BSGP in different food matrices and its effects on the sensory, physicochemical, rheological, and functional properties of developed food products [144]. Consumer acceptance is a key determinant of the successful development and market uptake of functional foods. However, surveys indicate that acceptance of BSGP-enriched products is conditional, with taste remaining a primary factor alongside quality, price, convenience, and the credibility of associated health claims [132, 145]. Accordingly, while functional benefits can enhance the overall value of BSGP-fortified foods, both health functionality and sensory quality are important, highlighting the need for continued research to develop products that effectively integrate both attributes.

Overall, functional foods continue to gain widespread consumer acceptance, reflecting their growing role in addressing health and lifestyle-related concerns and supporting their long-term relevance within a diversified and evolving market. Although challenges remain in terms of formulation, safety validation, and consumer acceptance, the valorisation of agro-industrial by-products such as brewers' spent grain (BSG) represents a promising and sustainable approach within the functional food sector. In particular, the incorporation of BSG as a functional ingredient offers significant potential to enhance nutritional quality while contributing to waste reduction, resource efficiency, and circular economy strategies. Continued research and innovation are therefore essential to overcome current limitations and fully realise the environmental, economic, and health-related benefits associated with BSG-derived functional foods.

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Conflict of interest

The authors declare no conflict of interest.

CRediT authorship contribution statement

Jiao, Zhang: Writing, editing, investigation, conceptualisation; Ana Esmeralda Herrero: Writing, review, editing, publishing submission; Ariane Perez-Gavilan: conceptualisation, supervision, funding acquisition; Adriana Cunha Neves: conceptualisation, supervision, funding acquisition.

6 References

1. FAO, I., UNICEF, WFP, &. WHO.: The State of Food Security and Nutrition in the World 2024, FAO; IFAD; UNICEF; WFP; WHO;, 20[1]FAO, I., UNICEF, WFP, &. WHO.: The State of Food Security and Nutrition in the World 2024, FAO; IFAD; UNICEF; WFP; WHO;, 2024.
2. Kumar, M.; Tomar, M.; Potkule, J.; Verma, R.; Punia, S.; Mahapatra, A.; Belwal, T.; Dahuja, A.; Joshi, S.; Berwal, M.K.; Satankar, V.; Bhoite,

A.G.; Amarowicz, R.; Kaur, C. and Kennedy, J.F.: Advances in the plant protein extraction: Mechanism and recommendations, Food Hydrocolloids, **115** (2021), pp. 106595–106595.
3. Marcus, J.B.: Protein Basics: Animal and Vegetable Proteins in Food and Health, Culinary Nutrition, Elsevier, 2013, pp. 189–230.
4. United Nations, D.P.D.: World Population Prospects2022.
5. Henchion, M.; Hayes, M.; Mullen, A.; Fenelon, M. and Tiwari, B.: Future Protein Supply and Demand: Strategies and Factors Influencing a Sustainable Equilibrium, Foods, **6** (2017), no. 7, pp. 53–53.
6. Kumar, R.; Hegde, A.S.; Sharma, K.; Parmar, P. and Srivatsan, V.: Microalgae as a sustainable source of edible proteins and bioactive peptides – Current trends and future prospects, Food Research International, **157** (2022), pp. 111338–111338.
7. Hadidi, M.; Aghababaei, F.; Gonzalez-Serrano, D.J.; Goksen, G.; Trif, M.; McClements, D.J. and Moreno, A.: Plant-based proteins from agro-industrial waste and by-products: Towards a more circular economy, International Journal of Biological Macromolecules, **261** (2024), pp. 129576–129576.
8. Neves, A.C.; Harnedy, P.A.; O'Keeffe, M.B.; Alashi, M.A.; Aluko, R.E. and FitzGerald, R.J.: Peptide identification in a salmon gelatin hydrolysate with antihypertensive, dipeptidyl peptidase IV inhibitory and antioxidant activities, Food Research International, **100** (2017), pp. 112–120.
9. Zhang, J.; Perez-Gavilan, A. and Neves, A.C.: Evaluation of the *In Vitro* Bioactivities' Profiles of Brewers' Spent Grain Protein and Hydrolysates with and without Cellulase Pretreatment, Nutraceuticals, **2** (2022), no. 3, pp. 218–233.
10. Zhang, J.; Perez-Gavilan, A. and Cunha Neves, A.: Determination of Functionalities of Proteins and Their Corresponding Hydrolysates from Brewers' Spent Grain, Journal of the American Society of Brewing Chemists, **83** (2024), no. 2, pp. 203–214.
11. Devnani, B.; Moran, G.C. and Grossmann, L.: Extraction, Composition, Functionality, and Utilization of Brewer's Spent Grain Protein in Food Formulations, Foods, **12** (2023), no. 7, pp. 1543–1543.
12. Dancker, P.; Glas, K. and Gastl, M.: Potential utilisation methods for brewer's spent grain: a review, International Journal of Food Science and Technology, **60** (2025), no. 1.
13. Carella, A. and Lamacchia, C.: Drying techniques for the valorization of brewer's spent grains: Impacts on nutritional quality, sensory properties, and process efficiency. A review, Applied Food Research, **5** (2025), no. 2, pp. 101429–101429.
