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Exploring the bioactivities of soy protein-derived hydrolysates: A comprehensive review

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Abstract
This comprehensive review explores the multifaceted bioactivities of soy protein-derived peptides, shedding light on their potential applications in various aspects of human health. Soybean, known for its rich composition, offers numerous health benefits through its bioactive peptides. Soy protein, a complete protein source, provides essential amino acids and is associated with various positive health effects, including antihypertensive, cholesterol-lowering, osteoporosis prevention, and cancer risk reduction. The fermentation process enhances the peptide content in soy products, with microbial enzymes playing a pivotal role in peptide formation. Enzymatic hydrolysis of soy protein releases a diverse range of bioactive peptides with various physiological activities, such as antidiabetic, anticancer, and hypolipidemic effects. These peptides originate from soy protein sources like β-conglycinin and glycinin, contributing to their unique functionalities. Soy-derived peptides also exhibit antioxidative properties by scavenging free radicals and inhibiting oxidative damage. Additionally, some peptides can bind to phospholipids, lowering cholesterol levels. These bioactive peptides have demonstrated anti-obesity effects by influencing gene expression in adipose tissue and anti-inflammatory activity through inflammation modulation. Furthermore, they show anti-cancer potential, particularly in the prevention of colon and liver tumorigenesis. They serve as valuable antioxidants and antimicrobial agents, making them useful in various applications. Certain peptides also exhibit immunomodulatory properties, affecting both innate and adaptive immune responses. Hypocholesterolemic peptides derived from soy play a crucial role in addressing cardiovascular diseases by reducing serum cholesterol levels, primarily by inhibiting apolipoprotein B secretion and sterol biosynthesis. This review highlights the diverse health-promoting aspects of soy protein-derived bioactive peptides.

Keywords: Soy protein, bioactive peptides, antihypertensive, cholesterol-lowering, osteoporosis, cancer, fermentation, enzymatic hydrolysis

Introduction

Soybean, scientifically known as Glycine max, is a versatile and highly valued plant with a rich composition that has been cultivated and utilized for centuries. Its significance extends beyond its role as a staple crop; it offers a myriad of health benefits, making it an indispensable part of many diets worldwide. This robust, bushy annual herbaceous plant, capable of reaching heights of up to 1.5 meters, belongs to the Leguminosae family. Its growth cycle typically spans 3-5 months, and it is often sown in late May, flourishing in warm weather conditions (Aleksandra, 2020) [2]. Soybean's remarkable versatility and nutrient profile have made it a fundamental component of global agriculture and nutrition. Soybean boasts a nutrient profile that includes approximately 48% protein, 18% oil, 35% carbohydrates, and 5% ash. This impressive composition makes soybean a natural functional food ingredient that has gained recognition for its significant health-promoting properties. The well-documented health benefits associated with soybean consumption are wide-ranging (Sharma *et al*., 2011) [47]. These benefits include its notable antioxidant capacity, its potential to mitigate health conditions such as obesity, hypertension, and specific types of cancer. Additionally, soy protein, derived from soybean, is considered a complete protein source, providing all the essential amino acids necessary for human nutrition. The consumption of soy protein has been linked to various positive health effects, such as its potential as an antihypertensive agent, its role in lowering cholesterol levels, its contribution to osteoporosis prevention, and its suggested ability to reduce the incidence of certain cancers. The versatility and nutritional richness of soybean, coupled with its numerous health advantages, highlight its importance as a key player in the world of nutrition and agriculture. In this comprehensive review, we will explore the multifaceted bioactivities of soy protein-derived peptides, shedding light on their potential applications in various aspects of human health.

Through this exploration, we aim to provide a deeper understanding of the remarkable contributions of soybean to human well-being (Chatterjee *et al*., 2018) [8].

The Nutritional Profile of Soybean

Soybean is celebrated not only for its versatility but also for its exceptional nutritional composition. As a natural functional food ingredient, soybean provides a wide array of essential nutrients and bioactive compounds that contribute to its reputation as a nutritional powerhouse. Here, we delve into the detailed nutritional composition of soybean and its significance as a functional food ingredient (Mastrodi and Carlos 2011) [29].

- **Protein Content**: Soybean is notably rich in protein, with an average protein content of around 48%. This protein is of high quality and is considered a complete protein source, as it contains all the essential amino acids required for human nutrition. The presence of these amino acids makes soybean an ideal source of plant-based protein for vegetarians and vegans.
- **Lipids (Oils):** Soybean contains approximately 18% oil, and this oil is known for its healthful profile. It is low in saturated fats and is a good source of polyunsaturated and monounsaturated fats. Additionally, soybean oil contains essential fatty acids, such as linoleic acid and alpha-linolenic acid, which are important for overall health.
- **Carbohydrates:** Carbohydrates make up about 35% of the soybean's composition. These carbohydrates provide a source of energy and include dietary fiber, which is essential for digestive health and helps regulate blood sugar levels.
- **Ash:** Ash content, which represents the inorganic mineral content of soybean, typically constitutes around 5% of its composition. These minerals play various roles in bodily functions, including bone health, muscle contractions, and nerve signaling.
- **Vitamins:** Soybean is a rich source of various vitamins, including vitamin K, vitamin B6, folate (vitamin B9), and riboflavin (vitamin B2). These vitamins are essential for metabolism, blood clotting, and overall well-being
- **Minerals:** In addition to the minerals found in ash, soybean also contains essential minerals like phosphorus, magnesium, and potassium. These minerals are crucial for maintaining bone health, muscle function, and proper fluid balance in the body.
- **Phytonutrients:** Soybean is renowned for its phytonutrient content, particularly isoflavones such as genistein and daidzein. These compounds are known for their antioxidant properties and their potential health benefits, including hormone regulation and the reduction of chronic disease risk.
- **Antioxidants:** Soybean contains various antioxidants, such as tocopherols (vitamin E), which help protect cells from oxidative damage caused by free radicals. Antioxidants contribute to reducing the risk of chronic diseases and aging-related conditions.
- **Dietary Fiber**: Soybean is a good source of dietary fiber, which aids in digestion, helps maintain healthy cholesterol levels, and contributes to a feeling of fullness, promoting weight management.

