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Extraction, bioactive function and application of wheat germ protein/peptides: A review

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ABSTRACT

The aging population and high incidence of age-related diseases are major global societal issues. Consuming bioactive substances as part of our diet is increasingly recognized as essential for ensuring a healthy life for older adults. Wheat germ protein has a reasonable peptide structure and amino acid ratio but has not been fully utilized and exploited, resulting in wasted wheat germ resources. This review summarizes reformational extraction methods of wheat germ protein/peptides (WGPs), of which different methods can be selected to obtain various WGPs. Interestingly, except for some bioactive activities found earlier, WGPs display potential anti-aging activity, with possible mechanisms including antioxidant, immunomodulatory and intestinal flora regulation. However, there are missing *in vitro* and *in vivo* bioactivity assessments of WGPs. WGPs possess physicochemical properties of good foamability, emulsification and water retention and are used as raw materials or additives to improve food quality. Based on the above, further studies designing methods to isolate particular types of WGPs, determining their nutritional and bioactive mechanisms and verifying their activity *in vivo* in humans are crucial for using WGPs to improve human health.

1. Introduction

Aging is a complex degenerative process, susceptible to multiple factors and can be associated with several negative manifestations of the body, including antioxidative damage, inflammation, dysbacteriosis and cognitive disorders (Liaoa et al., 2023). According to the United Nations Department of Economic and Social Affairs statistics, the number of people aged 60 or older in 2017 was 963 million and is expected to increase to 2.1 billion by 2050 and 3.1 billion by 2100 (World Health Organization, 2018). Thus, identifying effective intervention strategies for preventing and treating aging and age-related diseases is clearly in the public's interest. The role of diet is increasingly seen as a crucial element of human well-being, including aging. The vital role of the diet is inseparable from the nutritional properties of food, which have been clarified gradually in recent years. A precision diet or selectively eating food based on its function is becoming an important aid in medicine and improving a healthy life. Thus, it is essential to increase research efforts and focus on food production and the associated health benefits realized from specialized diets (Vatskel et al., 2021).

In the 21st century, wheat germ remains very popular because it is an indispensable part of healthy diets. The inclusion of wheat germ in a daily diet is considered to be the basis of "correct" and "healthy" nutrition systems (Vatskel et al., 2021). Wheat germ is the part to breed new life in wheat and accounts for 2%–3% of the total weight (Brandolini and Hidalgo, 2012). Wheat germ contains 26%–35% protein, 10%–15% lipids, ~10%–14% dietary fiber and ~4% minerals. However, during actual production, unsaturated fatty acids, oxidants and hydrolases in wheat germ affect flour quality and shorten the shelf life. Then, wheat germ is often removed during flour production, thus representing an important by-product in the flour-producing industry.

Noteworthy, wheat germ contains up to 30% wheat germ protein/peptides (WGPs) and is a comprehensive source of plant protein. However, WGPs have not been fully exploited because wheat germ is typically used for wheat germ oil extraction, generating a partial resource waste (Majzoobi et al., 2022). WGPs contain eight essential amino acids with relatively high levels of lysine, isoleucine, valine and leucine. The

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composition ratio of all types of amino acids is very close to the standard recommended by FAO/WHO. WGPs are readily absorbed and have high nutritional value (Boukid et al., 2018; Chen et al., 2021). Moreover, WGPs contain less glutenin than whole wheat, thus representing a good choice for gluten-intolerant suffers (Zhu et al., 2006). The non-protein nitrogen content is also high in wheat germ, which mainly contains asparagine, choline, lecithin and glutathione. Choline plays a key role in many physiological processes, such as signal transduction, biosynthesis and the integrity of cell membranes (Zhang et al., 2017). Glutathione is an active tripeptide condensed from glutamic acid, cysteine and glycine that protects cells from oxides and toxic substance damage (Gu et al., 2012). In addition to glutathione, other amino acid sequences in WGPs also have bioactivities. For example, peptides AREGETVVPG and PVLGPVRGPFPLL have antioxidant, lipid-lowering and anti-inflammatory functions.

WGPs contain various subtypes that can be extracted or hydrolyzed from wheat germ for human consumption. These peptides can be used as additives in food processing to improve the properties and quality of food. WGPs have good physicochemical properties, such as emulsifying activity, foaming property and water retention. As one of the most promising sources of plant nitrogen, studying the extraction, function and application of WGPs is important. This review presents the above features of WGPs, focusing primarily on the development of extraction methods and specific characteristics of each method, the bioactive functions of WGPs in reducing the onset of age-related health issues and applications in several food industries.

2. Extraction of WGPs

Currently, the leading technologies for extracting WGPs from defatted wheat germ include alkali extraction, microwave- or ultrasonic-assisted alkali extraction, subcritical water extraction, enzymatic extraction and reverse micelle extraction (Fig. 1) (Amagliani et al., 2017; Wen et al., 2019; Leal et al., 2021). Besides these chemical methods, microbial fermentation has also been an effective method in recent years.

2.1. Microwave- and ultrasonic-assisted alkaline extraction method

Chemical hydrolysis of proteins produces bioactive hydrolysates and peptides from proteins by breaking peptide bonds under acid-base catalysis. Alkaline hydrolysis is used widely in commercial protein hydrolysis because of its low cost and simple operation. Traditional protein extraction from defatted wheat germ using the alkali method showed that the optimal extraction conditions of 60 °C, pH 10 and a material-liquid ratio with 1:16 (g/mL) yielded 35.4% WGPs. Although alkali extraction is simple, the low extraction rate and a large amount of alkaline wastewater make this process a serious environmental issue (Zhu et al., 2006). In addition, WGPs were typically denatured following alkaline extraction, leading to unstable peptides and low functional activity. Some unexpected secondary products, including D- and L-amino acids contained in the products, were also difficult to remove. Because of the above disadvantages, obtaining WGPs using a chemical hydrolysis process seemed impractical and uneconomical.

In recent years, microwave- and ultrasonic-assisted extraction have become methods to extract substances from plant materials. Using these methods effectively improved the extraction efficiency and shortened the extraction time (Jerkovic et al., 2007; Kim et al., 2007; Rostagno et al., 2007; Stanisavljevic et al., 2007). The extraction rate of WGPs reached 75.88% when the ultrasonic power was 80 W and pH 9 were used (Sun and Ren, 2015). Thus, the extraction rate of WGPs by microwave- and ultrasonic-assisted alkaline methods improved significantly compared with the rate with the conventional alkaline method. However, microwave- and ultrasonic-related operations are more difficult than the conventional method and require expensive equipment, which limits their applications in factory production. Moreover, microwave- and ultrasonic-assisted extraction can affect proteins. Appropriate microwave assistance improves the emulsifying activity and foaming property of WGPs, but decreases foam stability, whereas ultrasonic assistance improves foam stability but reduces the foaming and emulsifying properties of proteins.

