



Health Applications of Soy Protein Hydrolysates

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Abstract

Numerous health benefits have been attributed to protein hydrolysates including antihypertensive, anti-obesity, anti-inflammatory, antioxidant, anti-cancer, hypocholesterolemic and immunomodulatory effects as well as alleviation or risk reduction of many chronic diseases. However very rare reviews have solely explored the numerous potentials of soy protein hydrolysates (SPHs). A lot of varying pieces of information on the physiological functions of soy proteins suggest their hydrolysates as functional or bioactive. In this regard, different methods have been used to prepare SPHs. An overview of documented immunomodulatory properties of SPHs and their mechanistic proclivity were presented. This was coupled with the most promising potentials of SPHs, bearing in mind that landmark advancement in elucidating soy protein bioactivity and functionalities have been achieved. Nonetheless, further investigations are recommended for the identification of main organs affected by SPHs, and confirmatory studies to underscore their action mechanisms. Attainment of these criteria will validate the potentialities of SPHs in the emerging functional foods, nutraceutical and pharmaceutical markets.

Keywords Soy protein · Soy protein hydrolysate · Functional property · Bioactive peptide · Immunomodulation · Functional foods

Introduction

Until the last two centuries, soy or soybeans have existed and been cultivated for about five millennia only in Asian countries, including China, Korea and Japan. The western world and others started cultivation of soy in the twentieth Century. Soy originally supplied rich dietary protein and peptides in Asian foods, with wide applications. These include tofu, natto and miso (Nishinari et al. 2014). The protein consists of all essential amino acids and many bioactive nutritional components (Ashaolu and Yupanqui 2017). The hydrolysates or bio-peptides prepared from soy protein confers numerous biological functions, which have preventive or therapeutic applications such as treating obesity, cancer and cardiovascular diseases (Ashaolu 2019a).

Soy protein hydrolysates (SPHs) are produced from soy proteins through an array of protein processing technologies such as hydrolysis and thermal treatments, or through biological processes including gastrointestinal digestion and microbial fermentation. It is important to look into these treatment options because each of them have different effects on the function or property of educed hydrolysates. There are several studies reported on protein hydrolysates and bioactive peptides from plant sources, but very rare reviews on SPHs, especially regarding their biological functions. Thus, it is intended in this present work to summarize recent research and potential benefits of SPHs, in relation to its biological/health applications, after reviewing the methods of processing soy protein into hydrolysates.

Protein Hydrolysis

Hydrolysis is a reaction, either by chemical, biological or physical means, of splitting (lysis) water (hydro) or a larger compound into smaller compounds/components, and molecules of water are added in this process. The addition of H^+ and OH^- to the compound in question are in different proportions. Thus, acids (e.g. concentrated sulfuric acid,

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H₂SO₄, or hydrochloric acid, HCl), bases (such as sodium hydroxide, NaOH), metal salts and enzymes are employed in catalyzing hydrolytic reactions. A protein hydrolysate is the product of protein hydrolysis, as it is broken into smaller peptides and free amino acids usable by the cells. Protein hydrolysates have been prepared from numerous sources including mung bean, wheat, soybean, shrimp, fish, sesame and some food wastes (Zhang et al. 2014; Lee et al. 2016; Holkar et al. 2016; Bhavsar et al. 2017; Hong et al. 2018, 2019). Methods involved in hydrolyzing food proteins have included physical, chemical or biological processes (da Silva et al. 2015; Bouhamed and Kechaou 2017; Hong et al. 2018; Callegaro et al. 2019).

Chemical Hydrolysis

Acid or base may be used and rid of after hydrolysis. The process involves a thermal application of up to 110 °C and within a period of more than 20 h but not exceeding 4 days (Gao et al. 2006). The rate at which peptide bonds are cleaved is of high importance, making the choice of acid type significant. Hydrochloric acid is often used since it cleaves peptide bonds faster than sulfuric acid, even at the same concentration of 6 M. Optimum timing condition for 6 M HCl is 24 h. Other than that, organoleptic properties of the hydrolysates prepared with hydrochloric acid possess higher acceptability values. At the end of hydrolysis, the residual acid is removed by evaporation (Gao et al. 2006). Acidic hydrolysis of protein has long been recognized as an effective way to produce peptides and amino acids. Acid hydrolysis can be performed with inorganic acids, such as hydrochloric acid (HCl), as well as organic acids, such as acetic acid, citric acid, lactic acid, and formic acid. HCl is commonly used to analyze the amino acid composition of proteins, but the downside of HCl hydrolysis is that it partially or totally destroys tryptophan, tyrosine, serine, and threonine under severe conditions, that is, 6 M, 110 °C for 20 h (Fountoulakis and Lahm 1998; Hong et al. 2019). Formic acid was favoured over other acids for collagen hydrolysis because it more easily penetrated into collagen molecules. It is also less corrosive to equipment than HCl and sulfuric acid, and it specifically cleaved Asp (D) residues that largely locate on cross-linked telopeptides. Formic acid inactivate viruses and bacteria in raw material, and it is easy to separate for reuse. Finally, it does not introduce additional inorganic salts into the hydrolysate (Hong et al. 2018).