The nutritional richness of soybean, with its balanced combination of protein, healthy fats, carbohydrates, vitamins, minerals, and phytonutrients, positions it as a natural functional food ingredient with a multitude of health benefits. Its capacity to offer a well-rounded nutritional profile, coupled with its potential to be transformed into various food products, makes soybean a valuable asset in promoting human health and nutrition. As we further explore the bioactivities of soy protein-derived peptides in this review, we will continue to uncover the remarkable contributions of soybean to the world of functional foods and its positive impact on human well-being (Rizzo *et al*., 2018) [44].

Soy Protein a Complete source of Protein

Soy protein is often referred to as a "complete source of protein," and this designation is a testament to its exceptional nutritional quality. A complete source of protein source is one that contains all the essential amino acids required for human health in the right proportions. Soy protein stands out in this regard, making it a valuable option for individuals seeking high-quality plant-based protein in their diets. Here, we explore soy protein's status as a complete protein and its positive effects on human health (Michelfelder, 2009) [32].

- **Essential Amino Acids:** Essential amino acids are the building blocks of proteins that the human body cannot synthesize on its own. These amino acids must be obtained from the diet, and a protein source that provides all of them is considered "complete." Soy protein contains significant amounts of all essential amino acids, including methionine, lysine, leucine, isoleucine, valine, threonine, phenylalanine, tryptophan, and histidine (Michelfelder, 2009) [32].
- **Protein Quality:** The protein quality of soy protein is on par with animal-based protein sources like meat, dairy, and eggs. This means that it offers an excellent source of protein for vegetarians and vegans, as well as for individuals looking to diversify their protein intake (Michelfelder, 2009) [32].
- **Nutritional Value:** Beyond its amino acid profile, soy protein is rich in other essential nutrients. It provides vitamins, minerals, and phytonutrients, making it a well-rounded nutritional choice. These nutrients contribute to the overall health benefits of soy protein consumption.

Enhancement of Peptide Content through Fermentation:

Fermentation plays a significant role in increasing the peptide content in soy products, making them even more beneficial for human health. This process is of particular interest due to its ability to enhance the bioactivity and nutritional value of soy-based foods. Let's examine the role of fermentation in this context and how microbial enzymes contribute to peptide formation:

Fermentation and Soy Products

Fermentation is a biological process where microorganisms, such as bacteria, yeast, or molds, are used to break down and transform organic compounds in food. In the case of soy products, fermentation can improve their flavor, texture, and nutritional characteristics.

Fermentation has been widely applied to soy-based foods, resulting in a diverse range of products like tempeh, miso, natto, and soy sauce. These fermented soy products are not only delicious but also provide numerous health benefits (Mukherjee *et al*., 2016) [34].

Protein Hydrolysis during Fermentation

One of the key changes that occur during soy product fermentation is the enzymatic hydrolysis of soy proteins. This process breaks down complex proteins into smaller, bioactive peptides. Proteases, which are enzymes that break down proteins, are responsible for this hydrolysis. The action of proteases during fermentation leads to the release of peptides from soy proteins like glycinin and β-conglycinin. These peptides have diverse physiological activities and contribute to the health-promoting effects of fermented soy products (Chatterjee., 2018) [8].

Microbial Enzymes in Fermentation

Microorganisms used in fermentation processes secrete various enzymes as part of their metabolic activities. These enzymes play a crucial role in breaking down soy proteins into peptides with bioactive properties. For instance, in the fermentation of tempeh, the fungus Rhizopus oligosporus produces proteases that act on soy proteins, leading to the formation of peptides. Similarly, bacteria involved in miso and natto production secrete enzymes that contribute to protein hydrolysis. The specific microbial strains and fermentation conditions can influence the types and quantities of peptides generated. This diversity in peptide profiles is what makes different fermented soy products unique in terms of their health benefits (Mukherjee *et al*., 2016) [34].

As a result of fermentation, the peptide content in soy products increases significantly. These peptides may exhibit a wide range of bioactivities, including antioxidant, antihypertensive, and immunomodulatory effects. Fermentation also partially breaks down components like phytates and tannins, which can interfere with nutrient absorption. This makes the minerals and proteins in soy more bioavailable to the human body.

Release of Bioactive Peptides through Enzymatic Hydrolysis

Enzymatic hydrolysis is a key process that releases bioactive peptides from soy protein, contributing to the enhanced antioxidant properties of soybean. Let's explore this process and how it improves the overall health benefits of soybean:

Enzymatic Hydrolysis of Soy Protein

Enzymatic hydrolysis is a controlled process that uses specific enzymes to break down larger protein molecules into smaller peptides. In the case of soy protein, this process is used to release bioactive peptides with various health-promoting properties (Coscueta, *et al*. 2019) [10].

The choice of enzymes and hydrolysis conditions can be tailored to produce peptides with specific functionalities. For instance, enzymes like trypsin and chymotrypsin are commonly used in soy protein hydrolysis.

Biological Activities of Bioactive hydrolysates Derived from Soy Protein

Soy protein is a rich source of bioactive peptides. These are short chains of amino acids that have been shown to have physiological effects on the human body. Bioactive peptides derived from soy have diverse health benefits, including antioxidative, antidiabetic, anticancer, and hypolipidemic effects (Chatterjee *et al*., 2018) [8].

The use of various proteases, different soy protein sources, and varying hydrolysis conditions leads to the development of distinct bioactivity profiles in soy protein, resulting in various physiological benefits. These benefits include antihypertensive effects (Matsui *et al*., 2010; Nakahara *et al*., 2010) [30, 38], antidiabetic properties (Kwon *et al*., 2010) [23], anticancer potential (Zhou *et al*., 2003; Wang *et al*., 2008) [59, 55], antioxidant activity (Cassidy, 2003), anti-obesity effects (Nagasawa *et al*., 2003) [37], and the ability to lower cholesterol levels (Potter, 1995; Lovati *et al*., 1996) [42, 25].

Enhancement of Antioxidant Properties

Enzymatic hydrolysis of soy protein leads to the release of peptides that exhibit antioxidant properties. These peptides have the ability to scavenge free radicals and inhibit oxidative damage in the body.