14. Terefe, G.: Preservation techniques and their effect on nutritional values and microbial population of brewer's spent grain: a review, CABI Agriculture and Bioscience, **3** (2022), no. 1, pp. 51–51.
15. El-Shafey, E.I.; Gameiro, M.L.F.; Correia, P.F.M. and Carvalho, J.M.R. de: Dewatering of Brewer's Spent Grain Using a Membrane Filter Press: A Pilot Plant Study, Separation Science and Technology, **39** (2004), no. 14, pp. 3237–3261.
16. Braidot, M.; Sarnataro, C.; Romanzin, A.; Piani, B. and Spanghero, M.: Improve the quality of ensiled brewers' grains by pressing raw material and including additives, Animal Feed Science and Technology, **328** (2025), pp. 116465–116465.
17. Zeko-Pivač, A.; Tišma, M.; Žnidaršič-Plazl, P.; Kulisic, B.; Sakellaris, G.; Hao, J. and Planinić, M.: The Potential of Brewer's Spent Grain in the Circular Bioeconomy: State of the Art and Future Perspectives, Frontiers in Bioengineering and Biotechnology, 10 (2022).
18. Henkin, J.M.; Mainali, K.; Sharma, B.K.; Yadav, M.P.; Ngo, H. and Sarker, M.I.: A Review of Chemical and Physical Analysis, Processing, and Repurposing of Brewers' Spent Grain, Biomass, **5** (2025), no. 3, pp. 42–42.
19. Nargotra, P.; Sharma, V.; Tsai, M.-L.; Hsieh, S.-L.; Dong, C.-D.; Wang, H.-M.D. and Kuo, C.-H.: Recent Advancements in the Valorization of Agro-Industrial Food Waste for the Production of Nanocellulose, Applied Sciences, **13** (2023), no. 10, pp. 6159–6159.
20. Zhang, Jiao; Herrero, Ana E.; Perez-Gavilan, Ariane; Cunha Neves, Adriana; Solid-state fermentation for enhanced brew-ers' spent grain protein valorisation: A com-prehensive review of conventional and emerging techniques, Brewing Science, **79** (2026), no. 5/6, pp. 64–78.
21. Jaeger, A.; Zannini, E.; Sahin, A.W. and Arendt, E.K.: Barley Protein Properties, Extraction and Applications, with a Focus on Brewers' Spent Grain Protein, Foods, **10** (2021), no. 6, pp. 1389–1389.
22. Rodriguez, L.M.; Camina, J.L.; Borroni, V. and Pérez, E.E.: Protein recovery from brewery solid wastes, Food Chemistry, **407** (2023), pp. 134810–134810.
23. Yousefi, N. and Abbasi, S.: Food proteins: Solubility & thermal stability improvement techniques, Food Chemistry Advances, **1** (2022), pp. 100090–100090.
24. Grossmann, L. and McClements, D.J.: Current insights into protein solubility: A review of its importance for alternative proteins, Food Hydrocolloids, **137** (2023), pp. 108416–108416.
25. Connolly, A.; Piggott, C.O. and FitzGerald, R.J.: Characterisation of protei-rich isolates and antioxidative phenolic extracts from pale and black brewers' spent grain, International Journal of Food Science & Technology, **48** (2013), no. 8, pp. 1670–1681.
26. Celus, I.; Brijs, K. and Delcour, J.A.: The effects of malting and mashing on barley protein extractability, Journal of Cereal Science, **44** (2006), no. 2, pp. 203–211.
27. Niemi, P.; Martins, D.; Buchert, J. and Faulds, C.B.: Pre-hydrolysis with carbohydrases facilitates the release of protein from brewer's spent grain, Bioresource Technology, **136** (2013), pp. 529–534.
28. Connolly, A.; Piggott, C.O. and FitzGerald, R.J.: Technofunctional properties of a brewers' spent grain protein-enriched isolate and its associated enzymatic hydrolysates, LWT - Food Science and Technology, **59** (2014), no. 2, pp. 1061–1067.
29. Celus, I.; Brijs, K. and Delcour, J.A.: Enzymatic Hydrolysis of Brewers' Spent Grain Proteins and Technofunctional Properties of the Resulting Hydrolysates, Journal of Agricultural and Food Chemistry, **55** (2007), no. 21, pp. 8703–8710.
30. Chen, L.; Chen, J.; Ren, J. and Zhao, M.: Modifications of soy protein isolates using combined extrusion pre-treatment and controlled enzymatic hydrolysis for improved emulsifying properties, Food Hydrocolloids, **25** (2011), no. 5, pp. 887–897.
31. Day, L.: Proteins from land plants – Potential resources for human nutrition and food security, Trends in Food Science & Technology, **32** (2013), no. 1, pp. 25–42.
32. Karabulut, G.; Kapoor, R. and Feng, H.: Biotransformation approaches using solid-state fermentation and germination with high-intensity ultrasound to produce added-value hemp protein nanoaggregates, Food Bioscience, **56** (2023), pp. 103400–103400.
33. Olukomaiya, O.O.; Fernando, W.C.; Mereddy, R.; Li, X. and Sultanbawa, Y.: Solid-state fermentation of canola meal with *Aspergillus sojae*, *Aspergillus ficuum* and their co-cultures: Effects on physicochemical, microbiological and functional properties, LWT, **127** (2020), pp. 109362–109362.
