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# Bioactive Peptides from Legumes and Their Bioavailability

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## Abstract

Bioactive peptides (BPs) isolated from legumes have functional properties as healthy foods. These functional effects depend on their stability and bioavailability in the gastrointestinal tract before reaching the target organs. Therefore, it is necessary to disclose the factors that influence it and discuss the technical processing to develop its utilisation. This chapter discusses and summarises the bioactive activities of BPs from various legumes, factors and mechanisms related to the bio-assessability, stability, bio-availability and bioactivity of BPs. Furthermore, the development of BPs bioseparation was also discussed. The results show that the nature of BPs varies greatly depending on the legume source and the production method. Factors that influenced the bio-availability of BPs include molecular weight, charge, amino acid sequence, the presence of specific residues and hydrophobic amino acids, and resistance to the action of peptidase while in the digestive tract. However, some BPs showed increased bio-accessibility and bio-availability after being hydrolyzed by digestive enzymes. Processing technologies such as encapsulation allowing BPs to enter the body and undergo release and degradation by enzymes digestion. Further studies are required to understand the increase in the bioavailability of BPs, the safety of the food components produced, and their use in producing functional foods.

**Keywords:** Legumes, bioactive peptides, fermentation, germination, health benefits, bioavailability

## 1. Introduction

Legumes are a cheap and healthy source of nutrition because of their high protein content and complete constituent components, such as fats, essential amino acids, complex carbohydrates, vitamins, and minerals or dietary fibre. This high protein content plays an essential role in producing functional compounds such as bioactive peptides (BPs) that benefit the health and treatment of chronic diseases. For example, BP from legumes is used as an antioxidant compound to prevent degenerative diseases such as atherosclerosis, coronary heart disease, diabetes mellitus, and cancer [1, 2]. The number of deaths due to NCDs (non-communicable diseases), especially cardiovascular disease, cancer, chronic respiratory diseases, and diabetes, increases globally, both in low-income and rich countries. As a result, NCDs are still the cause of most global deaths each year [3]. One way to reduce the risk of NCD is controlling hypertension, regulating diet and obesity.

Protease enzymes have an essential role in producing BP as a result of protein hydrolysis. Food processing, microbial fermentation, germination, or other process involving protease enzymes are examples of proteolytic processes. The involvement

of protease enzymes in producing BP is significant because BP is mainly composed of 2–20 amino acids [4]. Some countries have healthy food products from legumes. Examples of fermented foods such as natto [5], douchi [6], tempe [7], and others, have antihypertensive activity. Fermented foods represent, on average, one-third of total food consumption [8]. Fermented food has a delicious taste, easy to digest, nutritious and has beneficial properties. Such as antidiabetic, hypocholesterolemic, and anti-inflammatory activities [8, 9].

Many researchers have proven (both in vitro and in vivo using experimental animals) that BP from legumes has functional properties as a healthy food. However, this functional effect depends on the stability of BP to withstand the action of digestive enzymes while in the digestive tract on its way to reach the target organs [10]. In this target organ, BP will act to provide health effects for the body. One of the determining factors to be bioavailable is the number of amino acids, hydrophobic amino acid content, and resistance to digestive enzyme activity [11]. This chapter aims to describe the quality of various legumes, the BP of legumes and their effects on health. Also, an explanation of the factors that affect the stability, absorption, bioavailability and bio-activity of BP and food technology to develop functional food.

## 2. Legume, as a source of bioactive peptides

One of the excellent sources of essential amino acids and protein is legumes. As a source of bioactive peptides (BPs), an ingredient must have a high enough protein content. In addition, legumes also contain many components needed for body health, such as antioxidant compounds, resistant starch, dietary fibre and others [12]. However, it is a fact that the nutritional content and phytochemical composition among legumes vary widely, as shown in **Table 1**. The differences in their genetics, varieties, geographical location and climatic conditions may cause the nutritional content variation [18].

In general, the protein content of legumes ranged from 17.0 to 39.8% (w/w). Soybean is the legumes that have the highest protein content. Soybean is also the most studied legume regarding its function on health. According to FAO [22] world soybean production in 2019 was 333,671,692 tonnes (the highest among the types of legumes produced), of which Brazil produced 34.25% as the world's No. 1 producer country. Apart from soybean, some legumes also have a high protein content as a source of BPs, such as jack beans, velvet beans, lima beans, mung beans, and kidney beans (**Table 1**).

In addition to the protein content, it is also necessary to pay attention to the amino acid composition in choosing ingredients. Peptides with hydrophobic amino acids (Tyr, Phe, Trp, Ala, Ile, Val, and Met), positively charged amino acids (Arg and Lys), or contain Pro at the C end will have higher biological activity. For example, inhibition of ACE enzymes [23], Diabetes mellitus type 2 (T2DM) inhibitory activity [24], or other biological functions. Angiotensin-Converting Enzyme Inhibitor (ACEI), is a BP that affects lowering blood pressure. Meanwhile, DPP-IV inhibitors are compounds that can inhibit dipeptidyl peptidase-IV, an enzyme associated with T2DM disease [25]. So the presence of hydrophobic amino acids in short-chain peptides (between 2 and 20 amino acids in length) [4] is related to biological activities beneficial to health. Soybean, jack bean, velvet bean and mung bean are legumes that have high hydrophobic amino acid content (**Table 1**). Enzymatic breakdown through food processing produces short-chain peptides. For example, fermentation (to produce tempeh, soy sauce, natto, miso, douche, or other legume fermented products), germination (mung bean sprouts, soybean sprouts, or other sprouts), or other processes can break the polypeptide chain.