2.2. Subcritical water extraction method

Subcritical water extraction of WGPs involves placing water in a subcritical state under certain pressure and temperature conditions, and then the water polarity is changed to extract target substances. The optimal extraction conditions for the response surface method were pH 9.4, solid-liquid ratio of 1:20 (g/mL), 130 °C and 15 min. Under these conditions, the protein yield was 42.25% (Zhang et al., 2019). Compared with the findings with the conventional alkaline method, the extraction time of WGPs with subcritical water was shorter, and the extraction rate was higher (Budrat and Shotipruk, 2009; Simsek Kus, 2012). In addition, WGPs obtained under the above conditions displayed good characteristics, with emulsion stability of 82% and foam stability of 36%. The water absorption rate, oil absorption rate and solubility were 4.21%, 4.49% and 60.93%, respectively (Zhang et al., 2019).

2.3. Reverse micelle extraction method

Reverse micelles are nano-sized aggregates of surfactant molecules containing water molecules in nonpolar solvents. The extraction process of proteins using reverse micelles involves two steps: forward extraction and reverse extraction. During the forward extraction process, proteins

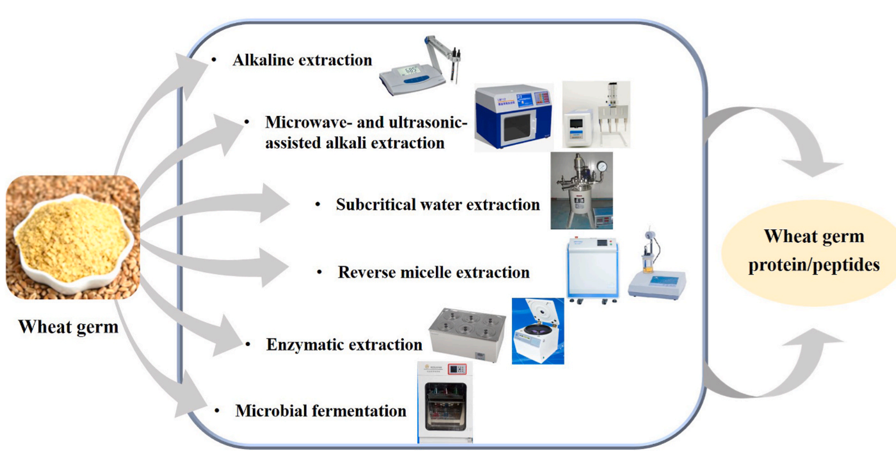

Fig. 1. The extraction methods of wheat germ protein/peptides from (defatted) wheat germ.

are dissolved in a reverse micelle solution of defatted wheat germ, whereas in the reverse extraction process, the dissolved proteins are recovered from the reverse micelle solution.

When combining ultrasonic-assisted extraction with reverse micelles and optimizing by a response surface method, the forward extraction efficiency of WGPs increased from 37% to 57% (protein extraction efficiency 1 = total protein in reverse micelle system/total protein in defatted wheat germ × 100%) (Zhu et al., 2009). A new backward extraction method was developed by changing the pH value and ionic strength of the aqueous phase (Sun et al., 2009). The backward extraction method combined with the response surface method was used to optimize the concentration and additive amount of a KCl solution and the pH of the vehicle. Under optimal conditions, the peeling efficiency of WGPs reached 80% (protein extraction efficiency 2 = precipitated total protein/total protein of reverse micelle system × 100%), and the final protein extraction efficiency of reverse micelles reached 45.6% (45.6% = 57% × 80%), which yielded higher purity of the target protein compared with the findings with alkaline extraction.

The ultrasonic-assisted reverse micelle method greatly improved the forward extraction efficiency of WGPs. Compared with those of plant proteins extracted by traditional methods, the amino acid composition, content and structure of plant proteins extracted by the reverse micellar method can be ensured to a greater extent, which ensures the biological activity of proteins is maintained at a lower cost because the surfactant and organic solvent can be reused in the reverse micelle method. However, the total extraction efficiency of the reverse micellar method is lower than that of ultrasonic-assisted alkaline extraction and enzymatic extraction.

2.4. Enzymatic extraction method

The structural and functional characteristics of WGPs vary under different extraction conditions. Chemical hydrolysis can easily disrupt peptide bonds, but it is rarely used in the industrial production of active peptides because of uncontrolled processing, which yields various products. In addition, the function of the products following chemical extraction is uncertain. Therefore, the extraction rate and the influence of the extraction methods on the quality of WGPs should be considered. Enzymatic hydrolysis breaks specific peptide bonds under mild conditions to give specific target peptide segments with defined biological activities.

Enzymatic extraction of wheat germ protein is an enzyme application technology. Under 50 °C and pH 8, proteins were extracted from wheat germ by alkaline protease, and the extraction rate reached 80% after hydrolysis for 5 h (Claver and Zhou, 2005). Furthermore, the enzyme can be immobilized for easy control and reuse during the reaction. Additionally, the reaction conditions are milder, yielding proteins/peptides of high quality.

Many impurities, such as carbohydrates and pigments, are often present and difficult to remove when defatted wheat germ protein is further used as raw material directly in product synthesis. Interestingly, it was found that most of the protein hydrolysate had high antioxidant activity. Thus, defatted wheat germ protein is usually taken to prepare highly active peptide products. Wheat germ peptides can be obtained by hydrolyzing defatted wheat germ protein with enzymes, such as alkaline protease, pepsin, protease K or papain (Liao et al., 2022). The hydrolysis degree of alkaline protease hydrolysate WGPs was high after 3 h, and the obtained peptides had good metal chelating activity at the 4-h time point. The hydrolysis rate slowed when extending the incubation time, and the calcium-binding activity of WGPs decreased (Zhu et al., 2015).

WGPs can also be extracted using alkaline protease combined with ultrasonic treatment. The structure of WGPs can change after 30 min of ultrasonic treatment with 1200 W power at 20 °C, presenting exposed hydrophobic groups. This change in protein structure made WGPs more susceptible to alkaline protease and thus greatly reduced the required hydrolysis time (Du et al., 2022). When evaluating the extraction efficiency by indicators of the hydrolysis degree of wheat germ albumin and the clearance rate of 1, 1-diphenyl-2-picryhydrazyl (DPPH) radical, microwave-assisted papain hydrolysis polypeptides showed increased solubility and reduced viscosity and foam stability with loose structures and rounded surrounding blocks. In particular, the proportions of proline, histidine, glycine, lysine and glutamic acid in the enzymatic hydrolysis products increased, whereas the proportions of leucine, phenylalanine, arginine and isoleucine decreased compared with the findings in the amino acids of wheat germ albumin (Tian et al., 2022).