34. Zadeike, D.; Gaizauskaite, Z.; Svazas, M.; Gruzauskas, R.; Gruzauskas, V.; Damasius, J. and Juodeikiene, G.: Application of Solid-State

Fermentation for the Improving of Extruded Corn Dry-Milling By-Products and Their Protein Functional Properties, Life, **12** (2022), no. 11, pp. 1909–1909.
35. Mills, E.N.C.; Huang, L.; Noel, T.R.; Gunning, A.P. and Morris, V.J.: Formation of thermally induced aggregates of the soya globulin β-conglycinin, Biochimica et Biophysica Acta (BBA) - Protein Structure and Molecular Enzymology, **1547** (2001), no. 2, pp. 339–350.
36. Zha, F.; Rao, J. and Chen, B.: Modification of pulse proteins for improved functionality and flavor profile: A comprehensive review, Comprehensive Reviews in Food Science and Food Safety, **20** (2021), no. 3, pp. 3036–3060.
37. McSweeney, S.L.; Mulvihill, D.M. and O'Callaghan, D.M.: The influence of pH on the heat-induced aggregation of model milk protein ingredient systems and model infant formula emulsions stabilized by milk protein ingredients, Food Hydrocolloids, **18** (2004), no. 1, pp. 109–125.
38. Ryan, M.; McEvoy, E.; Duignan, S.; Crowley, C.; Fenelon, M.; O'Callaghan, D.M. and FitzGerald, R.J.: Thermal stability of soy protein isolate and hydrolysate ingredients, Food Chemistry, **108** (2008), no. 2, pp. 503–510.
39. Hellebois, T.; Gaiani, C.; Planchon, S.; Renaut, J. and Soukoulis, C.: Impact of heat treatment on the acid induced gelation of brewers' spent grain protein isolate, Food Hydrocolloids, **113** (2021), pp. 106531–106531.
40. Kim, S.Y.; Park, P.S.W. and Rhee, K.C.: Functional properties of proteolytic enzyme modified soy protein isolate, Journal of Agricultural and Food Chemistry, **38** (1990), no. 3, pp. 651–656.
41. BLANSHARD, J.: Colloids in foodBy E. Dickinson and G. Stainsby. Applied Science Publishers Ltd, London. 1982, xiv + 533 pp., ISBN 0-85334-153-2. Price: £48.00 (US$98.50), Carbohydrate Polymers, **3** (1983), no. 3, pp. 243–244.
42. Lam, L.H.; Shimamura, T.; Manabe, S.; Ishiyama, M. and Ukeda, H.: Assay of Angiotensin I-converting Enzyme-inhibiting Activity Based on the Detection of 3-Hydroxybutyrate with Water-soluble Tetrazolium Salt, Analytical Sciences, **24** (2008), no. 8, pp. 1057–1060.
43. Bengoechea, C.; Romero, A.; Aguilar, J.M.; Cordobés, F. and Guerrero, A.: Temperature and pH as factors influencing droplet size distribution and linear viscoelasticity of O/W emulsions stabilised by soy and gluten proteins, Food Hydrocolloids, **24** (2010), no. 8, pp. 783–791.
44. Hu, M.; McClements, D.J. and Decker, E.A.: Lipid Oxidation in Corn Oil-in-Water Emulsions Stabilized by Casein, Whey Protein Isolate, and Soy Protein Isolate, Journal of Agricultural and Food Chemistry, **51** (2003), no. 6, pp. 1696–1700.
45. Kimura, A.; Fukuda, T.; Zhang, M.; Motoyama, S.; Maruyama, N. and Utsumi, S.: Comparison of Physicochemical Properties of 7S and 11S Globulins from Pea, Fava Bean, Cowpea, and French Bean with Those of Soybean—French Bean 7S Globulin Exhibits Excellent Properties, Journal of Agricultural and Food Chemistry, **56** (2008), no. 21, pp. 10273–10279.
46. Lam, R.S.H. and Nickerson, M.T.: Food proteins: A review on their emulsifying properties using a structure–function approach, Food Chemistry, **141** (2013), no. 2, pp. 975–984.
47. Shevkani, K.; Singh, N.; Kaur, A. and Rana, J.C.: Structural and functional characterization of kidney bean and field pea protein isolates: A comparative study, Food Hydrocolloids, **43** (2015), pp. 679–689.
48. Karaca, A.C.; Low, N. and Nickerson, M.: Emulsifying properties of chickpea, faba bean, lentil and pea proteins produced by isoelectric precipitation and salt extraction, Food Research International, **44** (2011), no. 9, pp. 2742–2750.
49. Burger, T.G. and Zhang, Y.: Recent progress in the utilization of pea protein as an emulsifier for food applications, Trends in Food Science & Technology, **86** (2019), pp. 25–33.
50. Chin, Y.L.; Chai, K.F. and Chen, W.N.: Upcycling of brewers' spent grains via solid-state fermentation for the production of protein hydrolysates with antioxidant and techno-functional properties, Food Chemistry: X, **13** (2022), pp. 100184–100184.
51. Park, S.; Chang, K.H. and Cho, S.J.: Enhanced Emulsifying and Calcium-binding Properties of Fermented Soybean Meal, Biotechnology and Bioprocess Engineering, **24** (2019), no. 1, pp. 183–190.