| No | Legumes sources | Protein | | Amino acid hydrophobic | |
|----|-----------------|---------|-----------|------------------------|-----------|
| | | (db, % w/w) | Reference | (% w/w protein) | Reference |
| 1 | Soybean, *Glycine max* | 35.35–39.80 | [13] | 37.70 | [14] |
| 2 | Jack bean, white, *Canavalia ensiformis* L. DC | 22.80–35.30 | [14] | 8.60–43.50 | [14] |
| 3 | Sword bean, red, *Canavalia gladiata* jacq. DC | 32.4–35.0 | [15] | 26.62–29.47 | [15] |
| 4 | Cowpea, *Vigna unguiculata* L. Walp | 20.90–24.70 | [16] | 8.46 | [16] |
| 5 | Bambara groundnut, *Vigna subterranea* L. Verde | 17.00–17.30 | [16] | 6.45 | [16] |
| 6 | Velvet bean, White, *Mucuna pruriens var. utilis* | 28.82 ± 0.14 | [17] | 34.14 | [17] |
| 7 | Velvet bean, Black, *Mucuna pruriens var. utilis* | 26.26 ± 0.07 | [17] | 32.79 | [17] |
| 8 | Kidney beans, *Phaseolus vulgaris* L. | 21.80–29.20 | [18] | 7.96 | [16] |
| 9 | Lima beans, brown, Phaseolus luna tus | 28.06 | [7] | 4.68 | [7] |
| 10 | Mung bean, Phaseolus radiata | 26.80 | [19] | 39.75 | [20] |
| 11 | Chick pea, Cicer Arieti num | 19.68–22.75 | [16] | 35.3 | [21] |

*The level of hydrophobic amino acids is the sum of the data available in the reference sources used.*

**Table 1.**
*Protein and hydrophobic amino acid content of several legumes.*

Apart from these benefits, legumes contain substances that are considered anti-nutritional compounds [26]. Despite having a high protein content, some toxic anti-nutritional substances limit the use of legumes. Food processing, such as soaking (hydration), cooking, autoclaving, germination, and combination, could reduce or eliminate anti-nutritional compounds [27, 28]; these processes can increase the digestibility value of protein ingredients. **Table 2** shows some of the anti-nutritional compounds present in some legumes. Kalpanadevi and Mohan [26] said that the soaking process and continued germination was less effective in removing anti-nutrients. However, the process would be effective if the germination process was extended (96 hours) or continued with the heating process (or autoclaving process). With this combination process, the effect of anti-nutritional compounds can be eliminated, such as phenolics, tannins, hydrogen cyanide, phytic acid, trypsin inhibitors, oligosaccharides and Phyto-hemagglutination activity.

Other researchers stated that the fermentation process is also very effective for reducing/removing anti-nutritional compounds because the fermentation process is a combination of several processes, including soaking, heating, and proteolytic hydrolysis by starter microbes [31]. For example, the process of fermenting koro bean tempe (*Canavalia ensiformis*) for 48 hours can eliminate 100% of concanavalin-A (Con-A) and reduce almost 99% of its HCN content [31]. Moreover, the fermentation process causes the percentage of peptides with MW <1 kDa to increase to the highest [33]. Short-chain peptides (between 2 and 20 amino acids) are associated with biological activities beneficial to health [4]. As shown by peptides from *Phaseolus lunatus* and *Mucuna pruriens*, peptides with MW < 1 kDa have higher ACE inhibitory activity [7, 34].

| No | Legume sources | Antinutritional compound | | | | | | | | References |
|----|----------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|
| | | Phenol (%) | Tannin (%) | L-DOPA (%) | Phytic acid (%) | Trypsin inhibitor (TIU/mg protein) | HCN (ppm) | Oxalate (%) | Total oligosaccharide (%) | |
| 1 | Cowpea, *Vigna unguiculata* L. Walp. | 1.21 | 0.38 | 2.46 | 0.4 | 26.48 | 2.2 | NA | NA | [26] |
| 2 | Velvet bean, Black, *Mucuna pruriens var. utilis* | 2.84 | 0.26 | 3.64 | 0.45 | 43.1 | 3.1 | NA | NA | [17] |
| 3 | Velvet bean, White, *Mucuna pruriens var. utilis* | 3.13 | 0.34 | 3.24 | 0.42 | 43.5 | 2.1 | NA | 4.49–6.08 | [17, 29] |
| 4 | Sword Bean, red, Canavalia gladiate jacq DC. | 1.21–1.41 | 0.16–0.20 | 2.32–2.64 | NA | 30.43–34.34 | NA | NA | NA | [16] |
| 5 | Kidney bean, *Phaseolus vulgaris* L. | NA | 0.54–2.88 | NA | 1.68–2.41 | 3.65 | NA | NA | 12.51 | [19, 30] |
| 6 | Chick pea, *Cicer arietinum* | NA | 0.01–0.05 | NA | NA | NA | 13.5–40.5 | 0.18–0.45 | NA | [17] |
| 7 | Jack Bean, white, *Canavalia ensiformis* L. DC. | 3, 83 | 0.083 | 1.7 | 0.90 | NA | 59.95 | NA | 8.17 | [28, 31] |
| 8 | Soybean, *Glycine max* | 1.40–3.62 | 1.11–1.88 | NA | 0.51–2.45 | 23.67 | NA | NA | 8.30–10.11 | [27, 32] |

*NA, data not available in the references used.*
*Data is prepared and recalculated so that it has the same units.*

**Table 2.**
*Anti-nutritional compounds in legumes.*

## 3. Bioactive peptides

Bioactive peptides (BPs) are tiny fragments of dietary protein, consisting of 2–20 amino acids, have a molecular weight of less than three kDa, and promote health benefits. After entering the body, BPs can be absorbed in the intestine, carry out various metabolic pathways, and perform various physiological functions [4, 35, 36]. Several researchers have reported that legumes have various biological activities good for body health (**Table 3**).