The use of base for hydrolysis (such as sodium hydroxide, NaOH, or potassium hydroxide, KOH), also referred to saponification is carried out at 180 °C (Gao et al. 2006). These strong bases are used to decompose not only proteins into amino acids, but also utilized in breaking down other macromolecules like carbohydrates and lipids into sugars and soaps, respectively (Gao et al. 2006). It is actually

employed in ester-linked lipid hydrolysis, and total lipid extracts. Alkaline hydrolysis is also applied to biomass, sediments and extracted residues. For protein hydrolysis, other chemicals like sodium, calcium and potassium are employed to solubilize already heated protein at 130 °C. Both acid and alkaline hydrolyses are cost and time effective with industrial applications and simple operations (Gao et al. 2006), but the use of these strong chemicals reduce the food products' nutritional qualities and are ecologically unacceptable. Alkaline hydrolysis is used to digest a wide variety of protein to analyze their amino acid compositions, especially in the determination of tryptophan, which will be destroyed by acid hydrolysis (Fountoulakis and Lahm 1998). A fast and sensitive method to quantify proteins such as collagen with alkaline hydrolysis, using a NaOH concentration of 7 mol l⁻¹ as a standard was established recently (da Silva et al. 2015). Also, the dilemma for chemical hydrolysis of proteins is that increasing the acid or alkaline conditions to reach a lower molecular weight usually means a higher yield of free amino acids. In addition, both alkali and acid methods are strongly corrosive to equipment and produce a high salt content in the final product after the neutralization (Hong et al. 2019).

All chemical processes employ alkali, strong acids or other reagents (such as reducing agents), frequently under high temperatures and pressure (Bouhamed and Kechaou 2017; Coward-Kelly et al. 2006; Lee et al. 2016; Sinkiewicz et al. 2017; Stiborova et al. 2016). Chemical hydrolysis is usually faster and simpler than enzymatic/microbial processes, with high hydrolysis yields (Callegaro et al. 2019). However, chemical hydrolysis occurs in a non-specific manner, leads to the potential destruction of amino acids and products with a high ash content (Adler et al. 2018; Sinkiewicz et al. 2018; Taskin and Kurbanoglu 2011). Additionally, some chemicals (thiols, sulfites and peroxides) present increased toxicity, severe hydrolysis conditions (high temperature and/or pressure) demand high-energy inputs and the treatment of process streams might be problematic. Novel approaches have been investigated for the hydrolysis of protein-rich materials, aiming to avoid or minimize the negative aspects of alkaline/acid hydrolyses (Callegaro et al. 2019). Among these are alternative hydrothermal treatments, such as the utilization of superheated water and steam flash explosion and the use of ultrasound or microwaves to assist chemical hydrolysis (Bhavsar et al. 2017; Holkar et al. 2016; Lee et al. 2016; Zhang et al. 2014; Callegaro et al. 2019).

It is noteworthy that the use of strong chemical treatment (Acids and Bases) alters the appearance, solubility, flavour and biochemical safety of the hydrolysates. Hence, the use of these chemicals have not been applied to soy protein hydrolysis, rather enzymatic hydrolysis has been majorly applied. Recently, formation of chlorohydrins [including 3-chloro-1, 2-propanediol (MCPD) and 1, 3-dichloro-2-propanol (DCP)] in vegetable protein hydrolysis led to a serious clinical concern in

many countries; and now, researchers and manufacturers apply a safe and mild method like enzymatic hydrolysis to replace strong chemicals hydrolysis (Ashaolu 2019b).

Biological Hydrolysis

The biological method may be classified into fermentation, enzymatic, and gastrointestinal digestive processes. Fermentation is an eco-friendly and cost-effective process that can stabilize and retain the nutritional quality of by-products. Fermentation plays a crucial role in texture and flavor development (Singh et al. 2014). Fermentative bacteria have been used over the decades mostly in Asian countries, to prepare fermented soy foods including miso, tempeh, natto and soy sauce (Ashaolu 2019c). Among the fermentative microbes used are the lactic acid bacteria, which are abundantly present in the upper gut, and often break down soy proteins into bio-peptides.