52. Li, J.; Yang, X.; Swallah, M.S.; Fu, H.; Ji, L.; Meng, X.; Yu, H. and Lyu, B.: Soy protein isolate: an overview on foaming properties and air–liquid interface, International Journal of Food Science & Technology, **57** (2021), no. 1, pp. 188–200.
53. Yang, X.; Su, Y. and Li, L.: Study of soybean gel induced by Lactobacillus plantarum: Protein structure and intermolecular interaction, LWT, **119** (2020), pp. 108794–108794.
54. Damodaran, S.: Protein Stabilization of Emulsions and Foams, Journal of Food Science, **70** (2005), no. 3.
55. Amagliani, L.; Silva, J.V.C.; Saffon, M. and Dombrowski, J.: On the foaming properties of plant proteins: Current status and future opportunities, Trends in Food Science & Technology, **118** (2021), pp. 261–272.
56. Drenckhan, W. and Saint-Jalmes, A.: The science of foaming, Advances in Colloid and Interface Science, **222** (2015), pp. 228–259.
57. Meinlschmidt, P.; Ueberham, E.; Lehmann, J.; Schweiggert-Weisz, U. and Eisner, P.: Immunoreactivity, sensory and physicochemical properties of fermented soy protein isolate, Food Chemistry, **205** (2016), pp. 229–238.
58. Emkani, M.; Oliete, B. and Saurel, R.: Effect of Lactic Acid Fermentation on Legume Protein Properties, a Review, Fermentation, **8** (2022), no. 6, pp. 244–244.
59. SATHE, S.K.; DESHPANDE, S.S. and SALUNKHE, D.K.: Functional Properties of Winged Bean [Psophocarpus tetragonolobus (L.) DC] Proteins, Journal of Food Science, **47** (1982), no. 2, pp. 503–509.
60. Naibaho, J. and Korzeniowska, M.: The variability of physico-chemical properties of brewery spent grain from 8 different breweries, Heliyon, **7** (2021), no. 3, pp. e06583–e06583.
61. Yu, J.; Ahmedna, M. and Goktepe, I.: Peanut protein concentrate: Production and functional properties as affected by processing, Food Chemistry, **103** (2007), no. 1, pp. 121–129.
62. Silva, A.M.M. da; Almeida, F.S.; Silva, M.F. da; Goldbeck, R. and Sato, A.C.K.: How do pH and temperature influence extraction yield, physicochemical, functional, and rheological characteristics of brewer spent grain protein concentrates?, Food and Bioproducts Processing, **139** (2023), pp. 34–45.
63. Du, M.; Xie, J.; Gong, B.; Xu, X.; Tang, W.; Li, X.; Li, C. and Xie, M.: Extraction, physicochemical characteristics and functional properties of Mung bean protein, Food Hydrocolloids, **76** (2018), pp. 131–140.
64. Udenigwe, C.C. and Aluko, R.E.: Food Protein Derived Bioactive Peptides: Production, Processing, and Potential Health Benefits, Journal of Food Science, **77** (2011), no. 1.
65. Wen, C.; Zhang, J.; Duan, Y.; Zhang, H. and Ma, H.: A Mini-Review on Brewer's Spent Grain Protein: Isolation, Physicochemical Properties, Application of Protein, and Functional Properties of Hydrolysates, Journal of Food Science, **84** (2019), no. 12, pp. 3330–3340.

66. Nyhan, L.; Sahin, A.W.; Schmitz, H.H.; Siegel, J.B. and Arendt, E.K.: Brewers' Spent Grain: An Unprecedented Opportunity to Develop Sustainable Plant-Based Nutrition Ingredients Addressing Global Malnutrition Challenges, Journal of Agricultural and Food Chemistry, **71** (2023), no. 28, pp. 10543–10564.
67. Korhonen, H. and Pihlanto, A.: Bioactive peptides: Production and functionality, International Dairy Journal, **16** (2006), no. 9, pp. 945–960.
68. Aguilar-Toalá, J.E.; Cruz-Narváez, Y.; Quintanar-Guerrero, D.; Liceaga, A.M. and Zambrano-Zaragoza, M.L.: In silico bioactivity analysis of peptide fractions derived from brewer's spent grain hydrolysates, International Journal of Food Science & Technology, **59** (2024), no. 4, pp. 2804–2815.
69. Connolly, A.; O'Keeffe, M.B.; Nongonierma, A.B.; Piggott, C.O. and FitzGerald, R.J.: Isolation of peptides from a novel brewers spent grain protein isolate with potential to modulate glycaemic response, International Journal of Food Science & Technology, **52** (2016), no. 1, pp. 146–153.
70. Kotlar, C.E.; Ponce, A.G. and Roura, S.I.: Improvement of functional and antimicrobial properties of brewery byproduct hydrolysed enzymatically, LWT – Food Science and Technology, **50** (2013), no. 2, pp. 378–385.
71. McCarthy, A.L.; O'Callaghan, Y.C.; Connolly, A.; Piggott, C.O.; FitzGerald, R.J. and O'Brien, N.M.: *In vitro* antioxidant and anti-inflammatory effects of brewers' spent grain protein rich isolate and its associated hydrolysates, Food Research International, **50** (2013), no. 1, pp. 205–212.