The hydrolysis of legumes protein can produce these BPs. The enzymatic hydrolysis process occurs in the food processing process, for example, in the fruit ripening process, the fermentation process (producing soy sauce, tempe, natto, and other fermented products), or the germination process (producing soybean sprouts, green bean sprouts, and sprouts products others). In addition, the protein breakdown process can also be carried out by in vitro enzymatic hydrolysis, for example, using the alcalase in legume protein. Some examples of enzymatic hydrolysis are soybean hydrolysis (*Glycine max*) or mung bean hydrolysis (*Vigna radiata*), which produces BP hydrolysate as an ACE inhibitor [40, 45]. The in vitro enzymatic hydrolysis process will produce peptides with enormous structural diversity. Bioinformatics techniques using in silico studies can help select suitable peptide sources. Simulations of biological processes, such as enzyme hydrolysis, can use these in silico studies and further characterise processes and products using software/computers [61].

BPs can perform their activities and roles based on their structural properties, composition and amino acid sequence [62]. Biologically, the active peptides have similar structural properties, including the length of the amino acids, containing hydrophobic amino acids, and resistance to proteolysis [11]. For example, BPs with antioxidant activity have a length of 5–16 amino acids [63]. The structure of ACE inhibiting BPs contains arginine or lysine residues at the C-terminal will affect their activity [11]. Therefore, selecting the protease enzyme to form BPs is essential to produce biologically active peptides. For example, the Carlsberg enzyme subtilysin will hydrolyse peptide bonds with broad specificity to produce peptides with C terminal in the form of hydrophobic amino acids such as Phe, Tyr, Trp, Leu, Ile, Val and Met [64]. In addition, because of their relatively small size and high specificity, BPs can inhibit protein–protein interactions [65]. Some examples of the functional properties possessed by BPs are anti-hypertensive [66], antioxidants [67], hypo-cholesterolemia [68], antimicrobials [69], anti-inflammatory [70, 71], anti-cancer [59], and other functional properties. One type of BPs can have more than one functional property [9, 65]. To date, researchers are still developing comprehensive studies and reviews to confirm the therapeutic effect of BPs. This chapter will discuss the BPs of legumes and their functions.

### 3.1 Antidiabetic

Increased blood sugar levels are signs of diabetes caused by decreased insulin secretion, impaired insulin function, or both. In patients with T2DM, the body does not respond adequately to insulin action and the blood glucose level increases, a condition known as hyperglycemia [72]. Changing diet is one way of treating diabetes, besides losing weight, exercising, or taking drugs to increase glucose homeostasis [25]. Side effects from synthetic antidiabetic drugs are gastrointestinal disorders [73]. Other side effects are hypoglycemia and weight gain [74].

Meanwhile, some patients are intolerant of the drug [75]. Therefore, research to find BPs from food as a safe antidiabetic has recently increased to overcome these side effects [76]. Measuring the inhibitory activity of DPP-IV is one way to

| | Legumes | Amino acid sequence | Reference |
|---|---|---|---|
| **a. Antidiabetic activity** | | | |
| | Kidney bean, *Phaseolus vulgaris* (L), Fermented | INEGSLLLPH | [9] |
| | Soybean, germinated | NNDDRDS, LSSTEAQQS, NAENNQRN, QQQQQGGSQSQ, EEPQQPQQ, IKSQSES | [37] |
| | Cowpea, *Vigna unguiculata*, germinated and enzymatic hydrolysis | TTAGLLE | [38] |
| | Black bean, *Phaseolus vulgaris*, hydrolysate | AKSPLF, ATNPLF, FEELN, LSVSVL | [39] |
| **b. Antihypertensive activity** | | | |
| | Kidney bean, *Phaseolus vulgaris* (L), Fermented | FVVAEQAGNEEGFE | [9] |
| | Mung Bean, *Vigna radiata*, Hydrolysate | KDYRL, VTPALR, KLPAGTLF | [40] |
| | Soybean, *Glycine max*, Hydrolysis | VLIVP, LAIPVNKP, LPHF, NVVGPLV, YLAGNQ, IPPGVPYWT, DQTPRVF, ASYDTKF, DTKF, PNNKPFQ, RPSYT | [41–45] |
| | Soybean protein, *Glycine max*, Hydrolysis | IVF, LLF, LNF, LSW, LEF | [46] |
| | Soybean germinated | RNLQGENEEEDSGA | [37] |
| | Fermented soybean, natto | VAHINVGK, YVWK | [5] |
| | Fermented soybean, soy sauce | GY, SY | [47] |
| | Garden pea, *Pisum sativum*, in silico | LRW | [48] |
| | Pigeon pea, in silico | VVSLSIPR | [49] |
| **c. Hypocholestcrolcmic activity** | | | |
| | Kidney bean, *Phaseolus vulgaris* L, Fermented | SGGGGGGVAGAATASR | [9] |