2.5. Preparation of WGPs by the microbial fermentation method

Microbial fermentation is also a potential method to prepare biologically active peptides, especially using natural sources such as wheat germ. Microbial fermentation involves enzymatic hydrolysis of a natural source using particular microbe strains. For the food industry, wheat germ fermentation can enhance physiological characteristics of WGPs due to the mild fermentative condition. During fermentation, *Bacillus subtilis*, lactic acid bacteria and several fungi can release a variety of peptides, and some of these peptides possess bioactive properties.

The preparation of antioxidant peptides from defatted wheat germ has been achieved by *B. subtilis* fermentation (Niu et al., 2013). After fermentation, the molecular weight of the peptide product was less than 1 kDa with good sensory properties (no bitter amino acids produced). Fermentation using *B. subtilis* and *Enterococcus faecium* has been reported to increase the levels of small peptides and crude proteins compared with levels in the defatted wheat germ (Shi et al., 2017). Although proteins or peptides produced by microbial fermentation have no bitterness, clarifying the fermentation mechanism and controlling the fermentation time to obtain suitable products with a high yield still needs to be examined.

Hydrolyzed fermentation peptides show potential antioxidant activity against free radicals. The role of lactic acid bacteria and yeast in producing WGPs has also attracted the attention of researchers. Wheat germ fermented with lactic acid bacterial strains *Lactobacillus plantarum LB1* and *L. rosella LB5* yielded bioactive substances, which inhibited the growth of germ cell tumors, colon carcinoma cells and ovarian carcinoma cells (Rizzello et al., 2013).

In addition, yeast fermentation of wheat germ extracts using *Saccharomyces cerevisiae* also obtained products with anticancer and immune-stimulating activities (Otto et al., 2016). However, in general, *Lactobacillus* fermentation appears to be more competitive than yeast. WGPs fermented by *L. plantarum* strain 299v were reported to afford a maximum DPPH radical scavenging rate of 88.95%, which is higher than that obtained by fermenting *S. cerevisiae* 5022 (80.19%–85.73%) during a 48 h treatment time at pH 6 (Bayat et al., 2022). Interestingly, the concentrations of peptides (532.50 μg/mL) and γ-aminobutyric acid (13.68 g/kg) produced by *S. cerevisiae* 5022 fermentation were lower than the concentrations of peptides (607 g/mL) and γ-aminobutyric acid (19.98 g/kg) produced by *L. plantarum* under optimal fermentation conditions (Bayat et al., 2022). In contrast, after the fermentation of a wheat embryo by another fungus, *Aspergillus niger*, the DPPH scavenging capacity of the fermentation broth was 75.68%, whereas the protein content in the fermentation broth reached 21.92 mg/mL (Feng et al., 2022). Thus, similar yields of WGPs were achieved by either *L. plantarum* or *A. niger* fermentation. Although variation in the antioxidant capacity has been observed, potential applications still require further in-depth analysis combined with examining the fermentation characteristics of different microorganisms.

3. Biological activities of WGPs

Studying the biological activities of WGPs can promote the use of wheat germ and draw a blueprint for developing functional WGPs healthcare products and pharmaceutical raw materials, especially in delaying aging (Fig. 2).

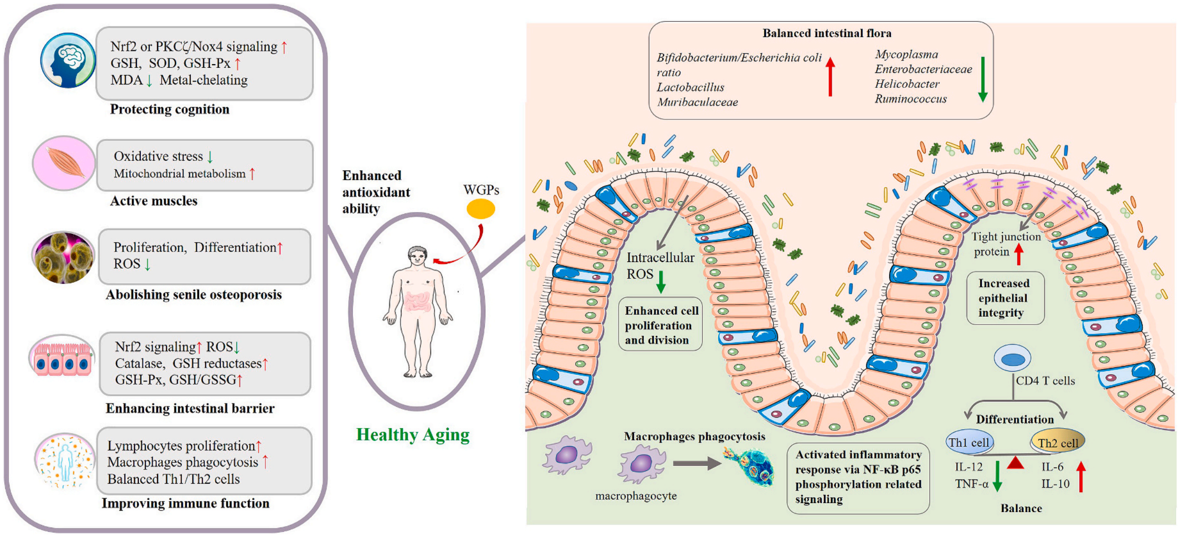

Fig. 2. Potential anti-aging biological activity of WGPs.
Abbreviations: WGPs, wheat germ protein/peptides; Nrf2, nuclear factor erythroid-2 related factor 2; PKCζ, anti-phospho-protein kinase ζ; Nox4, nicotinamide adenine dinucleotide phosphate oxidase 4; GSH, glutathione; SOD, superoxide dismutase; GSH-Px, glutathione peroxidase; MDA, malondiadehyde; ROS, reactive oxygen species; GSSG, oxidized glutathione; NF-κB p65, nuclear factor-κB p65; IL-12, interleukin-12; TNF-α, tumor necrosis factor-alpha; IL-6, interleukin-6; IL-10, interleukin-10.