Hydrolysis by enzymes (proteases) assist in the preparation of protein hydrolysates without any chemical or physical induced severe treatment. The nutritional and functional values of the hydrolyzed products are also retained (Gao et al. 2006). Temperature, pH, enzyme–substrate ratio, and type of the enzyme used are some factors that may affect enzymatic hydrolysis. Proteases used are enzymes that digest proteins into smaller peptides and free amino acids by breaking their peptide bonds. They are either endo- or exo-proteases. Endo-proteases degrade the protein molecule by hydrolyzing the peptide bonds randomly while exo-proteases break the peptide bonds at the terminals, releasing the amino acids at the N or C sides. Proteolysis affords reduced waste generation, faster hydrolytic reaction, milder conditions of reaction, and nominalized or avoidance of unwanted reactions. The drawbacks are cost of enzymes, recovery and off or bitter taste among others (Gao et al. 2006; Sun 2011). The human digestive enzymes naturally digest food proteins when ingested. These enzymes are found in the stomach, the intestines and pancreas, and include pepsin, trypsin, chymotrypsin and pancreatin. Numerous works have employed the use of these enzymes for soy protein hydrolysis, including bromelain, papain, alcalase, flavourzyme, neutrase, protemax, transglutaminase among others (Lopes-da-Silva and Monteiro 2019; Zhong et al. 2007b; Ding et al. 2019). Soy bio-peptides are produced in this same manner, and absorption of the smaller peptides occurs through the intestinal walls into the bloodstream from where they can target specific tissues and confer systemic effects (Ashaolu 2019a).

Soy Protein Hydrolysates and Bioactive Peptides

Protein supplements and peptide markets largely depend on soy and dairy industries. Milk and soy proteins are even described as the most important sources of hormone-like

peptides (Teschemacher 2003). Bio-peptides confer physiological benefits due to their readily available amino acid sequences, after consumption. While protein hydrolysates consist of amino acids and peptides mixture, peptides are pure amino acid sequences. A schematic production flow of bioactive peptides is shown in Fig. 1. BioPD (bioactive polypeptide database) has reported more than 1250 peptides with different functional significance, and more than 1500 different bioactive peptides also reported in a database named ‘Biopep’ (Dziuba et al. 2003). Soy bio-peptides may influence cardiovascular, digestive, nervous and immune systems of the body, based on their amino acids sequence. Bioactive peptides ranging from 2 to 30 amino acids in length (with some few exceptions like lunasin, which has 43 amino acids), are usually less than 6 kDa in size (Sarmadi and Ismail 2010). Many bioactive peptides are specific in their manner of functionality such as lunasin that possesses anticancer property.

Several bioactive peptides are isolated from animal and plant sources like milk, meat, egg, fish, oyster, cereals (rice, wheat, soybean, buckwheat, barley, and corn), soybean, and radish seeds (Lafarga and Hayes 2014) including marine macroalgae (seaweeds or sea vegetables) and microalgae (Ko et al. 2012). The bio-peptides have been identified with antimicrobial (Andavan and Lemmens-Gruber 2010), immunomodulatory (Ashaolu et al. 2017), opioid (Gómez-Ruiz et al. 2002), angiotensin converting enzyme (ACE)-inhibitory (Wijesekara and Kim 2010), renin-inhibitory (Fitzgerald et al. 2012), antioxidant (Lule et al. 2015) and anticoagulant (Kim and Wijesekara 2010) properties. The peptides must be cleaved from their parent protein sequences before they can become bioactive, by using methods such as chemical, enzymatic or fermentative hydrolysis, as well as gastrointestinal digestion, thermal and autolysis methods, as stated earlier.