|  | Legumes | Amino acid sequence | Reference |
|---|---|---|---|
|  | Soybean, *Glycine max*, Hydrolysate | LPYP, IAVPGEVA, IAVPTGVA, YVVNPDNDEN, YVVNPDNNEN, LPYPR | [50–52] |
|  | Soybean protein, *Glycine max*, in silico | SFGYVAE | [53] |
|  | Black bean and cowpea, in silico | YAAAT | [54] |
| **d. Antioxidant activity** |  |  |  |
|  | Soybean, *Glycine max*, Hydrolysis | LLPHHADADY, LLPHH, LVNPH, DHQN, TTYY, LQSGDALRVPSGTTYY | [42] |
|  | Soybean, *Glycine max*, Hydrolysis | RPSYT | [43] |
| **e. Antimicrobial activity** |  |  |  |
|  | Soybean, *Glycine max*, Hydrolysis | IIVVQGKGAIGF, ASRGIRVNGVAPGPVWTPIQPA, IIIAQGKGALGV, | [42] |
|  | Soybean, *Glycine max* | IKAFKEATKVDKVVVLWTA, PGTAVFK | [55] |
| **f. Anti-inflammatory activity** |  |  |  |
|  | Black bean (*Phaseolus vulgaris* L), hydrolysis | IAISISGLL, CNKY, YETN, QAEEEF, MSAMSNAAA, DLPYSCR, ATL, NLG, EDAY, GYDHPMGGL, PVNF, EEAK, LGAL, DLK, LVVL, VPTK, TGVI, TTW, MEL, FNL, GFTPL, KYGDKSVY, IPVL, KTCENL, GGSSDKR | [56] |
|  | Soybean (Glycine mox) | Lunasin | [57] |
| **g. Anti-cancer activity** |  |  |  |
|  | Soybean (*Glycine max*) | Lunasin | [58] |
|  | Common beans (*Phaseolus vulgaris*), Extra long Autumn Purple Bean cultivar | ANEIYFSFQRFNETNLILQR | [59] |
|  | Chickpea (Cicer anetinum) | ARQSHFANAQP | [60] |

**Table 3.**
*The amino acid sequence of several bioactive peptides from legumes.*

determine whether BPs have an antidiabetic activity or not. The role of the DPP-IV enzyme is to inactivate incretins, especially GLP-1 and GIP. GLP-1 is a glucagon-like peptide, while GIP is a glucose-dependent autotrophic insulin peptide. Incretin is a hormone that vitalising insulin secretion. So the mechanism commonly used to control T2DM is to measure how much DPP-IV inhibition is [77].

Several low molecular weight BPs can induce insulin stimulation in blood intake, for example, the peptides present in fermented soybeans [78] or fermented kidney beans [9]. Some BPs that are isolated from black bean (*Phaseolus vulgaris* L) protein hydrolyzate effectively inhibits glucose transporter 2 (GLUT2) and glucose transporter, which depends on sodium 1 (SGLT1), which functions to lower blood glucose levels [39]. The BPs found in legumes (such as kidney beans, *Phaseolus vulgaris* L with ten amino acids) have antidiabetic properties [9] (**Table 3**). The table states that the process can produce antidiabetic peptides, including fermentation, germination, or enzymatic hydrolysis.

## 3.2 Anti-hypertensives

Controlling hypertension is essential to reduce the risk of cardiovascular complications such as coronary heart disease (which causes heart disease) and stroke, congestive heart failure, irregular heart rhythm, and renal failure [3, 79]. A healthy diet is a way to control hypertension. Eating foods high in BPs is very healthy. Several studies have shown that food ingredients derived from legumes have an anti-hypertensive function. The preparation of BPs uses three ways: fermentation of materials into fermented products, germination, and enzymatic hydrolysis. **Table 3** shows some of the research results.

The anti-hypertensive activity was measured by measuring the inhibitory activity of the ACE (Angiotensin I-converting Enzyme). The ACE will cut angiotensin I to produce angiotensin II (vasoactive peptide). This angiotensin II compound will bind to receptors on the walls of blood vessels causing contraction of blood vessels so that blood pressure rises [80]. The presence of BPs will bind to the ACE enzyme, thus inhibiting the action of ACE, and as a result, blood pressure can drop. Some legumes that are recognised to contain BPs that lower blood pressure include garden beans (*Pisum sativum*) [48], green beans [20, 40], soy (*Glycine max*) glycinin [41], kidney bean [9], and pigeon pea [49]. Fermented products also have anti-hypertensive activity, such as douchi, a traditional Chinese food fermented soybean [8]. Other fermented products are Korean soybean paste fermented with mixed cultures of bacteria [81, 82], and many other products from legumes.

Research on anti-hypertensive BPs from food is still being studied [65]. Anti-hypertensive BPs (isolated from food) have a higher tissue binding affinity than synthetic drugs, resulting in slower tissue loss [83]. For vigorous anti-hypertensive activity, the position of specific amino acid residues is critical. For example, valine and isoleucine are essential for ACE inhibition [84]. Increased ACE inhibitory activity occurs when the C-terminal is Proline [84]. Therefore, the strategy to produce peptides with high anti-hypertensive activity is to hydrolyse protein to produce proline containing peptides.