3.1. Anti-aging via antioxidant activity

Oxidative stress is a major change in aging metabolism (Panyard et al., 2022). Particular peptides have good antioxidant activity because of their ability to scavenge free radicals (Ding et al., 2019), inhibit lipid peroxidation and chelate metals (Taheri et al., 2014). Therefore, peptide supplementation may promote anti-aging of the body. Peptides with low molecular weights obtained by hydrolysis of proteins usually have excellent antioxidant activity. Generally, hydrophobic amino acids, including Tyr, Try, Phe, Leu, Ile and Ala, can act as hydrogen donors to block the free radical peroxidation chain reaction. Among these amino acids, Tyr, Try and Phe are aromatic amino acids with the strongest antioxidant activity (Lapsongphon and Yongsawatdigul, 2013). WGPs containing amino acids Asp, Glu, Ser, Pro, Ala, Ile, Leu, Phe and Tyr can chelate ferrous ions and scavenge various free ions (Zhu et al., 2006). In addition, the molecular weight of WGPs from hydrolysates hydrolyzed by alkaline protease is less than 1.5 kDa and mainly composed of GPF, GPE and FGE, which are passively absorbed by the apical compartment of the intestinal epithelium and show effective antioxidant activity in a Caco-2 cell model (Zhu et al., 2006; Zhang et al., 2019).

GSH, glutathione disulfide and the GSH/oxidized glutathione (GSSG) ratio were found to decrease with age, indicating that these antioxidant-related peptides may be attenuated with age (Panyard et al., 2022). D-galactose is a reducing sugar that readily reacts with free amino acid amines in peptides and proteins, and excessive accumulation of these aberrant metabolites can induce oxidative stress to cause aging. Thus, D-galactose is often used as an inducer in cell and animal aging models. WGPs (wheat germ globulin) treatment has been reported to protect D-galactose and aluminum chloride co-induced cognitive impairment in aging rats, with increases in the activity of GSH and anti-oxidase superoxide dismutase (SOD) observed (Zheng et al., 2021). Intragastric administration of WGPs for 45 days also successfully delayed the aging process of mice induced by D-galactose. In the group treated with WGPs, the indicators of glutathione peroxidase (GSH-Px) and SOD activities increased significantly in serum, heart, liver and brain tissues (Zhao et al., 2021b). By studying the protective mechanism of WGPs (wheat germ globulin) on alcoholic liver-injured mice, it was also shown that treatment with WGPs increased the content of GSH and SOD, thus enhancing the antioxidant capacity of the liver (Yang et al., 2021). Various trace elements may be contained in WGPs, of which Fe^{2+} and Cu^{2+} chelate and inhibit lipid peroxidation, thus partially delaying aging.

As a part of physical manifestations during aging, oxidative stress is also a consequence of mitochondrial dysfunction. Generated free radicals, reactive oxygen species (ROS) and oxidative stress are all partly induced by mitochondrial dysfunction (Panyard et al., 2022). A type of WGPs, wheat embryo albumin, enhanced the mRNA expression of biogenic mitochondrial factors in skeletal muscle of mice (Li et al., 2022b), indicating the utilization potential for energy food with anti-fatigue and health benefits. The peptide AREGETVVPG from wheat germ albumin prepared by double enzymatic hydrolysis and separation by mass spectrometry showed a protective effect against oxidative stress induced by high glucose (Chen et al., 2017). These WGPs inhibit the PKCζ/Nox4 signaling pathway in vascular smooth muscle cells. In addition, in some obesity or diabetes models induced by a high-fat diet, WGPs were found to regulate protein expression in oxidative stress-related signaling and improve mitochondrial energy metabolism (Ojo et al., 2019). Therefore, the strong antioxidant capacity of WGPs may provide a basis for their development in an anti-aging diet.

A prominent manifestation of aging is brain dysfunction, resulting in decreased cognition, such as language capabilities, memory and ability to recognize, as described by hippocampal atrophy. It was found that a kind of WGPs, wheat germ globulin, protected damage to the brain in D-galactose-induced aging mice. This WGPs increased total antioxidant capacity, SOD and GSH-Px activities in the liver and brain of aging mice and reduced malondialdehyde (MDA, a lipid peroxide) content, indicating an effect on delaying the aging process (Liaoa et al., 2023). In addition, rats with Alzheimer's disease co-treated with D-galactose and aluminum chloride were protected from cognitive impairment, neuronal damage and oxidative stress by treatment with WGPs (Zheng et al., 2021). The total antioxidant capacity was enhanced, as indicated by a decrease in the level of MDA and an increase in GSH and SOD levels. The key observation was that changes in the acetylcholine system were positive, presenting as a decrease in acetylcholinesterase activity, an increase in acetyltransferase activity and elevated choline acetylcholine content. In another food contaminant, lead-induced neurotoxic PC12 cell model, WGPs including KKLNYPPY, HWPLHKVHAP and KKSPA antagonized oxidative stress by inhibiting ROS and MDA levels and increasing antioxidant substances SOD, catalase, glutathione reductases, GSH-Px and GSH/GSSG. Notably, the upregulated Nrf2 (a crucial redox-sensitive transcription factor) protein expression-related signaling pathway played a key role in the protective process of WGPs (Li et al., 2022a).

Additionally, the peptide ADWGGPLPH obtained from wheat germ effectively abolished senile osteoporosis. This peptide mainly acts on osteoblasts to promote proliferation and differentiation by reducing oxidative stress to improve the microstructure and bone mineral density in senile osteoporosis rats (Wang et al., 2022b). This peptide was also found to significantly prevent high glucose-induced proliferation in vascular smooth muscle cells, acting similarly by enhancing antioxidant activity and reducing intracellular ROS generation (Wang et al., 2020). Celiac disease is an allergic intestinal disease found widely in the population, which can be aggravated with aging. Three WGPs (YDWPGGRN, TGP, QPYPQQPQ) were found to significantly enhance the antioxidant capacity of cells and greatly alleviate the damage of Caco-2 cells induced by α-gliadin peptide (a sensitinogen for celiac disease). These three WGPs activate the Nrf2 signaling pathway and upregulate the glutamate-cysteine ligase catalytic subunit to increase the levels of antioxidant-related enzymes (catalase, glutathione reductases, GSH-Px and GSH/GSSG) and reduce ROS to normal levels (Wang et al., 2022a).

3.2. Anti-inflammatory and immune-protective activities

Inflammation is a protective physiological process involving immune cells removing harmful substances from the body (Weng et al., 2021). Inflammation-related metabolites are observed to change with age. Moreover, the content of pro-inflammatory factors in older adults is 2–4 times that of young people. Accelerated aging can occur in people suffering from chronic inflammation with disordered inflammatory factors.

Macrophages and lymphocytes (T and B lymphocytes) are crucial in inflammatory responses. These cells produce inflammatory enzymes, inducible nitric oxide synthase and cyclooxygenase-2, and cytokines such as tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6), resulting in the occurrence of inflammation (Gallo et al., 2020; Wang et al., 2022a). These inflammatory factors can attract microorganisms, organize blood immune cells, activate endothelial cells and affect the differentiation of helper T cells. In addition, macrophages will restrict inflammatory activation by inducing the production of anti-inflammatory cytokines.