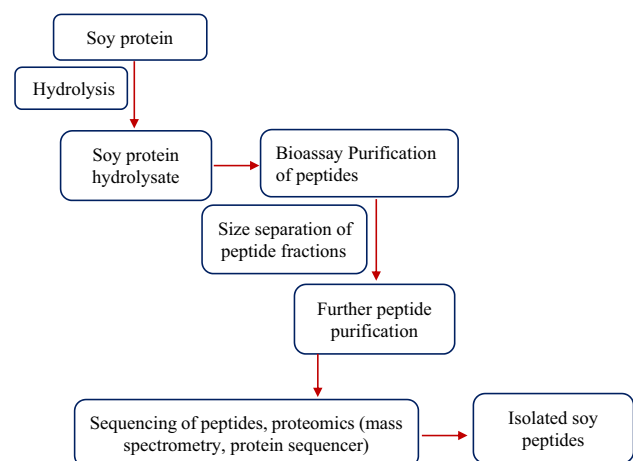


Fig. 1 General overflow of soy peptide production

Glycinin and β -conglycinin that account for 65–80% of total soy proteins, often serve as the precursor of soy peptides isolation. Soy protein contains the entire essential amino acids, including its peptic hydrolysates (Ashaolu and Yupanqui 2017), just as found in animal proteins. This makes its nutritional and biological values comparable to animal protein because the protein digestibility-corrected amino acid score (PDCAAS) of casein, egg and soy protein are same in this regard (Singh et al. 2014). From another viewpoint, soy protein is a suitable food for lactose-intolerant consumers, vegetarians and milk-allergy patients, as it is free of cholesterol, gluten and lactose; and its enzymatically hydrolyzed forms are hypoallergenic (Ashaolu and Yupanqui 2017, 2018). Several bioactive peptides have been isolated, purified and characterized from soy sources with ACE-inhibitory, hypocholesterolemic, immunomodulatory and anticancer activities among others (Table 1). According to an approved health claim, an intake of 25 g of soybean protein per day can lower serum cholesterol levels by 5–10%, and soy peptides/hydrolysates may promote cardiovascular health by aiding the treatment or prevention of hyperlipidaemia or hypercholesterolaemia (Zhong et al. 2007a). Aglycin is a natural bioactive peptide isolated from soy, possesses antidiabetic potential and it is stable in digestive enzymes. Lu et al. (2012) reported that oral administration of aglycin could reduce or stop hyperglycemia by up-regulating insulin receptor signaling pathway in the skeletal muscle of streptozotocin/high-fat-diet-induced diabetic mice. Nagarajan et al. (2008) also reported that bioactive peptides from soy glycinin and β -conglycinin played atheroprotective role through absorption from the intestinal tract walls.

Originally, SPHs, like other bioactive peptides, are dormant within the parent protein (SPI) sequence, unless released for activity by fermentation, gastrointestinal digestion, enzymatic or food processing (Kwon et al. 2010). The intestines easily absorb hydrolyzed SPI more efficiently, and then rapidly passed into the blood circulation since the peptides are usually 2–30 amino acids in length. This can confer systemic or local functional effects in target tissues or organs (Singh et al. 2014).

Applications of SPHs in Health

Selected biological functions of SPHs are presented in Table 2.

ACE and Renin Inhibition

As earlier explained, ACE plays a crucial role in the regulation of blood pressure. Since the discovery of ACE inhibitors in snake venom (Ferreira et al. 1970), studies have been directed at several food protein hydrolysates including those

from casein, rapeseed, whey, porcine and soy (Vermeirssen et al. 2004; Silva and Malcata 2005). This has led to several discoveries of ACE-inhibitory peptides isolated from soy sources, such as soy protein, soy sauce, soy paste, tempeh and natto (Norris et al. 2012; Saha and Hayashi 2001; Kitts and Weiler 2003). For instance, Val-Ala-His-Ile-Asn-Val-Gly-Lys and Tyr-Val-Trp-Lys peptides from fermented soybean were found to possess ACE-inhibitory properties in simulated gut digestion (Hernandez et al. 2004). In addition, hypertension is a risk factor of cardiovascular disease, and most frequently isolated and investigated bio-peptides from soy and other foods are antihypertensive peptides (Wang et al. 2008). This includes a peptide (HHL) derived from Korean soybean paste, reported to reduce hypertension in rats (Saha and Hayashi 2001). Furthermore, renin-inhibitory peptides have been isolated from plant sources including soybeans (FitzGerald et al. 2012).

Antioxidative Effects

Abnormal tissue growth results from immunosuppressive pro-oxidative and pro-inflammatory mechanisms. SPHs contain antioxidative peptides. It is generally accepted that the antioxidant activities of peptides are synergistic, as found in the mode of antioxidant action of soy peptides, which involve multiple mechanisms including hydrogen donation, scavenging of hydroxyl radicals, transition-metal ion chelation and active-oxygen quenching (Chatterjee et al. 2018).

Studies with histidine-containing peptides have demonstrated that they can act as metal-ion chelators, active-oxygen quenchers, and hydroxyl radical scavengers. Soy peptides possess these qualities because they consist of 3–16 amino acids including hydrophobic amino acids, valine or leucine, at the N-terminal positions, and proline, histidine or tyrosine in the sequence. Both anti-inflammatory and antioxidative potentials of lunasin gives rise to its anti-cancer property (Lule et al. 2015). As an anti-oxidant, lunasin inhibits 2,20-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt radical scavenger, production of ROS, and pro-inflammatory cytokines (TNF- α and IL-6) secretion in mouse RAW 264.7 macrophages. It acts as a potent peroxyl and superoxide scavenger, and can prevent glutathione peroxidase and catalase activities (Chatterjee et al. 2018).