## 3.3 Hypo-cholesterolemic

Many researchers have studied and reviewed the ability of BPs as cholesterol-lowering agents [65]. The human body needs healthy cholesterol levels to produce vitamin D and steroid hormones, and bile acids. However, arteriosclerosis can occur when cholesterol in the blood forms plaque in the arteries. As a result, it can reduce oxygen supply to the heart, which leads to cardiovascular disease. While chemicals that lower blood cholesterol can cause liver damage or failure, myopathy [85] and diabetes [86, 87], or some people are sensitive to statins (cholesterol-lowering drugs) [88]. Therefore, the research for BPs that can lower cholesterol has increased over the years [65]. **Table 3** shows BPs in legumes (such as red beans and soybeans with 4–16 amino acids) with hypocholesterolemic activity.

Cholesterol reduction by peptides can occur due to inhibition of cholesterol micelle formation, inhibition of lipase activity and strong bile acid-binding [89]. Peptides from fermented soy milk show the ability to bind bile acids [90]. The solubility of cholesterol in lipid micelles will be reduced due to BPs [91], resulting in inhibition of cholesterol absorption in Caco-2 cells with one layer. For example, peptides from cowpeas can inhibit HMGCoA reductase and reduce the dissolution of cholesterol micelles in vitro [92]. A 36% reduction in plasma cholesterol levels could occur in the livers of rats consuming the a'-subunit. The tight binding of BPs with taurocholate, deoxytaurocholate, and glycodeoxycholate can also lead to decreased cholesterol absorption in the intestine [93]. Soybean peptides (LPYP, IAVPGEVA and IAVPTGVA) can activate the LDLR-SREBP 2 pathway to increase LDL uptake effectively. For moderate hypercholesterolemia, 30 g/ml lupine protein consumption effectively reduced the Proprotein Convertase Subtilisin/Kexin type 9 enzyme (PCSK9). Inhibiting HMGCoA reductase activity on HepG2 cells may explain the hypocholesterolemic effect of lupine protein hydrolysate [94]. In addition, peptides cause the regulation of lipoprotein b-VLDL cholesterol receptors to increase in rat liver [95].

### 3.4 Antioxidants

The antioxidant properties of peptides have more to do with their composition, structure, and hydrophobicity [62]. The amino acid sequence of these peptides can determine different biological activities. Amino acids Tyr, Trp, Met, Lys, Cys, and His are examples of amino acids that cause antioxidant activity [96]. BPs from some legumes have antioxidant properties, for example, soy peptides with 4–16 amino acids [42, 43] (**Table 3**). This table also shows that BP of Leu-Leu-Pro-His-His from soybean β-conglycinin hydrolysate has antioxidant properties. The amino acid leucine or proline at the N-end can increase its antioxidative activity [35]. Amino acids with aromatic residues can donate protons to electron-deficient radicals. This property enhances the radical scavenging character of amino acid residues. Amino acids in the C-terminal region can increase the antioxidant activity higher than in the N-terminal region. This increase in antioxidative activity relates to the nature of the electronic, hydrophobic, steric, and hydrogen bonding amino acids in the area [39]. Soy milk has significant antimutagenic and antioxidant activity. So fermented soy milk probably can prevent mutagenic and oxidative damage [97]. Consumption of douchi (fermented soy food) extract will increase the activity of SOD (Superoxide dismutase) in the liver and kidneys of mice. This consumption also reduces the serum TBARS (Thio Barbituric Acid Reactive Substance), which will increase catalase activity (CAT). These results may indicate the involvement of BPs and free amino acid components from douchi extract as antioxidants [98].

### 3.5 Antimicrobial

The ability of BPs as antimicrobial peptides (AMP) has also been widely researched and studied [65]. For example, Pina-Pérez and Ferrús-Pérez [55] studied AMP from several legumes against bacterial pathogens that cause foodborne diseases. AMP is generally active against a broad spectrum of microorganisms, including bacteria (Gram+ and Gram-), fungi, and viruses [99]. Some AMPs also show additional activity, such as antioxidant activity [100], immunomodulation [101] and wound healing activity [102]. Therefore, this AMP may be a better choice of antibiotics for pathogenic bacteria resistant to conventional antibiotics.

AMP has various characteristics, including amino acid length (between 12 and 50), amino acid composition, charge and position of disulfide bonds [103]. AMP isolated from soybeans showed that long-chain peptides had higher AMP activity

than short peptides [55]. AMP interacts with microbes due to positive charges or hydrophilic and hydrophobic (amphipathic) terminal amino acids, recognised as a prominent structural motif. The charge, hydrophobicity and length of cationic AMP are directly related to their potential as antimicrobials [103]. AMP will cause changes in permeability and osmotic disturbances in bacterial cell membranes [104]. AMP can directly kill bacteria by creating pores through the bacterial cell membrane [101] or interacting with macromolecules in microbial cells [105]. The structure and sequence of peptide amino acids are the main factors for whether or not it is effective as an antimicrobial [104]. Some AMPs are rich in positively charged amino acids (arginine and lysine). Such AMP can enter microbial cells by inducing energy-dependent endocytic pathways such as micropinocytosis [106]. **Table 3** shows some of the AMP amino acid sequences from soybeans.