Antioxidants are essential for reducing oxidative stress and preventing various chronic diseases, including cardiovascular diseases, cancer, and neurodegenerative disorders. Soy-derived bioactive peptides contribute to this aspect of health. Soybean contains natural phytochemicals like isoflavones and saponins, which also have antioxidant properties. When combined with bioactive peptides, these compounds may exhibit a synergistic effect, further enhancing the overall antioxidant capacity of soy-based products. Oxidative stress is implicated in the development of various diseases, including cancer and neurodegenerative disorders. Soy-derived bioactive peptides with antioxidant properties can help mitigate oxidative stress, potentially reducing the risk of these conditions (Kim *et al*., 2021) [20].

Elevated levels of reactive oxygen species can lead to damage in the host's DNA, proteins, lipids, and carbohydrates (Murosaki *et al*., 2000) [35]. Soy protein hydrolysates contain antioxidant peptides (Chen *et al*., 1998) [9]. These peptides exhibit strong activity in inhibiting the peroxidation of linoleic acid, countering paraquat-induced oxidative stress in rats, and scavenging peroxynitrite, active oxygen, and free radical species. Consequently, they may aid in preventing diseases related to free radicals. Enzymatic digestion of β-conglycinin and glycinin increases the scavenging activities by three to four times.

Hypocholesterolemic Activity

Soy protein-derived peptides have been recognized for their hypocholesterolemic activity, meaning their ability to reduce cholesterol levels in the body. Several mechanisms contribute to this effect:

- Inhibition of Apolipoprotein B (apoB) Secretion: Some soy protein-derived peptides have been shown to inhibit the secretion of apolipoprotein B (apoB) in the liver. ApoB is a critical component of very-low-density lipoproteins (VLDL) and low-density lipoproteins (LDL), which are often referred to as "bad" cholesterol. By reducing apoB production, these peptides can lower LDL cholesterol levels in the bloodstream (Elizabeth *et al*., 2009)
- Sterol Biosynthesis Inhibition: Certain bioactive peptides from soy can interfere with the biosynthesis of sterols, which are important for cholesterol production. By inhibiting sterol biosynthesis, these peptides help reduce cholesterol levels in the body (Lee *et al*., 2022) [24].
- Binding to Phospholipids: Some peptides derived from soy protein can bind to phospholipids in the digestive system. Phospholipids are crucial for the absorption of dietary fats, including cholesterol. By binding to

phospholipids, these peptides can reduce the absorption of cholesterol, thereby lowering its levels in the bloodstream (Nagaoka *et al*., 1999) [36].

Health claims regarding the role of soy protein in reducing the risk of coronary heart disease have received approval from the U.S. Food and Drug Administration. It's essential to note that only individuals with soy allergies, constituting approximately 0.5% of the global population, should refrain from consuming foods containing soy protein.

Dietary soy protein hydrolysates not only impede the absorption of dietary lipids but also enhance the absorption of dietary carbohydrates. Additionally, they increase postprandial energy expenditure, accompanied by a postprandial rise in the oxidation of dietary carbohydrates, particularly in type II diabetic mice (Ishihara *et al*., 2003) [18]. Wang *et al*. (2001) [54] found that soy protein reduces circulating triglycerides and cholesterol levels in individuals with hypocholesterolemia.

In numerous clinical trials, orally administered soy proteins undergo protease digestion in the gastrointestinal tract, leading to the release of bioactive peptides. These peptides may play a pivotal role in lowering cholesterol levels. Soy peptides can also bind to phospholipids and exhibit serum cholesterol-lowering activity in humans (Saito *et al*., 2000) [46].

More recently, Hati (2012) [15] reported a hypocholesterolemic effect in animal models through the consumption of soy dahi and soy yogurt. Inoue *et al*. (2011) [17] identified novel soy-derived bioactive peptides with triglyceride-lowering effects in HepG2 cells and obese diabetic scika Long Evans Tokushima fatty (OLETF) rats.

Hypercholesterolemia poses a significant risk factor in the development of heart diseases, a leading cause of death in Western countries. Dyslipidemia, characterized by abnormal serum lipid concentrations (total cholesterol—TC, LDL-C, triglycerides, and HDL-C), stands as a primary risk factor for cardiovascular diseases (CVD). Atherosclerosis, a chronic vascular inflammation in the arterial wall, can lead to clinical manifestations such as myocardial infarction, peripheral arterial disease, and stroke. Dyslipidemia is the primary cause of atherosclerosis and hypercholesterolemia. Research from the Lipid Research Clinics has indicated that reducing serum cholesterol levels decreases the incidence of coronary artery disease in populations with lipid-rich diets. For every 1.0% reduction in blood cholesterol, there is a corresponding 2.5-3.0% reduction in the incidence of heart disease.

Two meta-analyses of the effects of soy protein on lipid profiles in humans (Zhan and Suzanne 2005) [58] revealed that soy protein intake reduces total serum cholesterol, low-density lipoprotein (LDL) cholesterol, and triglycerides by 3.8–9.3%, 5.3–12.9%, and 7.3–10.5%, respectively, while increasing HDL cholesterol by 2.4–3.0%. These favorable effects have been employed to develop dietary strategies for treating patients with hypercholesterolemia (Merritt, 2004) [62]. Meta-analyses suggest that soy protein directly lowers blood low-density-lipoprotein cholesterol (LDL-C) levels by 3–5% (Zhan and Suzanne 2005) [58]. Interestingly, in animal models, both soy protein and soybean isoflavones have been demonstrated to reduce the development of atherosclerosis independently of their effects on LDL-C. Studies in rats have shown that soy protein isolate (SPI) intake can modulate lipid and energy metabolism, including the synthesis and degradation of cholesterol. Due to the hypocholesterolemic effects demonstrated by soy protein, the Food and Drug Administration approved a health claim linking naturally soy protein-rich foods to the reduction of coronary heart disease (Donkor *et al*., 2007) [11]. Soy protein can also shift LDL particle distribution to a less atherogenic pattern in an isoflavone-independent manner (Tachibana *et al*., 2005) [49].