Immunomodulatory

Lymphocytes and Macrophages

Kong et al. (2008) found alcalase-educed SPHs to stimulate the proliferation of murine splenic lymphocytes, and macrophage phagocytosis. Similarly, Ashaolu et al. (2017) reported these effects in murine cells, using peptic hydrolysates. A clue from this is that SPHs lymphocytic and

Table 1 Some bioactive peptides derived from soy

Source	Peptide	Bioactivity	Experimental model	References
Soybean	His-Cys-Gln-Arg-Pro-Arg and Gln-Arg-Pro-Arg	Phagocytosis stimulatory	Murine cells	Yoshikawa et al. (2000)
Soy protein	X-Met-Leu-Pro-Ser-Try-Ser-Pro-Try [50]	Anticancer	P388D1, a mouse monocyte macrophage cell line	Kim et al. (2000)
Soybean	SKWQHQQDSCRKQKQGVNLTP-CEKHIMEKIQGRGD	Anticancer, Antioxidative, anti-inflammatory and hypocholesterolemic	Murine embryo fibroblast and hepatoma cells; human breast cancer cells; Caco-2 cells, THP-1 macrophages	Galvez and Benito (1999), Wang et al. (2008), Pabona et al. (2013) and Lule et al. (2015)
Korean soybean paste	DDDDDDDD (lunasin)	Antihypertensive	Rats	Saha and Hayashi (2001)
Soy protein	HHL	Hypocholesterolemic	Mice	Yoshikawa et al. (2000)
Japan soy sauce	LPYPR	Antihypertensive	Rabbit lung	Kitts and Weiler (2003)
Fermented soybean	PGTAVFK	ACE inhibitory peptide	Simulated gut digestion	Hernandez et al. (2004)
Soy protein	Val-Ala-His-Ile-Asn-Val-Gly-Lys and Tyr-Val-Trp-Lys	Hypotensive	Hypertensive rats	Kodera and Nio (Kodera and Nio 2002)
Soy milk	Tyr-Val-Val-Phe-Lys and Ile-Pro-Pro-Gly-Val-Pro-Try-Trp-Thr	Anti-inflammatory	Murine macrophage cell line RAW 264.7	Dia et al. (2014)
Soy protein	RQRK and VIK	Antilipidaemic	3T3-L1 pre-adipocytes	Tsou et al. (2013)
Soy protein	ILL, LLL and VHVV	Immunomodulatory	fMLP receptor assay and murine models	Ohinata et al. (2007), Kaneko et al. (2010), Yamada et al. (2012) and Yoshikawa (2015)
Soy protein	MITLAIPVKNKPGR; soymetide-9 (MITLAIPVN); Soymetide-4 (MITL)	Appetite suppressant, anxiolytic and hypoglycemic	Diabetic mice and guinea pig ileum	Ohinata et al. (2007), Kaneko et al. (2010), Yamada et al. (2012) and Yoshikawa (2015)

Table 2 Overview of soy protein hydrolysates and their selected bio-functions

SPH used singly or combined with other substrates	Enzyme/treatment	Bioactivity/uses	References
Singly	Pepsin	Increased serum IgA and IgG levels	Ashaolu et al. (2017)
Singly	<i>R. oryzae</i> neutral protease	Increased <i>Mx1</i> , <i>Aqp8</i> and <i>Dmbt1</i> in Peyer's patch cells, increased cytotoxicity of spleen cells	Egusa and Otani (2009)
Singly	Alcalase, pepsin	Inhibited β -hexosaminidase release in IgE-antigen complex stimulated RBL-2H3 cells	Ashaolu and Yupanqui (2017)
Singly	Theroase, biopraxe, FP	Total lymphocyte and granulocyte numbers altered, and increased CD11b ⁺ and CD56 ⁺ cells	Horiguchi et al. (2005)
Singly	Pepsin	Modulated human colonic microbiota	Ashaolu et al. (2019)
Singly	Soy-derived peptides bound to phospholipids	Energy release; hypocholesterolemic	Hayes (2013)
Singly	Pepsin	Immunomodulation	Ashaolu and Yupanqui (2018)
Combined	Thermal	Immunomodulation	Kiewiet et al. (2018)

phagocytic activities may not be affected by enzyme type used. In light of which soybean peptides His-Cys-Gln-Arg-Pro-Arg and Gln-Arg-Pro-Arg were demonstrated to confer phagocytotic and stimulatory effects in murine cells (Yoshikawa et al. 2000).