### 3.6 Anti-inflammatory

Inflammation is a natural immune system reaction to fight disease. Inflammation is generally associated with cancer because it involves the interaction of various immune cells that can lead to signals of proliferation, growth and invasion of tumour cells [107, 108]. There are two pathways of inflammatory-cancer interaction, namely the extrinsic pathway (inflammation facilitates cancer development) and the intrinsic pathway (genetic changes causing cancer to stimulate the inflammatory process to support tumour development) [109]. Bastiaannet and co-workers [110] and Crawford [111] reported that anti-inflammatory therapy could reduce or prevent cancer risk. This report shows the interaction between inflammatory-cancer. So far, lunasin, VPY and -glutamyl peptides have anti-inflammatory activity [70]. Lunasin exerts an anti-inflammatory effect by inhibiting the Akt-mediated NF-kB pathway [57]. BPs from legumes, particularly soybeans, can regulate several inflammatory markers, which include prostaglandin E2 (PGE2), nitric oxide (NO), induced nitric oxide synthase (iNOS), cyclooxygenase 2 (COX2), cytokines, and chemokines [70]. BPs from *Phaseolus vulgaris* L (the result of hydrolysis of alcalase and digestive enzymes pepsin and pancreatin) also showed similar results, inhibiting important markers and mediators of the inflammatory process [71]. Therefore BPs from *Phaseolus vulgaris* L can assist in managing diseases associated with chronic inflammatory processes such as T2DM and cancer. Several peptides from *Phaseolus vulgaris* L with anti-inflammatory activity are low molecular weight (MW) and contain 3–11 amino acids (**Table 3**). A lunasin-like peptide with low MW (5 kDa) inhibited the most potent pro-inflammatory markers than peptides with MW 8 and 14 KDa [112]. Biopsy of the small intestine mucosa showed repair in intestinal inflammation after supplementation of tempe in the diet [8]. Tempe contains many easily digestible compounds such as peptides and free amino acids that are affecting intestinal growth.

### 3.7 Anti-cancer

Many researchers are experimenting with cancer therapy using BPs from various legumes. The results are more promising, cheaper, and safer than cancer treatment using surgery, chemotherapy, or radiotherapy, which have adverse side effects due to the emergence of drug resistance and radio-resistance [113]. Extensive exploration has shown that a high intake of legumes can significantly reduce the risk of colorectal adenoma [114, 115] BPs with anti-cancer activity have a relatively low molecular weight (**Table 3**), as isolated from black soybean by-products have the sequence Leu/Ile-Val-Pro-Lys [116]. In comparison, lunasin from soybeans contains 43 amino acids [58]. The hallmark of lunasin is the Arg-Gly-Asp sequence which

functions for adhesion to the extracellular matrix, and the 8 Asp sequence to bind chromatin [117]. Kim and co-workers [118] said that hydrophobic BPs isolated from soybeans could act as anti-cancer. Some legumes also have higher hydrophobic amino acids that are similar in levels to soybeans, such as mung bean, chickpea, and velvet bean (**Table 1**), thus potentially producing anti-cancer peptides.

The mechanism of inhibition of tumour growth by BPs varies depending on the variety of legume sources, namely by induction of extrinsic apoptosis [119], induction of chromatin condensation [59] or inhibition of inflammatory processes [120]. BP isolated from chickpea (*Cicer arietinum* L.) inhibits the proliferation of breast cancer cells effectively [60]; this BP has the sequence ARQSHFANAQP. Meanwhile, BP from *Phaseolus vulgaris* (cultivar extra-long autumn purple) can also inhibit the proliferation of human tumour cells by inducing apoptotic bodies and nitric oxide [59]. Apoptosis (programmed cell death) is a complex process coordinated by specific target proteins and, in many cases, possibly responsible for the potential anti-cancer effects [121]. Lunasin can reduce the incidence of skin tumours by 70% [58]. Also, it inhibits gastrointestinal cancer cells [122] and cardiovascular and immunological disorders [123]. The researcher reported that consumption of legumes could reduce the risk of 10 kinds of chronic diseases, including breast cancer, lung cancer and colon cancer [124]. Consumption of legumes in higher amounts will lead to a lower risk of death from cancer [125].

## 4. Bioacessability, stability, and bioavailability of bioactive peptides

Bioaccessibility, stability, and bioavailability are the main concerns in utilising bioactive peptides (BPs) from food ingredients to remain active in maintaining a healthy body. Bioaccessibility is the first step in the digestive system so that nutrients/BPs out of the food tissue and transported across the intestinal epithelial barrier into the blood circulation system. BP transport processes may involve passive transport (paracellular or passive diffusion) or active routes [126]. During the nutrient transport process, the stability of the material must be kept high, so the bioavailability of nutrients is maintained to be utilised by target cells or tissues. In the digestive tract, nutrients are released from the food matrix and converted into chemical forms that can bind to and enter intestinal cells or pass between them. Dietary factors can also affect the bioavailability of the BPs contained. Interactions between peptides and components of the food matrix can modulate their digestibility and alter the absorption route of the peptide [10]. The release of nutrients in the small intestine starts from chewing, which involves digestive enzymes in the mouth and then in the stomach mixed with acids and enzymes in gastric juices. This whole process is a process for making nutrients biologically accessible [127].

Although the number of active components in the food consumed is abundant, it cannot necessarily prevent disease because it depends on the amount available to function in target organs or tissues [128]. Bioavailability is the number of bioactive compounds that organisms can use effectively [129]. For example, when food contact with the mouth or gastrointestinal tract, various interactions can affect the bioavailability of food nutrients (e.g., the presence of fat can increase the bioavailability of quercetin in food) [130]. In studying the role of bioactive compounds in human health, several factors can inhibit the bioavailability of the active components for use in target organs or tissues [131]. For example, fruit antioxidants mixed with macromolecules form a food matrix such as carbohydrates, fats, and proteins [132].