Wheat germ has rich bioactive components with antioxidant and anti-inflammatory activities (Karami et al., 2019). Wheat germ supplementation can significantly increase the content of antibacterial peptides in the ileum of mice and the abundance of intestinal *Lactobacillus*, thereby regulating the transformation of intestinal $CD4^+$ T cells into an anti-inflammatory phenotype (Ojo et al., 2019). Wheat germ extract treated with citric acid was found to specifically inhibit LPS-stimulated inflammation via NF-κB p65 phosphorylation-related signaling, including the inhibition of pro-inflammatory factors TNF-α, IL-6 and IL-12, along with elevated anti-inflammatory factors IL-10 and heme oxygenase-1 (Jeong et al., 2017). In addition, wheat germ can help animals avoid acute and chronic inflammation. For example, a fermented wheat germ extract significantly lowered the inflammatory response in LPS-induced IPEC-J2 porcine intestinal epithelial cells, especially related to oxidative stress. Comparatively, treatment with 1%–2% wheat germ extract decreased the level of intracellular ROS significantly and reduced disruption to epithelial integrity (Karancsi et al., 2020). Four novel peptides identified from alcohol-soluble components of *Lactobacillus* fermented wheat germ and an apple compound possessed anti-colitis activity. Among them, the peptide PVLGPVRGPFPLL acted most remarkably by preventing tight junction protein loss, maintaining epithelial barrier integrity and promoting cell proliferation *in vitro* and *in vivo* (He et al., 2022).

WGPs are rich in Gln. Besides being used as a raw material for glutathione synthesis, it is primarily used as an energy supplement for rapid cell division and to promote the proliferation of lymphocytes when required to prevent invasion from a pathogen. WGPs can stimulate lymphocyte proliferation and enhance macrophage phagocytosis, thereby defending against pathogen invasion and regulating immune function in mice (Weng et al., 2021). Isolated active peptides from wheat germ using alkaline protease hydrolysis were found to stimulate the proliferation of mouse spleen lymphocytes (Zhang et al., 2015). Alkaline phosphatase hydrolysate has high hydrophobicity and a strong immunomodulatory effect on the proliferation of RAW 264.7 cells and the secretion of pro-inflammatory cytokines (Wu et al., 2016), indicating that the hydrophobic properties of hydrolyzed peptides may modulate its immunomodulatory activity. Therefore, WGPs used as an immune supplement in the food industry may be prepared by treating defatted wheat germ with an alkaline enzyme.

WGPs were also found to facilitate recovery of damaged mouse spleen and thymus induced by cyclophosphamide by increasing the ratio of $CD4^+/CD8^+$ cells and restoring the imbalance of Th1/Th2 cells to improve the immune system (Ji et al., 2017). A short WGP, ECFSTA, isolated from wheat germ globulin, also exhibited an immunoregulatory effect by enhancing the phagocytosis of RAW 264.7 cells (Wu et al., 2017b). Adding WGPs to the diet may increase IL-6 levels and slightly decrease the concentrations of TNF-α and IL-10 in the body, suggesting that WGPs may enhance the anti-inflammatory capacity by promoting the activation of monocytes and T cells (Yu et al., 2021). In addition, WGP treatment also upregulates the amount of IgG cells significantly and downregulates the amount of IgM and IgA cells, indicating an immune-enhancing effect on B cells (Yu et al., 2021).

3.3. Intestinal protection and microbial regulation

Intestinal flora homeostasis plays a key role in health. Maintaining a stable intestinal environment is a promising approach to slow aging (Vaiserman et al., 2017). Bioactive peptides can regulate the composition or distribution of intestinal flora and thus affect the intestinal and mucosal immune systems. Wheat germ contains various peptides and promotes the growth of beneficial bacteria (*Bifidobacterium*) and inhibits the proliferation of potentially pathogenic and harmful bacterial species (such as *Escherichia coli*) (Weng et al., 2021; Wu et al., 2017a; Wang et al., 2019). Wheat germ has also been shown to increase the overall abundance of intestinal flora, thereby reducing the severity of intestinal flora imbalance (Weng et al., 2021; Wu et al., 2017a; Wang et al., 2019). In dextran sulfate sodium-induced ulcerative colitis (an inflammatory bowel disease) mice, protease hydrolysates of wheat germ shifted the balance of the intestinal flora caused by inflammation and reversed gut dysbiosis (Zhao et al., 2021a). Because changes to the intestinal flora are an important cause of inflammatory bowel disease, the identified micro-regulatory functions of WGPs provide clues for treating this disease.

Adding wheat germ to bread can help regulate intestinal flora and promote intestinal health (Moreira-Rosario et al., 2020). A high ratio of *Bifidobacterium* to *E. coli* in the intestine is an important indicator of intestinal health, and individuals who eat bread rich in wheat germ possess a high *Bifidobacterium* to *E. coli* ratio. Consumption of fermented wheat germ may positively regulate the environment of intestinal flora by inhibiting pathogens (including *Mycoplasma*, *Enterobacteriaceae*, *Helicobacter* and *Ruminococcus*) and increasing probiotics (including *Lactobacillus* and *Muribaculaceae*) (Zhao et al., 2021b). Furthermore, WGP treatment has been shown to reduce the abundance of *Bacteroidetes* and increase *Firmicutes* in mice (Yu et al., 2021), indicating the key functional role of the protein and/or peptides contained in wheat germ.

Some studies have found that WGPs inhibit harmful bacteria *in vitro*. A small peptide in WGPs (EYF) inhibited the proliferation of bacteria to varying degrees (Yang et al., 2011). An ethanol extract from wheat germ fermentation also displayed an inhibitory effect on *E. coli*. Therefore, WGPs can reshape the intestinal flora and improve the immune capacity of humans. WGPs can potentially be used as a safe and functional food supplement and may also be an important source of future wheat economic benefits in agriculture.

3.4. Other bioactive functions

In addition, WGPs also show a variety of biological activities that prevent aging caused by some systemic diseases. The incidence of diabetes mellitus is increasing globally, and the total number of patients is estimated to reach 700 million by 2045. Among this group, the proportion of middle-aged and older adults is high. Once diagnosed with diabetes, long-term medicinal control of the blood glucose level largely affects the living quality of patients. An efficient approach to treating diabetes mellitus involves delaying the digestion of carbohydrates by inhibiting the activity of the carbohydrate digestive enzyme α-glucosidase (Brand-Miller and Buyken, 2020). WGPs were also found to display anti-diabetic activity with an IC_{50} of 6.87 mg/mL, and peptide fractions below 1 kDa inhibited α-glucosidase strongly with an IC_{50} of 2.10 mg/mL. A high content of C-terminal arginine residues in these peptides may explain their inhibition of α-glucosidase (Liu et al., 2021).