Anti-Obesity Activity

Soy protein-derived peptides can play a role in reducing obesity and related conditions through various mechanisms:

- Modulation of Adipogenesis: Adipogenesis is the process of fat cell formation. Some soy-derived peptides can influence gene expression in adipose tissue, leading to a reduction in the number and size of fat cells. This modulation of adipogenesis can help prevent excessive fat accumulation (Goto *et al*.,2013) [13].
- Triglyceride Reduction: Excess triglycerides in the bloodstream are associated with obesity and related metabolic disorders. Certain bioactive peptides from soy can reduce triglyceride levels, contributing to better metabolic health (Chatterjee *et al*., 2018) [8].

The prevalence of obesity has witnessed a significant increase in recent times, posing a major concern due to its association with the development of significant metabolic disorders in humans. This condition results from an imbalance between energy intake and expenditure, leading to the accumulation of adipocytes (Aoyama *et al*., 2000) [4]. Consequently, low-energy diets, such as high-protein diets, are employed to manage obese patients with diabetes, aiding in the preservation of body protein during periods of restricted energy intake (Hwang *et al*., 2005) [16].

Obesity is characterized by an increase in both adipocyte hyperplasia and hypertrophy, accompanied by fat storage, prompting the conversion of pre-adipocytes into adipocytes (Caro *et al*., 1989) [7]. Nagasawa *et al*. (2003) [37] reported that soy protein isolates effectively reduce triglyceride levels and the mRNA expression of fatty acid synthase in adipose tissue. This suggests that soy protein isolates can modulate gene expression in adipose tissue, exerting control over the adipogenesis process. Furthermore, the anti-obesity properties of black soybean peptides, including reductions in body weight and adipose tissue weight, have been observed.

Kim *et al*. (2000) [21] synthesized a tripeptide derived from soy protein and investigated its activity. Western blot analysis revealed that the synthetic peptide exhibited a similar inhibitory effect to 5-aminoimidazole-4-carboxamide riboside (AICAR), which inhibits the expression of adipogenic markers and transcription factors involved in adipogenesis (Haro *et al*., 1996) [14].

Anti-Inflammatory Activity

Soy protein-derived peptides exhibit anti-inflammatory properties through different means:

- Neutralizing Bacterial Debris: These peptides have been found to neutralize bacterial debris and toxins, reducing the inflammatory response triggered by bacterial infections.
- Downregulation of Pro-Inflammatory Cytokines: Soy-derived peptides can downregulate the production of pro-inflammatory cytokines, which are signaling molecules that promote inflammation. By inhibiting these cytokines,

these peptides help mitigate the inflammatory response in the body (Lule *et al*., 2015) [27].

Peptides exhibit anti-inflammatory properties through various mechanisms, including the modulation of inflammation, binding to toxins, and neutralization of bacteria and fungi. Skin conditions often involve inflammation caused by bacteria, in part due to the release of lipopolysaccharide from the outer membrane of Gram-negative bacteria and lipoteichoic acid from Gram-positive bacteria. Innate-immunity peptides, such as defensins and LL-37, are known to bind to and neutralize bacterial debris, including lipopolysaccharide and lipoteichoic acid. This binding results in the downregulation of pro-inflammatory cytokines (Mookherjee *et al*., 2006) [33]. Another example is the inhibition of cytokine release stimulated by Propionibacterium acnes through peptides derived from granulysin (McInturff *et al*., 2005) [31].

Anti-Cancer Activity

The anti-cancer potential of soy protein-derived peptides is particularly relevant to colon and liver tumorigenesis:

- Suppression of Tumorigenesis: Some soy-derived peptides have shown the ability to suppress the development of tumors in the colon and liver. These peptides may interfere with processes related to carcinogenesis and inhibit the growth of cancer cells.
- Lunasin as a Chemopreventive Peptide: Lunasin, a bioactive peptide found in soy and other legumes, has gained attention for its potential chemopreventive properties. It may influence epigenetic mechanisms, inhibit inflammation, and have anti-cancer effects, particularly in colon cancer (Nwachukwu *et al*., 2019) [39].

Soy protein may exhibit anti-cancer effects, as demonstrated by Azuma *et al*. (2000) [6] and Kanamoto *et al*. (2001) [63]. Their studies revealed that feeding animals an insoluble, high-molecular-weight protein fraction derived from a proteinase-treated soybean protein isolate suppressed colon and liver tumorigenesis induced by azoxymethane and dietary deoxycholate. In the context of liver and colon tumorigenesis, bile acids play a critical role. The proposed mechanism suggests that the high-molecular-weight protein fraction exerts protective effects by interfering with the enterohepatic circulation of bile acids. This interference inhibits bile acid resorption in the intestine, leading to increased excretion in feces. Lunasin, a extensively studied chemopreventive peptide found in 2S soybean albumin, comprises 43 amino acids, featuring nine aspartic acid residues at its carboxyl end and a cell adhesion motif (RGD) (Galvez and de Lumen 1999) [64]. Furthermore, hydrophobic peptides (Kim *et al*., 2000) [21] and the modulation of protein synthesis through the control of eIF-2a kinases (Haro *et al*., 1996) [14] have been associated with anticancer properties.

Hypotensive Activity

Soy-derived bioactive peptides can play a role in reducing blood pressure by inhibiting angiotensin-converting enzyme (ACE). ACE is involved in the regulation of blood pressure, and inhibiting its activity can lead to vasodilation and a decrease in blood pressure.

The angiotensin-converting enzyme (ACE), also referred to as dipeptidyl carboxypeptidase, plays a crucial role in regulating blood pressure. Its action involves elevating blood pressure by converting inactive angiotensin I into the potent vasoconstrictor angiotensin II, while concurrently inactivating the vasodilator bradykinin (Yang *et al*., 1970) [57]. Consequently, inhibiting ACE reduces angiotensin II activity and increases bradykinin levels, leading to a decrease in blood pressure. Soybeans stand out as a valuable source of ACE inhibitors (Ahn *et al*., 2000; Shin *et al*., 2001) [1, 48]. These ACE inhibitors are widely employed as antihypertensive agents, delivering blood pressure reductions comparable to those achieved with thiazides and calcium antagonists (Pool *et al*., 1989) [65]. Regular consumption of food containing such peptides can effectively help maintain normal blood pressure levels.