From a nutritional point of view, bioavailability refers to several nutrient fractions or bioactive compounds that are ingested and can reach the systemic circulation and can finally be utilised [133]. Besides that, bioavailability is the

fraction of a nutrient stored or available for a particular physiological function [134]. Another definition, bioavailability, is the amount of active metabolite from the oral dose fraction reaching systemic circulation [135]. The bioavailability of oral BPs is limited because their release from the plant matrix is affected by: solubility in GI fluids, permeability in intestinal epithelial cells, enzymatic and chemical reactions in the GI tract [136]. Four essential steps are required to absorb bioactive compounds effectively: (a) release from the food matrix; (b) incorporation into bile salt micelles; (c) absorption by epithelial cells; and finally; (d) incorporation into the cyclomicron secretion into the lymphatic system.

The biological effects of a BP depend on its capacity to survive until it reaches the target organ. Thus, the main requirement of a BP is its stability or resistance to gastrointestinal enzyme hydrolysis, brush border and serum peptidase. Experimental evidence shows that the length of the peptide chain determines the ability of BPs to pass through the intestinal epithelium in humans by different mechanisms. For small peptides, it is possible to transport through active basolateral, while for large peptides through a transport mechanism mediated by exocytotic-vesicles [137].

However, many peptides are biologically active but are unlikely to be absorbed in the gastrointestinal tract via local effects or receptors that release hormones and cell signalling in the gut. Such BPs affect gastric emptying, gastrointestinal transport, nutrient absorption (amino acids, glucose, lipids) and composition of the colon microflora. They may also regulate food intake [138].

In addition to the presence of specific residues, charge, and molecular weight, hydrogen bonding potential and amino acid hydrophobic tend to affect the bioavailability resistance of BPs to proteases and enzyme hydrolysing peptides [11, 139, 140]. Lunasin, a BP isolated from soybeans and cereal (wheat, barley and rice), has 43 amino acids (MW 5.4 kDa), displays a helical structure and contains nine aspartic acid residues in the C-terminal region. Lunasin is highly bioavailable, heat-stable (100°C, 10 min), and anti-cancer against carcinogenic chemicals. In vivo digestibility of lunasin-fortified soy protein was studied in mice fed for four weeks [141].

During transit in the central digestive tract, the structural properties of the peptide will influence the stability of BPs, including molecular weight, charge, amino acid sequence, and hydrophobicity [126]. Tests using Sprague Dawley rats showed that the highest absorption of ACE inhibitor BPs was in the jejunum [7]. The results showed that BPs with 2–6 amino acids were easy to absorb than proteins and free amino acids [142]. Small (di- and tripeptide) and large (10–51 amino acids) peptides can pass through the intestinal barrier and exhibit their biological function at the target tissue level. However, as the molecular weight of BPs increases, their chances of passing through the intestinal barrier decrease further [143]. The presence of proline and proline hydroxyl will result in resistance of BPs to digestive enzymes, especially a tripeptide with Pro-Pro at the C-terminal [144]. In another study, the number of peptides in human plasma increased depending on the dose of the BP administered. Thus, it concluded that the saturation of BP transporters could affect the number of peptides that can enter the peripheral blood [145].

Encryption of BPs in their natural protein structure may protect these BPs from gastric digestion. Another way to protect BPs is to modify structural proteins such as phosphorylation of serine, threonine, or tyrosine can prevent hydrolysis by digestive proteases. As a result, protein or peptides have a greater chance of being absorbed in target organs or tissues [146]. Stability also depends on the degree of hydrophobicity/hydrophilicity. The more hydrophobic the structure, the more difficult it is to attack by proteases [147].

Therefore, it explained that the difference in bioavailability of BPs between in vitro and in vivo tests (after oral consumption), which may be smaller or larger,

occurs due to an increase or decrease in BPs after being catalysed by gastrointestinal proteases. A simulation test of the gastrointestinal digestion process of several tempe legumes (*Phaseolus lunatus* L, *Canavalia ensiformis* L, *Mucuna pruriens*) showed that the proteolysis process by the digestive enzyme pepsin-pancreatin increased ACE inhibitory activity [7, 31, 34]. Another example is that BPs' anti-oxidant activity in vivo is more significant than in vitro [148]. The shape of the molecular structure also influences the stability of BPs. For example, a small BP (YPI) isolated from ovalbumin has additional stability when tested in GI digestive system simulation. BPs (YPI) and peptides containing P at the C end (RADHP and ADHP) are stable. If their structure is slightly modified by adding one or two amino acids to the C end (e.g. RADHPF, RADHPFL, FRADHPFL), they become unstable in simulated GI hydrolysis [149].

Finally, the use of BPs in nutraceutical and pharmacology for human health is still limited. For that, it is necessary to evaluate: (1) degradation of BPs by proteases in the digestive tract, which can affect bioaccessibility, stability, and bioavailability; (2) the existence of technology that allows modification of the structure of BPs such as (a) phosphorylation of amino acids in BPs to make them more resistant to hydrolysis by digestive enzymes; or (b) increase the amino acid hydrophobic at the N-terminal or C-terminal [150].

## 5. Technology for bioactive peptides

In general, protein-rich foods that undergo processing involving protease enzymes will produce peptides. However, not all peptides resulting from protein hydrolysis of foodstuffs will become bioactive peptides (BPs) beneficial to body health. The structural properties of BPs (composition, amino acid sequence, hydrophobic amino acid content, and resistance to digestive enzymes) will determine their beneficial functional properties [126], such as, example anti-diabetic, anti-hypertensive, cholesterol-lowering, antioxidant, and other functional properties.