WGPs, especially some DWGPH-derived peptides, have been shown to prevent the adhesion and infection of *Helicobacter pylori* on human gastric epithelial cells both *in vitro* and *in silico* using molecular analysis (Sun et al., 2020; Dang et al., 2022). The underlying mechanism of the anti-adhesive action of WGPs is possibly because these peptides act as receptor analogs that bind to *H. pylori* adhesin proteins. However, there is a paucity of data describing the nature and strength of this molecular interaction and the participating species and drug-likeness of WGPs. Therefore, WGPs can be used in research and developing healthcare products, providing ideas for preventing or treating different chronic diseases.

4. Cohort study of WGPs activities

All bioactivities found in WGPs *in vivo* or *in vitro* must be verified in humans, which is a basic procedure before marketing. Currently, comprehensive cohort studies concerning WGPs have not been reported. Nonetheless, a wheat germ supplement decreased the serum cholesterol concentration in a clinical trial of 80 participants with type 2 diabetes (Mohammadi et al., 2020). After treatment for 12 weeks, the volunteers in the intervention group given 20 g wheat germ exhibited a significant decrease in the serum total cholesterol concentration compared with those in the placebo group. Although the underlying effective ingredients contained in the wheat germ were not characterized fully, WGPs most likely played a key role because WGPs have been reported to decrease the serum concentration of total cholesterol and promote the expression of the low-density lipoprotein receptor (Liu et al., 2022). Patients with type 2 diabetes often suffer from elevated levels of pro-inflammatory cytokines and oxidative stress, which are symptoms present in aging people. Thus, WGPs offer significant potential to delay aging and increase human longevity. However, this potential can only be realized through sufficient larger-scale and multi-angle intervention clinical trials.

5. Application of WGPs in the food industry

WGPs have high nutritional and functional characteristics, which can improve the nutritional value of amino acids in food. WGPs can also be used as additives to change the properties of food. WGPs have good foamability, emulsification and water retention, and as a natural food additive are safer than common synthetic additives. Thus, WGPs have a high application value in food industries (Fig. 3).

5.1. Application of WGPs in flour products

WGPs are often used as a food additive to make bread and promote original oil and water materials to form a more stable emulsion in bread. By adding WGPs, the emulsion mixture is more uniform, and the bread taste and texture are more appealing (Ma et al., 2014). Adding WGPs to flour products improves the nutritional value (tends to whole protein quality) and promotes the appearance of flour products (elevates the hardness). Compared with ordinary flour products, flour products with 12% WGPs added can have a two-fold increase in lysine content, thus resulting in whole protein food (Teterycz et al., 2022). Flour with wheat germ also has good flavor and is easy to digest and absorb. In addition, the content of easily oxidized fat in flour products with WGPs is reduced, thus prolonging the storage period of flour products (Teterycz et al., 2022).

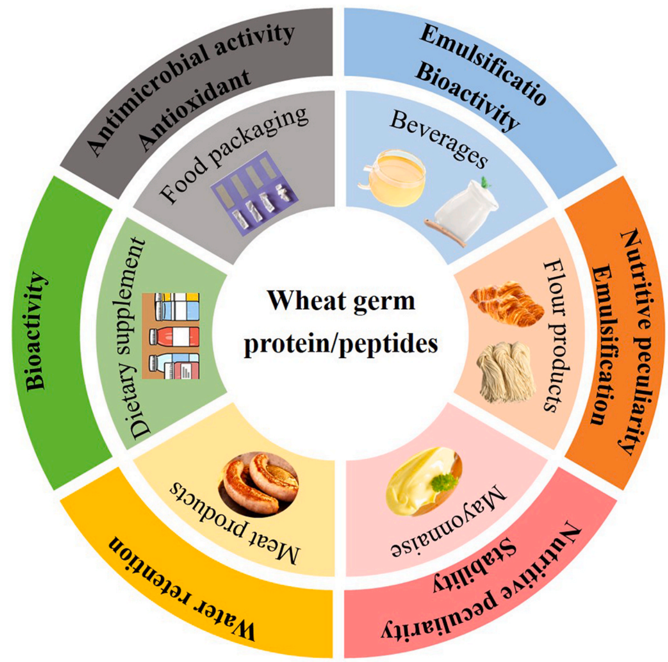

Fig. 3. The utilization of wheat germ protein/peptides in food industry.

5.2. Application of WGPs in beverages

Adding WGPs to beverages can generate a uniquely refreshing flavor with stable qualities and no stratification. Some researchers have used jujube, aloe and WGPs as raw materials to create healthy drinks. These functional beverages are rich in cellulose and minerals and contain a comprehensive composition of proteins. Alcalase hydrolysate of WGPs has excellent solubility, foaming and emulsion properties when compared with hydrolysis by flavourzyme. The addition of an appropriate content of the alcalase hydrolysate (i.e., less than 1.5%) to frozen yogurt exhibited a higher pH, viscosity, uranium, melting resistance and texture hardness than the control yogurt sample (Ghelich et al., 2022). Combining wheat germ and lactic acid bacteria to produce beverages has been proposed (Rice et al., 2020). The ferment of lactic acid bacteria may offer more nutritional and digestive components in beverages. This type of drink is rich in active lactic acid bacteria, proteins, carbohydrates and dietary fiber, which promotes intestinal digestion and absorption, improves the structure of intestinal flora and delays aging (Seleet et al., 2016).

5.3. Application of WGPs in mayonnaise

Research has examined the addition of WGPs to mayonnaise. Based on the amount of mayonnaise added, the best ratio of WGPs and xanthan gum was 7.87% and 0.2%, respectively, which improved the stability, viscosity and tissue properties of mayonnaise (Rahbari et al., 2015). In addition, the cholesterol content in WGPs is very low. Thus, wheat germ mayonnaise is cholesterol-low, with high acceptance.

5.4. Application of WGPs in meat products

An inevitable problem in cooking meat is that the water retention capacity of proteins weakens, leading to the discharge of liquid from muscles. Plant protein material is an important source of raw food material, which has swelling, wettability, water retention, gelation and surface properties. Plant protein materials can affect the quality of food products by changing the mechanical strength, elasticity, plasticity and fluidity. At pH 8.0 and 70 °C, the water retention capacity of WGPs was observed to be as high as 229.4% (Ge et al., 2000). Adding WGPs powder to ham instead of 3.5% lean pork decreased fat content and reduced cooking loss because of the water retention of WGPs (Gnanasambandam and Zayas, 1992).