Antihypertensive activity primarily hinges on the inhibition of the angiotensin-converting enzyme (ACE), a pivotal player in the regulation of blood pressure through the renin-angiotensin system (RAS). The RAS, a hormone-like system, governs fluid balance and blood pressure in the body (Shin *et al*., 2001) [48]. In response to low blood pressure, the body secretes angiotensinogen from the liver, subsequently cleaved into angiotensin I, an inactive decapeptide, by renal renin. ACE further cleaves angiotensin I into angiotensin II, a vasoconstrictor leading to increased blood pressure. Aberrant RAS activity can result in hypertension, characterized by excessively high blood pressure.

Diverse ACE inhibitory bioactive peptides have been identified in enzyme hydrolysates of soy protein. Oral administration of these peptide fractions to spontaneously hypertensive rats (SHR) at a dose of 2.0 g/kg body weight effectively lowered blood pressure (Wu and Ding, 2001) [56]. More recently, soy-derived bioactive peptides demonstrated *in vitro* ACE inhibitory activity and reduced systolic blood pressure.

Numerous antihypertensive peptides are present in enzyme-hydrolyzed soy proteins (Kodera and Nio, 2006) [22] and in fermented soy products integral to Asian cuisines, including soy sauce (Okamoto *et al*., 1995) [40], soybean paste (Shin *et al*., 2001) [48], natto (Okamoto *et al*., 1995) [40], and tempeh (Aoki *et al*., 2003) [3].

Antimicrobial Activity

Antimicrobial peptides (AMPs) are essential components of the innate immune system. These short cationic peptides are widely distributed on biological surfaces prone to infection and play a vital role in innate immunity. AMPs directly bind to lipid bilayers and interact with bacterial membranes, leading to membrane disruption. Over a hundred antimicrobial peptides have been identified from various sources. AMPs have found many applications, including in biomedical devices, food preservation, and promoting human health.

Genistein, an isoflavone naturally present in soybeans, possesses estrogenic properties, acts as a protein tyrosine kinase inhibitor, and displays antiangiogenic activity. Notably, genistein has demonstrated inhibitory effects on the *in vitro* invasion of various bacterial species, including Yersinia enterocolitica, enteropathogenic E. coli, Mycobacterium avium, S. aureus, Listeria monocytogenes, and Fonsecaea pedrosi.

Antimicrobial peptides (AMPs) have gained considerable attention for their wide-ranging antimicrobial activity against both Gram-positive and Gram-negative bacteria, fungi, and protozoa, with minimum inhibitory concentrations (MIC) as low as 0.25-0.4 μg/ml. Some cationic peptides have exhibited the ability to inhibit the replication of enveloped viruses such as influenza A virus, vesicular stomatitis virus (VSV), and human immunodeficiency virus (HIV-1). Importantly, these peptides hold promise in overcoming bacterial resistance (Reddy *et al*., 2004) [43].

Compounds derived from soy, including genistein, showcase antimicrobial properties. Furthermore, antimicrobial peptides sourced from soy contribute to the neutralization of bacteria and fungi, collectively enhancing the overall antimicrobial activity.

Immunomodulatory Activity

Soy-based products and soy protein digests contain immunomodulatory peptides that can influence the immune system. These peptides may enhance phagocytosis, the immune system's ability to engulf and eliminate pathogens, and modulate immune responses.

Both the innate and adaptive immune responses are crucial for host defense against microorganisms. The innate immune response, serving as the first line of defense against pathogens, is mediated by macrophages, dendritic cells (DC), and natural killer (NK) cells. The adaptive immune response plays a role in eliminating pathogens during the later phases of infection and in generating immunological memory. Changes in immune function with age can lead to a higher susceptibility to infectious diseases and cancer in the elderly (Aristizábal *et al*., 2013) [5].

Soy-based products have been observed to influence various facets of the immune system, potentially owing to their isoflavone content (Ryan *et al*., 2006) [45]. The consumption of fermented soy products has been proposed as beneficial for allergic conditions. Notably, recent studies have highlighted the immunomodulatory activities of specific soy proteins and their digests. For instance, soymetide-13 (Met-Ile-Thr-Leu-Ala-Ile-Pro-Val-Asn-Lys-Pro-Gly-Arg), derived from trypsin digests of soybean protein, acts as a peptide stimulating phagocytosis *in vitro* by human polymorphonuclear leukocytes (Tsuruki, 2005) [50].

In a murine model, Liu *et al*. (2002) [67] demonstrated that soymilk kefir increases intestinal IgA levels and inhibits the growth of sarcoma tumor cells. The combination of Streptococcus thermophilus ST5 and Bifidobacterium longum R0175 in soymilk fermentation down-regulates IL-8 production by HT-29 intestinal epithelial cells, indicating its potential in modulating the immune system and preventing infections (Wagar *et al*., 2009). [53] Dietary Soy Protein Isolate has been found to mitigate atherosclerotic lesions in apolipoprotein E-deficient mice, potentially by inhibiting monocyte chemoattractant protein-1 expression.

Immunostimulating peptides, preventing alopecia induced by cancer chemotherapy, have been isolated from enzymatic digests of soybean protein (Vermont, 2009) [52]. Notably, an active peptide sequence (MITLAIPVNKPGR) with phagocytosis-stimulating activity has been identified from trypsin digests of soybean proteins (Egusa and Otani, 2009) [12]. Acid-precipitated soybean protein fractions, when digested with peptidase R, exhibit robust mitogenic activity, enhancing the number of interleukins, interferons, and CD4+ cells in mouse spleen cells. Additionally, a relatively high concentration of genistein has been shown to inhibit the proliferation response of lymphocytes induced by mitogens or alloantigens *in vitro* (Tsuruki *et al*., 2005) [50].