Peyer's Patches

Gene expression of Peyer's patches derived cells were evaluated after feeding mice with SPHs (Egusa and Otani 2009), and was found that innate immunity related genes were up-regulated as well as the host defense genes. *Igh-4* and *Aqp8* genes known to improve phagocytic processes; and *Dmbt1*, *Slpi*, and *Mx1* genes known to serve as anti-viral and anti-bacterial components were up-regulated (Ligtenberg et al. 2007). Peyer's patches are known to be components of gut lymphoid tissues, and are crucial as the central immune sampling and signaling site in the gut (Kiewiet et al. 2018).

Across Gut Barriers

Apart from splenic lymphocytic or phagocytic immune responses to SPHs treatment, other systemic immune responses have been reported. Hydrolysates and smaller peptides of soy were detected in volunteers who consumed soy products, suggesting the passage of soy peptides through the gut barrier into systemic circulation and allowing first-hand immune cells impact (Dia et al. 2009). Ashaolu et al. (2017) observed increased IgG and IgA antibodies in the blood sera of Wistar rats after feeding them with peptic SPHs. This implies that SPHs may not only affect B cell differentiation, but also induce class-switching and antibody production (Kiewiet et al. 2018). In a human study, SPHs enhanced the increase of leukocytes and granulocytes, i.e. CD11b⁺ (macrophages and/or dendritic cells) and CD56⁺ cells (NK cells)

in the blood of a small group of volunteers, who consumed a single dose of hydrolyzed soy protein (Yimit et al. 2012). This implies the immunomodulatory activity of SPHs in humans, except that well-designed and comprehensive human studies are still recommended for validation.

Hypoallergenic and Inflammation Quenchers

Soy allergy is recognized as one of the eight most common food allergies globally (FARE 2018). Allergy mechanisms involved the exposure of linear protein to Th2 cells by the antigen-presenting cells (APC), which stimulate B cells to produce antigen-specific IgEs. The IgE receptors become bound to mast cells for degranulation and production of histamines and several mediators including cytokines, granulocyte macrophage colony-stimulating factor (GM-CSF), leukotrienes, heparin, as well as proteolytic enzymes (Stone et al. 2010). The substances produced are chemicals responsible for allergy symptoms. β -hexosaminidase is one of the enzymes latent in mast cells, and has become a recommended criterion for measuring the extent of degranulation (Guo et al. 2009). Peptic and alcalase hydrolyses of soy protein were found to suppress the degranulation of RBL-2H3 cells, and inhibit β -hexosaminidase release (Ashaolu and Yupanqui 2017).

Several peptides derived from soy are reported to possess anti-inflammatory property after being used up by the cell (Kiewiet et al. 2018). The process depends on the peptide transporter PepT1, which mediates the uptake of many di- and tri-peptides in intestinal epithelial cells and then transports them into the blood stream. A peptide from soy identified as VPY conferred anti-inflammatory effects on human monocytes (THP-1), and another one, KVP, also exerted anti-inflammatory property on T cells (Kovacs-Nolan et al. 2012). Interestingly, PepT1 transporter is expressed

on the two cell types, connoting anti-inflammatory prospects of bio-peptides in both innate and adaptive immune cells through same mechanism as found in epithelial cells (Kiewiet et al. 2018).

Receptors

Food protein hydrolysates do bind to opioid receptors, function as neurotransmitters and modulate innate and acquired immune responses (Stefanucci et al. 2018). Signals from opioid receptors help antibody synthesis and influence macrophage phagocytosis after administering protein hydrolysates (Cheido et al. 2014). Soy β -conglycinin A' subunit peptide stimulated neutrophilic phagocytosis, but without much affinity for *N*-formyl-methionyl-leucyl-phenylalanine (fMLP) receptor. After blocking the receptor, the phagocytic property was lost, implying that its poor affinity for fMLP receptor makes it a safe immunostimulant, without causing any inflammation (Tsuruki et al. 2003).

Endocytic Effects

Lunasin, a soy peptide is reported to employ endocytosis as a mechanism of modulating the immune cells (Cam et al. 2013). It interacts with the α V β 3 integrin, which inhibits α V β 3 integrin-mediated pro-inflammatory markers and thereby downregulates the Akt-mediated NF- κ B pathway. lunasin can be processed by integrin signaling, clathrin-coated structures, and macropinosomes via endocytosis with several inhibitors (Cam et al. 2013).