Furthermore, WGPs can change the microstructure of products and reduce the volume of fat globules, thus increasing the stability and quality of products. An extract from *L. plantarum* DY-1 fermented wheat germ was found to improve the oxidation stability of emulsified sausages by retarding the formation of thiobarbituric acid-reactive substances during seven days of storage at 4 °C. The enhancement in the antioxidant properties of this extract may arise from an increase in the soluble protein content during fermentation. However, the amount added to emulsified sausages should be less than 2.14% because a higher content caused a 41.4% reduction in hardness and a 78% increase in cooking loss (Wu et al., 2020).

5.5. Application of WGPs in food packaging

WGPs can even be used as a surface coating of a polylactic acid/ethyl cellulose blend film in food packaging. The WGPs obtained by enzymatic hydrolysis were found to have stronger antioxidant activity than an ordinary chitosan film coating and a chitosan-WGPs mixed coating. Furthermore, a WGPs-coated film reduced the growth of bacteria (*E. coli* and *Staphylococcus aureus*), displaying effective antimicrobial activity (Hosseini et al., 2022). Thus, the polylactic acid/ethyl cellulose blend film coated with WGPs represents a promising candidate for food packaging with antioxidant and antibacterial properties.

5.6. Application of WGPs in healthy food

Wheat germ hydrolyzed by proteases yields a variety of bioactive peptides that display various functions, including antioxidant, anti-fatigue, antibacterial and cognitive-protective activities. There are even cases of applying extracted WGPs to produce different health foods on the market. WGPs are a natural, cheap and healthy nutrient source that represent promising additives in functional food or even novel drugs for treating diseases. Thus, WGPs have broad application prospects in the food and biomedical fields.

6. Challenges and future prospects of WGPs

Many advanced technologies have been applied to extract WGPs from wheat germ. These technologies improve the extraction rate of WGPs, and some can maintain the structure and function of proteins or peptides. Although the alkaline hydrolysis method disrupts peptide bonds readily to yield a range of peptides, there are issues such as uncontrolled processing, environmental pressure and mediocre or poor bioactivity of these peptides when used as functional food ingredients. Some advanced methods, such as microwave- and ultrasonic-assisted alkaline extraction of WGPs, have high extraction rates, but these methods also affect the characteristics of the proteins or peptides. The extractant of the subcritical water extraction method is water, which can be extracted continuously with no adverse effects on protein quality and environmental safety. The surfactant and organic solvent used in the reverse micelle method can also be reused. Compared with that of the traditional alkaline method, the extraction rate of the reverse micelle method is higher. The native conformation and activity of proteins are maintained using the subcritical water extraction method. Various types of small peptides can be obtained by the enzymatic extraction method from wheat germ protein, displaying various bioactive properties. Enzymatic extraction can be combined with other methods, such as alkali extraction or fermentation. Although these methods can provide a range of bioactive peptides, to optimize production, the extraction method used to obtain WGPs should be fine-tuned to match the intended use.

Clinical evaluation of the bioactive functions of WGPs and their application as health foods is essential. Pre-clinical bioactivity and safety evaluations of WGPs are insufficient for determining suitability for human consumption. Currently, most reports on bioactive properties are carried out *in vivo* or *in vitro* (Table 1), which likely differ from humans. In addition, the pathogenesis of diseases that occur during aging varies and is often complex. Thus, defining a uniform anti-aging or disease-modifying mechanism of WGPs may not be applicable. Besides, the evaluation of specific fragments of WGPs using population-based cohort studies is missing. Thus, to develop and establish a WGP and confirm its bioactive effects at different doses, the action mechanism in humans will be required and represents a future hot topic.

Current products on the market are almost whole wheat germ with unseparated peptides. Moreover, the application of WGPs in meat products and food packaging is limited by technicalities. Based on the above application status of WGPs, further research on WGPs should focus on bioactivity and specific functional peptides and on improving related processing technologies to expand their usage scope in the food industry.

7. Conclusion

WGPs are a group of proteins or peptides present in wheat germ. Innovative methods to extract WGPs that maintain excellent physicochemical and bioactive properties are gradually being developed. WGPs display various functions, including those that slow aging through antioxidant, anti-inflammatory and intestine-protective capacities. WGPs are used widely in the food industry, including flour products, beverages, meat products, food packaging and health foods. Further studies should focus on analyzing the extraction of specific peptide fragments complemented with clinical evaluation of the extracted WGPs to maximize their use and service for human health benefits.

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CRediT authorship contribution statement

Zhi-hui Zhang: Conceptualization, Investigation, Software, Formal analysis, Visualization, Writing – original draft, Preparation. **Wei-long Cheng:** Software, Formal analysis, Visualization, Writing – review & editing. **Xiu-de Li:** Software, Visualization, Writing – review & editing. **Xin Wang:** Methodology, Resources, Writing – review & editing. **Fang-wei Yang:** Conceptualization, Methodology, Formal analysis, Writing – review & editing. **Jun-song Xiao:** Methodology, Resources, Validation, Writing – review & editing, Supervision. **Yi-xuan Li:** Conceptualization, Methodology, Formal analysis, Supervision, Writing – review & editing. **Guo-ping Zhao:** Project administration, Supervision, Funding acquisition, Methodology, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial

Table 1
Biological activities of WGPs reported in recent years.