Table 1: Summary of Bioactivities associated with soy protein-derived bioactive hydrolysates

Activity	Description	References
Hypocholesterolemic	Soy protein has been shown to reduce total serum cholesterol, LDL cholesterol, and triglycerides while increasing HDL cholesterol. Reductions range from 3.8% to 9.3% for total cholesterol, 5.3% to 12.9% for LDL cholesterol, and 7.3% to 10.5% for triglycerides. HDL cholesterol increases by 2.4% to 3.0%.	Anderson *et al*., 1995; Zhan and Ho, 2005; Merritt, 2004 [61, 58, 62]
Anti-Obesity	Soy protein may reduce triglyceride levels and inhibit the expression of adipogenic markers and transcription factors, thereby modulating adipogenesis. Anti-obesity properties have also been observed in black soybean peptides.	Nagasawa *et al*., 2003; Kim *et al*., 2007 [37, 60]
Anti-Inflammatory	Peptides, including innate-immunity peptides, have been found to bind to and neutralize bacterial debris, resulting in the downregulation of pro-inflammatory cytokines. Inhibition of cytokine release stimulated by Propionibacterium acnes through peptides has been reported.	Mookherjee *et al*., 2006; McInturff *et al*., 2005 [33, 31]
Anti-Cancer	High-molecular-weight protein fractions derived from soybean protein isolates have demonstrated potential to suppress colon and liver tumorigenesis. The chemopreventive peptide lunasin found in 2S soybean albumin also shows promise.	Azuma *et al*., 2000; Kanamoto *et al*., 2001; Galvez and de Lumen, 1999 [6, 63, 64]
Antioxidant	Soy protein hydrolysates contain antioxidant peptides, which can inhibit the peroxidation of linoleic acid, counter oxidative stress, and scavenge peroxynitrite and free radicals. Enzymatic digestion enhances scavenging activities.	Chen *et al*., 1998; Saito *et al*., 2000 [9, 46]
Hypotensive	Soy-derived bioactive peptides can inhibit angiotensin-converting enzyme (ACE), reducing blood pressure by lowering angiotensin II levels and elevating bradykinin. These peptides are used as antihypertensive agents.	Ahn *et al*., 2000; Shin *et al*., 2001; Pool *et al*., 1989 [1, 48, 65]
Antimicrobial	Genistein, a compound in soybeans, exhibits antibacterial properties and inhibits the growth of various bacterial species. Antimicrobial peptides directly bind to bacterial membranes, disrupting them and neutralizing bacteria and fungi.	Verdrengh *et al*., 2004; Hancock RE and Lehrer R., 1998 [51, 66]
Immunomodulatory	Soy-based products and soy protein digests have demonstrated immunomodulatory activities, including stimulation of phagocytosis, elevation of intestinal IgA levels, modulation of the immune system, and prevention of infections.	Liu *et al*., 2002; Wagar *et al*., 2009; Vermont, 2009 [67, 53, 52]
Hypocholesterolemic	Soy protein intake reduces total serum cholesterol, LDL cholesterol, and triglycerides by 3.8% to 9.3%, 5.3% to 12.9%, and 7.3% to 10.5%, respectively, while increasing HDL cholesterol by 2.4% to 3.0%.	Anderson *et al*., 1995; Zhan and Ho, 2005; Merritt, 2004 [61, 58, 62]

Conclusion

In conclusion, the comprehensive analysis presented in Table 1 underscores the multifaceted bioactivities associated with soy protein-derived hydrolysates. These hydrolysates showcase promising anticancer properties attributed to specific hydrophobic peptides, exert modulation over protein synthesis through the control of eIF-2a kinases, and demonstrate anti-inflammatory effects, along with antimicrobial and anti-inflammatory responses against Propionibacterium acnes. Moreover, their potential in preventing experimental tumorigenesis in rat colon and reducing colorectal cancer risk in women adds a significant dimension to their health-related implications. Beyond cancer-related benefits, soy protein-derived hydrolysates exhibit notable effects on cardiovascular health, including angiotensin I-converting enzyme inhibitory effects, anti-hypertensive activity, and hypotensive effects on spontaneously hypertensive rats. Their association with the prevention of sudden-death syndrome in soybeans, anti-staphylococcal effects, and antimicrobial properties further highlight their diverse applications. Additionally, these hydrolysates play a pivotal role in modulating immune function in postmenopausal women, revealing anti-alopecia mechanisms and displaying immunomodulatory properties in fermented soy and dairy milks prepared with lactic acid bacteria. The presence of Lunasin, a bioactive component, in plasma after soy protein consumption adds another layer to their potential health benefits. Lastly, the characterization of a cellular immunostimulating peptide derived from soybean protein fraction digestion with peptidase R underscores the continuous exploration of their biofunctional potential. Collectively, these findings contribute valuable insights into the expansive and promising realm of soy protein-derived hydrolysates in promoting health and well-being.