Neuromodulatory Effects

The human β -casomorphin-4 (YPFV) is an opioid peptide that has morphine-like activity. It has the same sequence of the β -subunit of soy β -conglycinin. As a result of this discovery, three peptides from soy with anxiolytic activities: soymorphin-5 (YPFVV), soymorphin-6 (YPFVVN), and soymorphin-7 (YPFVVNA) have been isolated (Ohinata et al. 2007). It was demonstrated that these peptides suppressed food intake and small intestinal transit due to the coupling of the receptor to neurotransmitters in mice (Kaneko et al. 2010). In addition, soymorphin-5 improved glucose and triglyceride levels in a KKAY diabetic mouse model by activating adiponectin and PPAR α , and promoting β -oxidation and energy expenditure (Yamada et al. 2012). These soy peptides exerted anxiolytic effects.

Hypocholesterolemic Property

One of the foremost soy protein benefits ever to be studied was its cardiovascular functions, to reduce levels of cholesterol in blood (Demejia and Delumen 2006). There is

a consensus that soy proteins serve hypocholesterolemic effects in humans, and that dietary intake of soy protein (at least 20 g) and isoflavones (at least 80 mg) for 5 weeks would be effective in reducing cardiovascular health diseases risk among high-risk, middle-aged men (Sagara et al. 2004). Hypercholesterolemic patients are rid of circulating triglycerides and cholesterol when they consumed soy peptides, with a less atherogenic pattern of low-density lipoproteins in an isoflavones independent manner (Wang et al. 2004). One hypothesis states that blood cholesterol levels may be lowered when re-absorption of bile acids is inhibited by soy protein binding. Another way to look at it is for soy peptides to target the organ from where cholesterol is produced (liver) and reduce or inhibit its production from there (Demejia and Delumen 2006). It suffices still to associate hypocholesterolemic effect of soy peptides to phospholipids binding (Saito et al. 2003). Meanwhile, Yoshikawa et al. (2000) had already reported a soy monopeptide (LPYPR) to reduce cholesterol levels in mice subjects.

Anti-cancer Potentials

Following its recent discovery, lunasin is famous for its anticancer property. However, another soy peptide, Kunitz trypsin inhibitor, also possesses this property. It was reported to block urokinase activity and thus mitigate proliferation of cancer cells in the ovary (Xiao et al. 2005). Other than these two examples, numerous soy hydrophobic peptides possess anticancer property, at least upon in vivo and in vitro experiments. González de Mejía et al. (2004) investigated the anticancer prospects of thermolase-educed peptides from defatted soy protein, which were purified with ethanol, and chromatographically fractionated. There was in vitro cytotoxicity on mouse monocyte macrophage cell line. At 1 mg/mL, the fraction significantly affected cell cycle progression by inhibiting the cells in G2/M phases. Further purification with C18 HPLC resulted in 1157 Da nonapeptide (X-Met-Leu-Pro-Ser-Tyr-SerPro-Tyr) (González de Mejía et al. 2004).

Some researchers reported the majority of anti-cancer soy peptides to belong to the 2S albumin fraction of isolated soy proteins, lunasin and Bowman-Birk inhibitor (BBI) (Pabona et al. 2013). The BBI is a protein with low molecular weight, and it inhibits trypsin and chymotrypsin activity, previously considered as an anti-nutrient. BBI has proven to be anti-carcinogenic in the ovary, colon, esophagus, liver, prostate, breast, and other tissues/organs in mammals. Its mechanism of action is via ROS-induced damage of the mitochondria. This apoptotic phenomenon results from the inhibition of proteasomes and angiogenesis (Cruz-Huerta et al. 2015). Thus, SPHs with the support of BBI confers anti-cancer effects.

Future Prospects

Special Nutrients and Diets

In general, SPHs proffer high potentials in special diets and nutritional effects. They are excellent protein sources for human nutrition and physiological functions, as they are easily absorbed in the gastrointestinal tract. The digestion and metabolism of soy di- and tripeptides is more effective than the native protein. Therefore, they are suitable for the production of elderly, infants and patients foods. Peptic hydrolysates of soy protein have been reported to possess hypo-allergenic functions (Ashaolu and Yupanqui 2017), providing amino acids requirement for infants and adults, and thus suitable for fortifying soluble formulas and as the only protein source in special medical diets. In addition, the use of specific bacterial enzymes or genetically transformed microorganisms is of interest for future research. Moreover, genetically modified proteins and peptides may be designed to carry multiple copies of bioactive sequences.