72. Chandimali, N.; Bak, S.G.; Park, E.H.; Lim, H.-J.; Won, Y.-S.; Kim, E.-K.; Park, S.-I. and Lee, S.J.: Free radicals and their impact on health and antioxidant defenses: a review, Cell Death Discovery, **11** (2025), no. 1.
73. Tumilaar, S.G.; Hardianto, A.; Dohi, H. and Kurnia, D.: A Comprehensive Review of Free Radicals, Oxidative Stress, and Antioxidants: Overview, Clinical Applications, Global Perspectives, Future Directions, and Mechanisms of Antioxidant Activity of Flavonoid Compounds, Journal of Chemistry, **2024** (2024), pp. 1–21.
74. Siti, H.N.; Kamisah, Y. and Kamsiah, J.: The role of oxidative stress, antioxidants and vascular inflammation in cardiovascular disease (a review), Vascular Pharmacology, **71** (2015), pp. 40–56.
75. MITTERER-DALTOÉ, M.; BORDIM, J.; LISE, C.; BREDA, L.; CASA-GRANDE, M. and LIMA, V.: Consumer awareness of food antioxidants. Synthetic vs. Natural, Food Science and Technology, **41** (2021), no. suppl 1, pp. 208–212.
76. Halliwell, B.: How to characterize an antioxidant: an update, Biochemical Society Symposia, **61** (1995), pp. 73–101.
77. Samaranayaka, A.G.P. and Li-Chan, E.C.Y.: Food-derived peptidic antioxidants: A review of their production, assessment, and potential applications, Journal of Functional Foods, **3** (2011), no. 4, pp. 229–254.
78. Freitas, L.C.; Barbosa, J.R.; da Costa, A.L.C.; Bezerra, F.W.F.; Pinto, R.H.H. and Carvalho Junior, R.N. de: From waste to sustainable industry: How can agro-industrial wastes help in the development of new products?, Resources, Conservation and Recycling, **169** (2021), pp. 105466–105466.
79. Vieira, E.F.; da Silva, D.D.; Carmo, H. and Ferreira, I.M.P.L.V.O.: Protective ability against oxidative stress of brewers' spent grain protein hydrolysates, Food Chemistry, **228** (2017), pp. 602–609.
80. Vieira, M.C.; Brandelli, A. and Thys, R.C.S.: Evaluation of the technological functional properties and antioxidant activity of protein hydrolysate obtained from brewers' spent grain, Journal of Food Processing and Preservation, **46** (2022), no. 7.
81. Chai, K.F. and Chen, W.N.: Recovery of antioxidative protein hydrolysates with functional properties from fermented brewer's spent grain via microwave-assisted three phase partitioning, Innovative Food Science & Emerging Technologies, **91** (2024), pp. 103551–103551.
82. Patrignani, M.; Brantsen, J.F.; Awika, J.M. and Conforti, P.A.: Application of a novel microwave energy treatment on brewers' spent grain (BSG): Effect on its functionality and chemical characteristics, Food Chemistry, **346** (2021), pp. 128935–128935.
83. McCarthy, A.L.; O'Callaghan, Y.C.; Connolly, A.; Piggott, C.O.; FitzGerald, R.J. and O'Brien, N.M.: Brewers' spent grain (BSG) protein hydrolysates decrease hydrogen peroxide (H_2O_2)-induced oxidative stress and concanavalin-A (con-A) stimulated IFN-γ production in cell culture, Food & Function, **4** (2013), no. 11, pp. 1709–1709.
84. Shobako, N.: Hypotensive peptides derived from plant proteins, Peptides, **142** (2021), pp. 170573–170573.
85. W.H.O.: Hypertension, . https://www.who.int/news-room/fact-sheets/detail/hypertension, (2023).
86. Carlos Sgarbieri, V.: Food Proteins and Bioactive Peptides, Functional Diets, Food Science & Nutrition, **3** (2017), no. 2, pp. 1–14.
87. Chen, Z.-Y.; Peng, C.; Jiao, R.; Wong, Y.M.; Yang, N. and Huang, Y.: Anti-hypertensive Nutraceuticals and Functional Foods, Journal of Agricultural and Food Chemistry, **57** (2009), no. 11, pp. 4485–4499.
88. Tom, B.; Dendorfer, A. and Jan Danser, A.H.: Bradykinin, angiotensin-(1–7), and ACE inhibitors: how do they interact?, The International Journal of Biochemistry & Cell Biology, **35** (2003), no. 6, pp. 792–801.
89. Fan, H.; Liu, H.; Zhang, Y.; Zhang, S.; Liu, T. and Wang, D.: Review on plant-derived bioactive peptides: biological activities, mechanism of action and utilizations in food development, Journal of Future Foods, **2** (2022), no. 2, pp. 143–159.
90. LOPONEN, J.: Angiotensin converting enzyme inhibitory peptides in Finnish cereals: a database survey, Agricultural and Food Science, **13** (2008), nos 1–2, pp. 39–39.
91. Connolly, A.; O'Keeffe, M.B.; Piggott, C.O.; Nongonierma, A.B. and FitzGerald, R.J.: Generation and identification of angiotensin converting enzyme (ACE) inhibitory peptides from a brewers' spent grain protein isolate, Food Chemistry, **176** (2015), pp. 64–71.