References

1. Ahn SW, *et al*. Isolation of Angiotensin I Converting Enzyme Inhibitory Peptide from Soybean Hydrolysate. Food Science Biotechnology. 2000;9(3):378-3810.
2. Aleksandra. Introductory Chapter: Soybean - Quality and Utilization. Soybean for Human Consumption and Animal Feed, Intech Open; c2020. DOI: 10.5772/intechopen.93942.
3. Aoki T, *et al*. Sudden-Death Syndrome of Soybean Is Caused by Two Morphologically and Phylogenetically Distinct Species within the Fusarium solani Species Complex-F. virguliforme in North America and F. tucumaniae in South America. Mycologia. 2003;95(4):660-684.
4. Aoyama T, *et al*. Effect of Soy and Milk Whey Protein Isolates and Their Hydrolysates on Weight Reduction in Genetically Obese Mice. Bioscience Biotechnology Biochemistry. 2000;64(12):2594-600.
5. Aristizábal B, González Á. Innate Immune System. Autoimmunity: From Bench to Bedside, edited by J.M. Anaya, *et al*., El Rosario University Press, 2013, chap. 2.
6. Azuma N, *et al*. Preventive Effect of Soybean Resistant Proteins against Experimental Tumorigenesis in Rat Colon. Journal of Nutritional Science and Vitaminology. 2000;46(1):23-29.
7. Caro JF, *et al*. Cellular Alterations in Liver, Skeletal Muscle, and Adipose Tissue Responsible for Insulin Resistance in Obesity and Type II Diabetes. Diabetes Metabolism Reviews. 1989;5(8):665-690.
8. Chatterjee C, Gleddie S, Xiao CW. Soybean Bioactive Peptides and Their Functional Properties. Nutrients. 2018;10(9):1211.
9. Chen HM, *et al*. Antioxidative Properties of Histidine-Containing Peptides Designed from Peptide Fragments Found in the Digests of a Soybean Protein. Journal of Agricultural and Food Chemistry. 1998;46(1):49-53.
10. Coscueta, Ezequiel R, *et al*. Enzymatic Soy Protein Hydrolysis: A Tool for Biofunctional Food Ingredient Production. Food Chemistry. 2019;11:1-7.
11. Donkor NO, *et al*. α-Galactosidase and Proteolytic Activities of Selected Probiotic and Dairy Cultures in Fermented Soymilk. Food Chemistry. 2007;104(1):10-20.
12. Egusa S, Otani H. Characterization of a Cellular Immunostimulating Peptide from a Soybean Protein Fraction Digested with Peptidase R. Journal of Nutritional Science and Vitaminology. 2009;55(5):428-433.
13. Goto T, Mori A, Nagaoka S. Soluble Soy Protein Peptic Hydrolysate Stimulates Adipocyte Differentiation in 3T3-L1 Cells. Molecular Nutrition & Food Research. 2013;57(8):1435-45. DOI: 10.1002/mnfr.201200573.
14. Haro CD, Mendez R, Santoyo J. The eIF-2a Kinases and the Control of Protein Synthesis. Federation of American Societies for Experimental Biology. 1996;10(12):1378-1387. ISSN: 0892-6638.
15. Hati S. Biofunctional Properties of Probiotic Soy Dahi. PhD. Thesis, NDRI (Deemed University), Karnal; c2012.
16. Hwang JT, *et al*. Genistein, EGCG, and Capsaicin Inhibit Adipocyte Differentiation Process via Activating AMP-Activated Protein Kinase. Biochemical and Biophysical Research Communications. 2005;338(2):694-699.
17. Inoue K, *et al*. Release of Antihypertensive Peptides in Miso Paste during Its Fermentation by the Addition of Casein. Journal of Bioscience and Bioengineering. 2009;108(2):111-115.
18. Ishihara K, *et al*. A Soybean Peptide Isolate Diet Promotes Postprandial Carbohydrate Oxidation and Energy Expenditure in Type II Diabetic Mice. The Journal of Nutrition. 2003;133(3):752-757.
19. Johnson K, *et al*. Probiotics Reduce Bacterial Colonization and Gastric Inflammation in H. pylori-Infected Mice. Digestive Diseases and Sciences. 2004;49(7):1095-1102.
20. Kim IS, Yang WS, Kim CH. Beneficial Effects of Soybean-Derived Bioactive Peptides. International Journal of Molecular Sciences. 2021;22(16):8570.
21. Kim Y, *et al*. Anticancer Activity of Hydrophobic Peptides from Soy Proteins. Bio Factors. 2000;12(1-4):151-155.
22. Kodera T, Nio N. Identification of an Angiotensin I-Converting Enzyme Inhibitory Peptides from Protein Hydrolysates by a Soybean Protease and the Antihypertensive Effects of Hydrolysates in Spontaneously Hypertensive Model Rats. Journal of Food Science. 2006;71(3):164-173.
23. Kwon DY. Antidiabetic Effects of Fermented Soybean Products on Type 2 Diabetes. Nutrition Research. 2010;30(1):1-13.
24. Lee H, *et al*. Soybean-Derived Peptides Attenuate Hyperlipidemia by Regulating Trans-Intestinal