Samples	Experimental model	Experimental design	Main effects	References
Antioxidant related actions				
wheat embryo globulin	Kungming male mice (6 w, 18–22 g)	60 mg/kg BW/d; 30 d	decreased levels of serum total cholesterol, and triglyceride; decreased liver malondialdehyde as well as the mRNA expression of CYP2E1; increased levels of serum high-density lipoprotein-cholesterol, reduced hepatic GSH, SOD and the mRNA expression of ADH2 and ALDH2	Yang et al. (2021)
fermented wheat germ	BALB/c mice (6 w)	20 mg/kg BW/d; 6 w	delayed aging with enhanced learning and memory function; decreased serum amount of total cholesterol, triglycerides, and glucose; reduced oxidative damage and increased levels of antioxidant enzymes, decreased malondialdehyde levels in different tissues; increased ratio of *Bacteroidetes/Firmicutes* as well as the number of beneficial bacteria	Zhao et al., 2021
wheat embryo globulin nutrient	Sprague-Dawley male rats (180–200 g)	300, 600, 900 mg/kg BW/d, 35 d	protect from cognitive impairment, neuronal damage, oxidative stress, and maintain choline function; decreased malondialdehyde levels and choline acetyltransferase activities; increased GSH, SOD, choline acetyltransferase activities, and choline acetylcholine content	Zheng et al. (2021)
wheat embryo albumin	male mice (6–8 w, 18–20 g)	25, 50, 75 mg/kg BW/d; 25 d	prolonged swimming time; reduced the accumulation of lactate dehydrogenase, blood urea nitrogen, and creatine kinase; increased glycogen storage in liver and muscle; enhanced the activities of SOD and malondialdehyde; decreased the level of malondialdehyde; enhanced the mRNA expression of mitochondrial biogenesis factors	Liu et al., 2022
peptide ADWGGPLPH	C57BL/6 male mice (6 w)	4 mg/kg BW/d; 1 w	enhanced antioxidant abilities and attenuated inflammatory cytokine generation in streptozotocin-induced diabetic mice	Wang et al. (2020)
peptide ADWGGPLPH	Sprague-Dawley female rats (9 m, 280–320 g)	1.5, 6.0 mg/kg BW/d; 12 m	reduced the oxidative stress levels and improved the microstructure and bone mineral density; improved proliferation and differentiation activity of osteoblasts	Wang et al., 2022
wheat embryo globulin	BALB/c male mice (18–22 g)	20 μg/kg•BW/d with 0.1, 0.2, 0.3 mL; 6 w	improved percentage of the platform quadrant, increased total antioxidant capacity, SOD, GSH-Px activities, and reduced malondialdehyde content in the liver and brains of aging mice	Liaoa et al., 2023
peptide fragments isolated from wheat germ albumin	Vascular smooth muscle cells	0.35 mg/mL; 15 d	prevented high glucose-induced cell growth; decreased generation of intracellular ROS; suppressed phosphorylation of PKCζ, AKT and Erk1/2; inhibited Nox4 protein expression	Chen et al. (2017)
wheat germ peptides prepared with subcritical water	Caco-2 Cells	0.125, 0.25, 0.5, 0.75, 1.0, 2.0 mg/mL; 24 h	high antioxidant activity; scavenged DPPH free radical possibly due to three small peptides with hydrophobic amino acids (GPF, GPE, FGE)	Zhang et al. (2019)
wheat germ peptides	Caco-2 cells	100 μM; 3 h	enhancd antioxidant level of cells; increased CAT, GR, GSH-Px, and GSH/oxidized GSH levels	Wang et al., 2022
Immunomodulatory and microbioprotective related actions				
wheat germ extracted with citric acid	peritoneal macrophages separated from Balb/c male mice	0, 100, 200, 400, 800, 1500, 3000 μg/mL; 24 h	inhibited secretion of pro-inflammatory cytokines TNF-α, IL-6, and IL-12 and inhibited synthesis of cyclooxygenase-2, increased levels of anti-inflammatory IL-10 and heme oxygenase-1; inhibited phosphorylation of NF-κB p65 and p38 kinase	Jeong et al. (2017)
wheat germ globulin	BALB/c male mice (6–8 w, 18–22 g)	10, 20, 40 mg/kg BW/d; 10 d	reduced immunosuppression in the spleen and thymus indexes; mitigated the damage caused by cyclophosphamide in the spleen and thymus; increased ratio of $CD4^+/CD8^+$ in the immunosuppressed mice, restored Th1/Th2 imbalance, enhanced contents of IL-2 and IL-4; reduced mRNA expression of T-Bet and GATA-3	Ji et al. (2017)
wheat germ	C57BL/6 male mice (6 w)	10% wheat germ-contained diet; 12 w	increased gene expression of the anti-inflammatory cytokine IL-10; reduced serum concentrations of the pro-inflammatory cytokines IL-1β, IL-6, IL-γ, and TNF-α selectively increased gut *Lactobacillaceae*, upregulated ileal antimicrobial peptides	Qjo et al., 2019
wheat germ protein hydrolysates	C57 bl/6 male mice (6–8 w, 18–24 g)	300, 600 mg/kg BW/d; 7 d	alleviate DSS-induced imbalance of flora and reduce the symptoms of inflammatory diseases; increased colon length; improved intestinal flora diversity; significantly differenced abundance of *Bacteroidetes*, *Proteobacteria*, and *Firmicutes* compared with those in DSS-induced group	Zhao et al., 2021
defatted wheat germ globulin	RAW 264.7 cells	5, 20, 80 μg/mL; 24 h	immunomodulatory activity with increased lymphocyte proliferation; phagocytosis of neutral red and secretion of pro-inflammatory cytokines	Wu et al. (2016)
glutelin	RAW 264.7 cells	50, 200, 800 mg/mL	regulated production of IL-6, TNF-α and IL-10; stimulated proliferation of splenocytes; increased IL-2 production, phagocytosis, and nitric oxide secretion	Wu et al., 2017
peptide ECFSTA	RAW 264.7 cells	10, 20, 40, 80, 160 μg/mL; 24 h	enhanced phagocytosis of cells; increased secretion of NO, IL-6, TNF-α, and ROS; activated TLR2 and TLR4	Wu et al., 2017
Others actions				
T24 (PVLGPVRGPFPLL)	C57BL/6J male mice (8 w)	30 mg/kg BW/d; 7 d	prevented tight junction protein loss, maintained epithelial barrier integrity, and promoted cell proliferation during by regulating mitogen-activated protein kinase signaling pathways	Dang et al., 2022
wheat germ peptides	ejunums from adult Sprague−Dawley rats	1, 2 mg/mL; 1.5 h	identified α-glucosidase inhibition activities, exhibiting an IC50 value of 6.87 mg/mL	Liu et al. (2021)

(continued on next page)

Table 1 (*continued*)

Samples	Experimental model	Experimental design	Main effects	References
aqueous extract of fermented wheat germ with *Lactobacillus plantarum* DY-1	HT-29 cells	0.2, 0.4, 0.8 mg/mL; 24, 48, 72 h	high antiproliferative effects; attenuated the progression from the G0−G1 to the G2−M phase of the cell cycle; promoted the activation of caspase-3	Zhang et al. (2015)
wheat germ protein hydrolysates	Human gastric epithelial cells	10 mg/mL; 90 min	inhibited *H. pylori* adhesion to gastric epithelial cells;	Sun et al. (2020)

Abbreviations: GSH, glutathione; SOD, superoxide dismutase; GSH-Px, glutathione peroxidase; ROS, reactive oxygen species; PKCζ, anti-phospho-protein kinase ζ; AKT, protein kinase B; Erk 1/2, extracellular regulated protein kinases 1/2; Nox4, nicotinamide adenine dinucleotide phosphate oxidase 4; DPPH, 1, 1-diphenyl-2-picryhydrazyl; CAT, catalase; GR, glutathione reductases; TNF-α, tumor necrosis factor-alpha; IL-6, interleukin-6; IL-12, interleukin-12; IL-10, interleukin-10; NF-κB p65, nuclear factor-κB p65; IL-2, interleukin-2; IL-4, interleukin-4; IL-1β, interleukin-1β; IL-γ, interleukin-γ; NO, nitric oxide; TLR2, toll-like receptor 2; TLR4, toll-like receptor 4.

interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

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

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