Food processing processes related to conventional BPs production include cooking, ripening, fermentation and germination. In principle, the processing involves protease enzymes, e.g., chymotrypsin, trypsin, papain, thermolysin, and others) [151], either in the form of free or immobilised enzymes. For food processing by fermentation, protease enzymes derived from microbes are used in the process, while for germination, the enzymes are from growing seeds. Production of BPs increased by regulating the types of enzymes, microbes used, and germination time. Combining these processes (enzymatic process followed by fermentation, or vice versa) will increase the production of BPs so that it is more optimal [152]. The conventional production of the BPs product was a low amount and purity, making it less effective for the industrial scale [153]. So this conventional method for producing BPs does not necessarily involve a separation and purification process, but the production of functional foods containing healthy BPs in the form of fermented food products [153].

The process technology used to produce functional or nutraceutical food will affect the functional, nutritional and biological properties of the protein in the food. Therefore, several things to pay attention to, namely: (1) the effect of using a thermal (or non-thermal) process on the components of the food produced, including its effect on its functional properties and preservation capabilities; (2) available extraction processes and formulations and their optimization; (3) innovative and sustainable applications that can be developed [127]. In addition, consideration of the choice of processing technology must also be based on the desired nutritional function and appearance and sensory properties (such as colour, texture, and taste in the mouth) to be attractive to consumers [154]. Thermal processes can encourage

non-enzymatic Maillard reactions between amino groups and reducing sugars [155]. This process will produce colour, sensory properties that affect consumer acceptance and reduce the activity of BPs [155, 156]. The use of thermal processes (e.g. boiling, cooking, blanching, frying, and sterilising) for softening cell walls and inactivate microorganisms and enzymes to make the shelf life longer [127]. The development of non-thermal processes has several weaknesses; for example, the use of nanofiltration membranes requires energy [157]. Freeze-drying, encapsulation, and solvent extraction techniques are costly. To overcome this limitation, food technology experts must develop new alternative technologies (technology that can maintain bio-accessibility, stability, bio-availability and bio-activity of active components). Including BPs, processed food ingredients and the form of pure isolates (capsules or nanocapsules).

The production of BPs has become more accessible, faster, and more effective with the development of science and technology. Production of BPs on an industrial scale usually uses an enzyme hydrolysis process. So the BPs production process uses computer equipment and database search algorithms to predict target peptides and their properties. By selecting the correct protease enzyme through the database, it is possible to select the protein-enzyme combination, in-silico hydrolysis, and the nature of the peptide to be produced [152, 153, 158]. This in-silico hydrolysis method is a functional and widely practised approach for producing legume BPs (**Table 3**).

The legume or various food peptides resulting from enzymatic hydrolysis was then fractionated and purified using a combination of various chromatographic techniques [158–160]. Isoelectric focusing and ultrafiltration are separate macromolecular compounds (such as protein and pectin). Meanwhile, extraction techniques use solvents or supercritical solutions to isolate small molecule bioactive compounds such as antioxidants [157, 161, 162]. This extraction technique, combined with thermal technology (e.g. pasteurisation or spray drying), has been applied to functional foods. This conventional food processing technology is well documented and well established, but its application for the isolation of BPs still needs development and improvement.

The weakness of current technology is that there is still a need for studies on product safety for health. For example, advanced technologies such as cold plasma, nanotechnology, ultrasound, and others, are thought to affect advanced lipid oxidation processes and cause cell tissue damage. For this reason, the effect of this advanced technology on the safety and health of the food components produced needs to be studied to obtain a complete understanding. In this case, it is necessary to adapt the product and technology to the desired functional properties of the active ingredient. For example, modification or interaction with other macronutrients (e.g. dietary fibre) can increase the bio-availability of bioactive compounds [163].

On the other hand, encapsulation technology using legume protein ingredients as a material is also a technique for providing chemical compounds found naturally in plants and other nutraceutical compounds (such as vitamins, minerals, BPs, or others). Thus this encapsulation allowing these compounds (including BPs) to enter the body and undergo release and degradation by enzymes digestion [164]. Other technologies used to protect the active ingredients or nutraceuticals (such as BPs and others) are encapsulation, edible films and coatings, and vacuum impregnation. One may be promising is nutrigenomics, where the active ingredients are given to individuals on a Taylor-made basis according to the genetic characteristics of each individual [165].

Although several researchers have evaluated and characterised BPs that BPs isolated from food have potential bioactive activity and therapeutic functions, and have high bio-availability (bio-accessibility) (due to the support of excellent and modern processing technology), however, all of them can only have a positive impact on human health when combined with healthy living habits [4].

## 6. Conclusions

Legumes have various biological activities that are good for body health, such as antihypertensive, anti-diabetic, anti-cancer, antioxidant, and others, but legumes also contain anti-nutritional compounds. Food processing is an effective process to remove anti-nutritional compounds and, at the same time, can produce BP compounds that are healthy for the body. Although the number of active components in food is abundant, it is not necessarily able to prevent disease because it is very dependent on the amount available to function in target organs or tissues. One of the contributing factors is the BPs enzyme in holding the action while in the digestive tract. Some legumes showed that hydrolysis by these enzymes increased their bio-accessibility and bio-availability in the digestive tract of rats (in antihypertensive testing). Due to the diverse nature of BP, it is necessary to develop technology that is following the desired functional properties of BP, for example, to protect it so that it is stable while passing through the digestive tract using microencapsulation, edible film and coating technology. Further research still needs to be developed related to the study of safety separation technology for the products produced. From the excellent stability and bioavailability of BPs from legumes, it is likely to be more promising to develop alternative healthy functional food products containing BPs and sensory properties that attract consumers.

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