ACE Inhibition

From another viewpoint of ACE-inhibition, Coscueta et al. (2019) wondered if the sequence of ACE inhibitory peptides obtained from SPHs fractions would remain stable in the gastrointestinal tract of humans or not, before entering the circulatory system. This is because an in vitro simulated gastrointestinal digestion of the peptides showed high ACE inhibitory and antioxidant properties, having oxygen radical scavenging, and ACE inhibition values of $3.9 \pm 0.1 \mu\text{mol TE/mg protein}$ and (IC_{50}) of $52 \pm 4 \mu\text{g protein/mL}$, respectively. This is another point for consideration into the dynamics of clinical experiments. The effects of SPH in the gastrointestinal tract may further be localized to specific compartments. For instance, Kiewiet et al. (2018) demonstrated that SPHs resisted duodenal digestion in vitro, but may have a great potential for strengthening the epithelial barrier in certain individuals. Moreover on this note, the hydrolytic process used in the preparation of SPHs will continue to redefine their functions as found in the investigations of Daliri et al. (2019), who used *L. rhamnosus* to ferment soy proteins upon prozyme treatment, to generate ACE inhibitory bio-peptides thereof. Although, the SPHs reduced blood pressure, the mechanisms were not yet established. Like other bioactive peptides, SPHs have the potentials of being applied in drug and therapeutic agents discovery (Lammi et al. 2019).

Immunomodulatory Prospects

Reports showing SPHs to possess immunomodulatory property suggest their applicability in nutraceutical and

functional food products. These products will possess same immune modulation effects found in their core hydrolysate component. The numerous immune health benefits ascribed to SPHs as mentioned earlier suggest that they are potential peptides for reducing the symptoms of inflammatory bowel diseases and irritable bowel syndrome. Apart from this, systemic immune disease patients may derive some benefits from SPHs consumption. Therapeutic containment of certain diseases is possible with the development of specific soy peptides and hydrolysates using modern innovative biotechnological methods, including sequencing and genetic modification of already developed soy peptides and hydrolysates fractions.

Prevention of specific diseases may be achieved by some age groups of individuals when they consume immunomodulatory SPHs. The aging process predisposes an individual to inflammations, infections and increased mortality rate arising from the weakening of the immune system. An altered cytokine production by monocytes and macrophages occurs, with a decreased phagocytic capacity and a reduced TLR expression in this process (Plowden et al. 2004). Available information on the immunomodulatory effects of SPHs suggest that they may improve the health and quality of life for elderly individuals, rendering healthcare costs inexpensive or inapplicable.

Immune related stress is experienced by many people, leading to more immune-related diseases (Corazon et al. 2018). Immune system impairment are induced by acute or chronic stress, and they later result in inflammation, autoimmune, and allergic diseases. These processes may be halted by SPHs. The production of short chain and branched chain fatty acids, which have a lot of health functions including immune boosting in individuals, might be achieved by SPHs (Ashaolu et al. 2019). However, investigations until now have mainly focused on immune studies in vitro and in animal models, with rare human interventions or follow up investigations. Human trials are essential for the development of immunomodulatory SPH products, for proper interpretation of their safety, bioavailability, and resultant effects in the human body. From thence, further investigations may be carried out.

Anti-diabetic Potentials

Recently, a tetra peptide obtained from SPHs, Val-His-Val-Val (VHVV), was reported to possess anti-apoptotic and anti-hypertrophic efficiency against high glucose (Marthandam Asokan et al. 2018). The study showed that the soy peptide could enhance insulin secretion in the pancreas of mice subjects, and thus potentiates as an effective anti-diabetic peptide as well as β -cell protector. The investigation showcases SPHs as resisting hyperglycemia progression because it facilitated blood glucose regulation. Other than

this report, α -glucosidase-inhibitory tri peptides, Gly-Ser-Arg and Glu-Ala-Lys, capable of mitigating symptoms of diabetes or hyperglycemia were reported in mice models (Jiang et al. 2018). They may serve as functional food ingredients for diabetic patients in no distant future. Nonetheless, further investigations are necessary for the identification of main organs affected by SPHs, and confirmatory studies to underscore their action mechanisms.

Conclusions

Soy protein is a nutritious and cheap protein source, making its hydrolysates very important in functional foods applications. Edge-cutting sequencing technologies as well as validating human trials are required to design effective dosages and safe SPHs. In addition, tailored hydrolysates meant for specific age or target groups can be developed as therapeutics to mitigate, suppress, or prevent the onset of a wide variety of immune-related conditions. These observations create big space for improved SPHs production, and identification methods, possibly for nutraceuticals or drug/therapeutics development.

Compliance with Ethical Standards

Conflict of interest There are no conflict of interest to declare.

Research Involving Human Participants and/or Animals No human participants or animals were used for this study.

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