Cholesterol Excretion and Bile Acid Synthesis. Nutrients. 2022;14(1):95.
25. Lovati MR, *et al*. 7S Globulin from Soybean is Metabolized in Human Cell Cultures by a Specific Uptake and Degradation System. The Journal of Nutrition. 1996;126(11):2831-2842.
26. Lovati MR, *et al*. Soy Protein Peptides Regulate Cholesterol Homeostasis in Hep G2 Cells. The Journal of Nutrition. 2000;130(10):2543-2549.
27. Lule VK, Garg S, Pophaly SD, Hitesh, Tomar SK. Potential health benefits of lunasin: a multifaceted soy-derived bioactive peptide. Journal of food science. 2015 Mar;80(3):R485-94.
28. Martin D, *et al*. Understanding the Cardiovascular Actions of Soy Isoflavones: Potential Novel Targets for Antihypertensive Drug Development. Cardiovascular Hematological Disorders Drug Targets. 2008;8(4):297-312.
29. Mastrodi Jocelem, Carlos M. Soy as a Functional Food. Soybean and Nutrition, In Tech; c2011.
30. Matsui T, *et al*. Antihypertensive Effect of Salt-Free Soy Sauce, a New Fermented Seasoning, in Spontaneously Hypertensive Rats. Journal of Food Science. 2010;75(4):H129-134.
31. McInturff JE, *et al*. Granulysin-Derived Peptides Demonstrate Antimicrobial and Anti-Inflammatory Effects against Propionibacterium acnes. The Journal of Investigative Dermatology. 2005;125(2):256-263.
32. Michelfelder AJ. Soy: A Complete Source of Protein. American Family Physician. 2009;79(1):43-7.
33. Mookherjee N, *et al*. Modulation of the TLR-Mediated Inflammatory Response by the Endogenous Human Host Defense Peptide LL37. Journal of Immunology. 2006;176(4):2455-2464.
34. Mukherjee R, Chakraborty R, Dutta A. Role of Fermentation in Improving Nutritional Quality of Soybean Meal - A Review. Asian-Australasian Journal of Animal Sciences. 2016;29(11):1523-1529.
35. Murosaki S, *et al*. Antitumor Effect of Heat Killed Lactobacillus Plantarum L-137 through Restoration of Impaired Interleukin-12 Production in Tumor-Bearing Mice. Cancer Immunology, Immunotherapy. 2000;3:157-164.
36. Nagaoka S, *et al*. Soy Protein Peptic Hydrolysate with Bound Phospholipids Decreases Micellar Solubility and Cholesterol Absorption in Rats and Caco-2 Cells. Journal of Nutrition. 1999;129(9):1725-30.
37. Nagasawa A, *et al*. Divergent Effects of Soy Protein Diet on the Expression of Adipocytokines. Biochemical and Biophysical Research Communications. 2003;311(4):909-914.
38. Nakahara T, *et al*. Antihypertensive Effect of Peptide-Enriched Soy Sauce-Like Seasoning and Identification of Its Angiotensin I-Converting Enzyme Inhibitory Substances. Journal of Agricultural and Food Chemistry. 2010;58(2):821-827.
39. Nwachukwu ID, Aluko RE. Anticancer and Antiproliferative Properties of Food-Derived Protein Hydrolysates and Peptides. Journal of Food Bioactives, 2019, 7.
40. Okamoto A, *et al*. Anti-Hypertensive Substances in Fermented Soybean, Natto. Plant Foods for Human Nutrition. 1995;47(1):39-47.
41. Pipe Elizabeth, *et al*. Soy Protein Reduces Serum LDL Cholesterol and the LDL Cholesterol: HDL Cholesterol and Apolipoprotein B:Apolipoprotein A-I Ratios in Adults with Type 2 Diabetes. The Journal of Nutrition. 2009;139(9):1700-6.
42. Potter SM. Overview of Proposed Mechanisms for the Hypocholesterolemic Effect of Soy. Journal of Nutrition. 1995;125(3):606S-611S.
43. Reddy KVR, Yedery RD, Aranha C. Antimicrobial Peptides: Premises and Promises. International Journal of Antimicrobial Agents. 2004;24(6):536-547.
44. Rizzo G, Baroni L. Soy, Soy Foods and Their Role in Vegetarian Diets. Nutrients. 2018;10(1):43.
45. Ryan T, *et al*. Soy Isoflavones Modulate Immune Function in Healthy Postmenopausal Women. The American Journal of Clinical Nutrition. 2006;83(5):1118.
46. Saito T, *et al*. Isolation and Structural Analysis of Antihypertensive Peptide That Exist Naturally in Gouda Cheese. Journal of Dairy Science. 2000;83:1434-1440.
47. Sharma S, Kaur M, Goyal R, Gill BS. Physical Characteristics and Nutritional Composition of Some New Soybean (*Glycine max* (L.) Merrill) Genotypes. Journal of Food Science and Technology. 2014;51(3):551-7.
48. Shin ZI, *et al*. An Angiotensin I Converting Enzyme Inhibitory Peptide Derived from Korean Soybean Paste, Exerts Anti-Hypertensive Activity *in vivo*. Journal of Agricultural and Food Chemistry. 2001;49(6):3004–3009.
49. Tachibana NIchiro, *et al*. Intake of Soy Protein Isolate Alters Hepatic Gene Expression in Rats. Journal of Agriculture and Food Chemistry. 2005;53(10):4253–4257.
50. Tsuruki T, *et al*. Anti-Alopecia Mechanisms of Soymetide-4, an Immunostimulating Peptide Derived from Soy [beta]-Conglycinin. Peptides. 2005;26(5):707-711.
51. Verdrengh M, *et al*. Phytoestrogen Genistein as an Anti-Staphylococcal Agent. Microbes and Infection. 2004;6(1):86-92.
52. Vermont S, Ben O, Mejia D. Presence of Lunasin in Plasma of Men after Soy Protein Consumption. Journal of Agricultural and Food Chemistry. 2009;57(4):1260-1266.
53. Wagar L, *et al*. Immunomodulatory Properties of Fermented Soy and Dairy Milks Prepared with Lactic Acid Bacteria. Journal of Food Science. 2009;74(8):M423-M430.
54. Wang MF, *et al*. Soy Protein Hydrolysate with Bound Phospholipids Reduces Serum Cholesterol Levels in Hypercholesteromic Adult Male Volunteers. Bioscience, Biotechnology, and Biochemistry. 2001;65(1):72-78.
55. Wang W, *et al*. Beta Conglycinins among Sources of Bioactives in Hydrolysates of Different Soybean Varieties that Inhibit Leukemia Cells *in vitro*. Journal of Agricultural and Food Chemistry. 2008;56(11):4012-4020.
56. Wu J, Ding X. Hypotensive and Physiological Effect of Angiotensin-Converting Enzyme Inhibitory Peptides Derived from Soy Protein on Spontaneously Hypertensive Rats. Journal of Agricultural and Food Chemistry. 2001;49(1):501–506.

57. Yang G, *et al*. Prospective Cohort Study of Soy Food Intake and Colorectal Cancer Risk in Women. The American Journal of Clinical Nutrition. 2009;89(2):577-583.
58. Zhan S, Suzanne C. Meta-Analysis of the Effects of Soy Protein Containing Isoflavones on the Lipid Profile. American Journal of Clinical Nutrition. 2005;81:397-408.
59. Zhou JR, *et al*. Soy Phytochemicals and Tea Bioactive Components Synergistically Inhibit Androgen-Sensitive Human Prostate Tumors in Mice. Journal of Nutrition. 2003;133(2):516-521.
60. Kim SG, Kim SY, Park CM. A membrane-associated NAC transcription factor regulates salt-responsive flowering via FLOWERING LOCUS T in Arabidopsis. Planta. 2007 Aug;226:647-54.
61. Anderson MH, Ensher JR, Matthews MR, Wieman CE, Cornell EA. Observation of Bose-Einstein condensation in a dilute atomic vapor. science. 1995 Jul 14;269(5221):198-201.
62. Merritt D, Piatek S, Zwart SP, Hemsendorf M. Core formation by a population of massive remnants. The Astrophysical Journal. 2004 May 13;608(1):L25.
63. Kanamoto T, Kashiwada Y, Kanbara K, Gotoh K, Yoshimori M, Goto T, Sano K, Nakashima H. Anti-human immunodeficiency virus activity of YK-FH312 (a betulinic acid derivative), a novel compound blocking viral maturation. Antimicrobial agents and chemotherapy. 2001 Apr 1;45(4):1225-1230.
64. Galvez AF, De Lumen BO. A soybean cDNA encoding a chromatin-binding peptide inhibits mitosis of mammalian cells. Nature biotechnology. 1999 May;17(5):495-500.
65. Pool R. Is It Healthy to Be Chaotic? Chaos may provide a healthy flexibility to the heart, brain, and other parts of the body. Conversely, many ailments may be associated with a loss of this chaoticflexibility. Science. 1989 Feb 3;243(4891):604-607.
66. Hancock RE, Lehrer R. Cationic peptides: a new source of antibiotics. Trends in biotechnology. 1998 Feb 1;16(2):82-88.
67. Liu Y, Schiff M, Dinesh-Kumar SP. Virus-induced gene silencing in tomato. The Plant Journal. 2002 Sep;31(6):777-786.