92. Garzón, A.G.; Ferreira, M. del R.; Cian, R.E.; Oliva, M.E.; D'Alessandro, M.E. and Drago, S.R.: Microencapsulated bioactive peptides from brewer's spent grain promotes antihypertensive and antidiabetogenic effects on a hypertensive and insulin-resistant rat model, Journal of Food Biochemistry, **46** (2022), no. 10.
93. Zhou, J.; Chen, M.; Wu, S.; Liao, X.; Wang, J.; Wu, Q.; Zhuang, M. and Ding, Y.: A review on mushroom-derived bioactive peptides: Preparation and biological activities, Food Research International, **134** (2020), pp. 109230–109230.
94. Valdez-Miramontes, C.E.; De Haro-Acosta, J.; Aréchiga-Flores, C.F.; Verdiguel-Fernández, L. and Rivas-Santiago, B.: Antimicrobial peptides in domestic animals and their applications in veterinary medicine, Peptides, **142** (2021), pp. 170576–170576.
95. Li, J.; Hu, S.; Jian, W.; Xie, C. and Yang, X.: Plant antimicrobial peptides: structures, functions, and applications, Botanical Studies, **62** (2021), no. 1.
96. Tan, Y.N.; Ayob, M.K. and Wan Yaacob, W.A.: Purification and characterisation of antibacterial peptide-containing compound

derived from palm kernel cake, Food Chemistry, **136** (2013), no. 1, pp. 279–284.
97. Freitas, C.S.; Vericimo, M.A.; da Silva, M.L.; da Costa, G.C.V.; Pereira, P.R.; Paschoalin, V.M.F. and Del Aguila, E.M.: Encrypted antimicrobial and antitumoral peptides recovered from a protein-rich soybean (Glycine max) by-product, Journal of Functional Foods, **54** (2019), pp. 187–198.
98. Li, L.; Zong, X.Y.; Zhang, J.; Wei, C.H.; Huang, Z.G. and Luo, H.B.: Antibacterial Activity of Brewer's Spent Grains Peptides to Staphylococcus aureus, Advanced Materials Research, **699** (2013), pp. 320–325.
99. Fakhriya Taha, S.S.M., S.M. Wagdy, Gamal Abdelhamid MohamedGamal Abdelhamid Mohamed: Antioxidant and antimicrobial activities of enzymatic hydrolysis products from sunflower protein isolate, World Applied Science Journal, **21** (2013), no. 5, pp. 651–658.
100. Wang, B.; Xie, N. and Li, B.: Influence of peptide characteristics on their stability, intestinal transport, and *in vitro* bioavailability: A review, Journal of Food Biochemistry, **43** (2018), no. 1, pp. e12571–e12571.
101. Ahmed, T.; Sun, X. and Udenigwe, C.C.: Role of structural properties of bioactive peptides in their stability during simulated gastrointestinal digestion: A systematic review, Trends in Food Science & Technology, **120** (2022), pp. 265–273.
102. Brodkorb, A.; Egger, L.; Alminger, M.; Alvito, P.; Assunção, R.; Ballance, S.; Bohn, T.; Bourlieu-Lacanal, C.; Boutrou, R.; Carrière, F.; Clemente, A.; Corredig, M.; Dupont, D.; Dufour, C.; Edwards, C.; Golding, M.; Karakaya, S.; Kirkhus, B.; Le Feunteun, S.; Lesmes, U.; Macierzanka, A.; Mackie, A.R.; Martins, C.; Marze, S.; McClements, D.J.; Ménard, O.; Minekus, M.; Portmann, R.; Santos, C.N.; Souchon, I.; Singh, R.P.; Vegarud, G.E.; Wickham, M.S.J.; Weitschies, W. and Recio, I.: INFOGEST static *in vitro* simulation of gastrointestinal food digestion, Nature Protocols, **14** (2019), no. 4, pp. 991–1014.
103. Silva do Nascimento, E.; Anaya, K.; de Oliveira, J.M.C.; de Lacerda, J.T.J.G.; Miller, M.E.; Dias, M.; Mendes, M.A.; de Azevedo Lima Pallone, J.; Weis Arns, C.; Juliano, M.A.; Santi Gadelha, T.; Bertoldo Pacheco, M.T. and de Almeida Gadelha, C.A.: Identification of bioactive peptides released from *in vitro* gastrointestinal digestion of yam proteins (Dioscorea cayennensis), Food Research International, **143** (2021), pp. 110286–110286.
104. Sánchez-Velázquez, O.A.; Cuevas-Rodríguez, E.O.; Mondor, M.; Ribéreau, S.; Arcand, Y.; Mackie, A. and Hernández-Álvarez, A.J.: Impact of *in vitro* gastrointestinal digestion on peptide profile and bioactivity of cooked and non-cooked oat protein concentrates, Current Research in Food Science, **4** (2021), pp. 93–104.
105. Nongonierma, A.B. and FitzGerald, R.J.: Dipeptidyl peptidase IV inhibitory properties of a whey protein hydrolysate: Influence of fractionation, stability to simulated gastrointestinal digestion and food–drug interaction, International Dairy Journal, **32** (2013), no. 1, pp. 33–39.
106. Mune Mune, M.A.; Minka, S.R. and Henle, T.: Investigation on antioxidant, angiotensin converting enzyme and dipeptidyl peptidase IV inhibitory activity of Bambara bean protein hydrolysates, Food Chemistry, **250** (2018), pp. 162–169.
107. Cermeño, M.; Connolly, A.; O'Keeffe, M.B.; Flynn, C.; Alashi, A.M.; Aluko, R.E. and FitzGerald, R.J.: Identification of bioactive peptides from brewers' spent grain and contribution of Leu/Ile to bioactive potency, Journal of Functional Foods, **60** (2019), pp. 103455–103455.
108. Cian, R.E.; Oliva, M.E.; Garzón, A.G.; Ferreira, M. del R.; D´Alessandro, M.E. and Drago, S.R.: *In vitro* and *in vivo* antithrombotic and antioxidant properties of microencapsulated brewers' spent grain peptides, International Journal of Food Science & Technology, **57** (2022), no. 6, pp. 3872–3879.
109. Ribeiro-Oliveira, R.; Martins, Z.E.; Faria, M.Â.; Sousa, J.B.; Ferreira, I.M.P.L.V.O. and Diniz, C.: Protein Hydrolysates from Brewing By-Products as Natural Alternatives to ACE-Inhibitory Drugs for Hypertension Management, Life, **12** (2022), no. 10, pp. 1554–1554.
110. McCarthy, A.; O'Callaghan, Y. and O'Brien, N.: Protein Hydrolysates from Agricultural Crops—Bioactivity and Potential for Functional Food Development, Agriculture, **3** (2013), no. 1, pp. 112–130.
111. Lister, D.L.; Jones, H.; Oliveira, H.R.; Petrie, C.A.; Liu, X.; Cockram, J.; Kneale, C.J.; Kovaleva, O. and Jones, M.K.: Barley heads east: Genetic analyses reveal routes of spread through diverse Eurasian landscapes, PLOS ONE, **13** (2018), no. 7, pp. e0196652–e0196652.
112. Teixeira, C.; Prykhodko, O.; Alminger, M.; Fåk Hållenius, F. and Nyman, M.: Barley Products of Different Fiber Composition Selectively Change Microbiota Composition in Rats, Molecular Nutrition & Food Research, **62** (2018), no. 19.
113. Kanauchi, O. and Agata, K.: Protein, and Dietary Fiber-rich New Foodstuff from Brewer's Spent Grain Increased Excretion of Feces and Jejunum Mucosal Protein Content in Rats, Bioscience, Biotechnology, and Biochemistry, **61** (1997), no. 1, pp. 29–33.
114. Zhang, J.-X.; Lundin, E.; Andersson, H.; Bosaeus, I.; Dahlgren, S.; Hallmans, G.; Stenling, R. and Åman, P.: Brewer's Spent Grain, Serum Lipids and Fecal Sterol Excretion in Human Subjects with Ileostomies, The Journal of Nutrition, **121** (1991), no. 6, pp. 778–784.
115. Mikola, J. and Suolinna, E.-M.: Purification and Properties of a Trypsin Inhibitor from Barley, European Journal of Biochemistry, **9** (1969), no. 4, pp. 555–560.
116. Gupta, R.K.; Gangoliya, S.S. and Singh, N.K.: Reduction of phytic acid and enhancement of bioavailable micronutrients in food grains, Journal of Food Science and Technology, **52** (2013), no. 2, pp. 676–684.
117. Popova, A. and Mihaylova, D.: Antinutrients in Plant-based Foods: A Review, The Open Biotechnology Journal, **13** (2019), no. 1, pp. 68–76.
118. F. S. A.: Labelling - Allergens. (Ireland), https://www.fsai.ie/business-advice/labelling/labelling-allergens, (2024).
119. THE EUROPEAN PARLIAMENT AND THE COUNCIL OF THE EUROPEAN UNION: REGULATION (EU) No 1169/2011 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL2011.
120. Jones, S.M.; Magnolfi, C.F.; Cooke, S.K. and Sampson, H.A.: Immunologic cross-reactivity among cereal grains and grasses in children with food hypersensitivity, Journal of Allergy and Clinical Immunology, **96** (1995), no. 3, pp. 341–351.
121. Nye-Wood, M.G. and Colgrave, M.L.: LC-MS/MS Reveals Hordeins Are Enriched in Brewers' Spent Grain, Journal of the American Society for Mass Spectrometry, **35** (2024), no. 3, pp. 409–412.
122. Das, R. and Biswas, S.: Nutraceutical-prophylactic and Therapeutic Role of Functional Food in Health, Journal of Nutrition & Food Sciences, **6** (2016), no. 4.
123. Liu, L.; Li, S.; Zheng, J.; Bu, T.; He, G. and Wu, J.: Safety considerations on food protein-derived bioactive peptides, Trends in Food Science & Technology, **96** (2020), pp. 199–207.
124. Hardy, G.: Nutraceuticals and functional foods: introduction and meaning, Nutrition, **16** (2000), nos 7–8, pp. 688–689.
125. Martirosyan, D. and Stratton, S.: Advancing functional food regulation, Bioactive Compounds in Health and Disease, **6** (2023), no. 7, pp. 166–166.
126. Coppens, P.; da Silva, M.F. and Pettman, S.: European regulations

on nutraceuticals, dietary supplements and functional foods: A framework based on safety, Toxicology, **221** (2006), no. 1, pp. 59–74.
127. Martirosyan, D.; Kanya, H. and Nadalet, C.: Can functional foods reduce the risk of disease? Advancement of functional food definition and steps to create functional food products, Functional Foods in Health and Disease, **11** (2021), no. 5, pp. 213–213.
128. Chopra, A.S.; Lordan, R.; Horbańczuk, O.K.; Atanasov, A.G.; Chopra, I.; Horbańczuk, J.O.; Jóźwik, A.; Huang, L.; Pirgozliev, V.; Banach, M.; Battino, M. and Arkells, N.: The current use and evolving landscape of nutraceuticals, Pharmacological Research, **175** (2022), pp. 106001–106001.
129. Kaur, S. and Das, M.: Functional foods: An overview, Food Science and Biotechnology, **20** (2011), no. 4, pp. 861–875.
130. Mohamadi, A.; Shiraseb, F.; Mirzababaei, A.; AkbarySedigh, A.; Ghorbani, M.; Clark, C.C.T.; Aali, Y. and Mirzaei, K.: The association between adherence to diet quality index and cardiometabolic risk factors in overweight and obese women: a cross-sectional study, Frontiers in Public Health, **11** (2023).
131. Topolska, K.; Florkiewicz, A. and Filipiak-Florkiewicz, A.: Functional Food—Consumer Motivations and Expectations, International Journal of Environmental Research and Public Health, **18** (2021), no. 10, pp. 5327–5327.
132. Siró, I.; Kápolna, E.; Kápolna, B. and Lugasi, A.: Functional food. Product development, marketing and consumer acceptance—A review, Appetite, **51** (2008), no. 3, pp. 456–467.
133. Chandrasekaran, P.; Weiskirchen, S. and Weiskirchen, R.: Effects of Probiotics on Gut Microbiota: An Overview, International Journal of Molecular Sciences, **25** (2024), no. 11, pp. 6022–6022.
134. Keller, C.: Trends in beverages and "Measurable Health", konference 'Proceedings of the third functional food net meeting', 2006.
135. Emmanuel, J.K.; Nganyira, P.D. and Shao, G.N.: Evaluating the potential applications of brewers' spent grain in biogas generation, food and biotechnology industry: A review, Heliyon, **8** (2022), no. 10, pp. e11140–e11140.
136. Wang, X.; Xu, Y.; Teo, S.Q.; Heng, C.W.; Lee, D.P.S.; Gan, A.X. and Kim, J.E.: Impact of solid-state fermented Brewer's spent grains incorporation in biscuits on nutritional, physical and sensorial properties, LWT, **182** (2023), pp. 114840–114840.
137. Shi, P.; Ng Yuen Kai, R.; Vijayan, P.; Lim, S.L. and Bhaskaran, K.: Valorization of spent barley grains: isolation of protein and fibers for starch-free noodles and its effect on glycemic response in healthy individuals, Frontiers in Sustainable Food Systems, **7** (2023).
138. Schettino, R.; Verni, M.; Acin-Albiac, M.; Vincentini, O.; Krona, A.; Knaapila, A.; Cagno, R.D.; Gobbetti, M.; Rizzello, C.G. and Coda, R.: Bioprocessed Brewers' Spent Grain Improves Nutritional and Antioxidant Properties of Pasta, Antioxidants, **10** (2021), no. 5, pp. 742–742.
139. Fărcaș, A.C.; Socaci, S.A.; Chiș, M.S.; Martínez-Monzó, J.; García-Segovia, P.; Becze, A.; Török, A.I.; Cadar, O.; Coldea, T.E. and Igual, M.: *In Vitro* Digestibility of Minerals and B Group Vitamins from Different Brewers' Spent Grains, Nutrients, **14** (2022), no. 17, pp. 3512–3512.
140. Annunziata, A. and Vecchio, R.: Agri-food Innovation and the Functional Food Market in Europe: Concerns and Challenges, EuroChoices, **12** (2013), no. 2, pp. 12–19.
141. Shi, J., Mazza, G.,.&. Le Maguer, M.: Functional Foods: Biochemical and Processing Aspects, CRC Press, 2016.
142. Granato, D.; Barba, F.J.; Bursać Kovačević, D.; Lorenzo, J.M.; Cruz, A.G. and Putnik, P.: Functional Foods: Product Development, Technological Trends, Efficacy Testing, and Safety, Annual Review of Food Science and Technology, **11** (2020), no. 1, pp. 93–118.
143. Lau, T.-C.; Chan, M.-W.; Tan, H.-P. and Kwek, C.-L.: Functional Food: A Growing Trend among the Health Conscious, Asian Social Science, **9** (2012), no. 1.
144. Cermeño, M.; Dermiki, M.; Kleekayai, T.; Cope, L.; McManus, R.; Ryan, C.; Felix, M.; Flynn, C. and FitzGerald, R.J.: Effect of enzymatically hydrolysed brewers' spent grain supplementation on the rheological, textural and sensory properties of muffins, Future Foods, **4** (2021), pp. 100085–100085.
145. Bazsefidpar, N.; Ghandehari Yazdi, A.P.; Karimi, A.; Yahyavi, M.; Amini, M.; Ahmadi Gavlighi, H. and Simal-Gandara, J.: Brewers spent grain protein hydrolysate as a functional ingredient for muffins: Antioxidant, antidiabetic, and sensory evaluation, Food Chemistry, **435** (2024), pp. 137565–137565.

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