Review Article

A Review on Factors and Processing Methods Affecting Antihypertensive Properties of Legumes, and Antihypertensive Properties of Selected Legumes.

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ABSTRACT

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Hypertension is one of the major risk factors which leads to cardiovascular diseases, and the typical treatment for hypertension is drug therapy. However, as there are side effects to drug therapy, there has been an increase in research on legume proteins as biopeptides have shown antihypertensive effects. Many species of legumes have been cultivated for consumption as they are a good source of protein, carbohydrates, minerals, vitamins, and dietary fiber. Bioactive compounds of legumes potentially improve factors that affect antihypertensive properties, such as angiotensin-converting enzyme (ACE) inhibition activity, renin inhibition activity, and γ-aminobutyric acid (GABA) production. The processing method of enzymatic hydrolysis can improve ACE inhibition activity, as can be seen with horse gram, pigeon pea, and lentil hydrolysates containing potent ACE inhibitory peptides of Thr-Val-Gly-Met-Thr-Ala-Lys-Phe, Val-Val-Ser-Leu-Ser-Ile-Pro-Arg, and Asn-Ser-Leu-Thr-Leu-Pro-Ile-Leu-Arg-Tyr-Leu, while pigeon pea and kidney bean hydrolysates have shown good renin inhibition activity. Fermentation can also be used to process legumes as potent ACE inhibitory peptide Val-Val-Ser-Leu-Ser-Ile-Pro-Arg was identified from fermented pigeon pea, while kidney beans and lentils demonstrated good GABA production through natural fermentation and fermentation with microbial cultures. Germination processing method could also help improve ACE inhibition activity as horse gram has shown good inhibition activity. *In vivo* study of pigeon pea, kidney bean, and lentils showed potential antihypertensive properties as a significant lowering of systolic blood pressure of test subject was observed. Research done on the structure and function of antihypertensive properties of legumes can help in the development of functional food products which will be beneficial to human health.

Keywords: antihypertensive property, legume, bioactive compound, inhibition activity

1. INTRODUCTION

Hypertension is the leading cause of premature death worldwide as it increases the risk of mainly heart, brain, and kidney diseases [1]. Hypertension occurs when a person's systolic blood pressure (SBP) is above 140mm Hg and diastolic blood pressure (DBP) is above 90mm Hg [2]. The first step to treating hypertension includes a diet of moderate sodium intake, reduced alcohol consumption, and increased physical activity [3]. However, if hypertension has already progressed to a more dangerous stage drug therapy would be necessary, and some commonly applied drugs include ACE (Angiotensin I-converting enzyme) inhibitors, angiotensin II receptor antagonists, calcium channel blockers, and β-blockers [3].

Although research on drug therapy has shown that it can treat hypertension, side effects of these drugs have also been reported in various studies [3]. For example, the usage of ACE inhibitors such as captopril has reported side effects on taste disturbances and skin rashes; calcium channel blockers such as nifedipine are used with caution due to hypotension risk, and β-blockers usage should be monitored as symptoms of depression and fatigue have been reported [3]. The negative side effects of drug treatments have encouraged research on bioactive compounds present in foods that contain potential antihypertensive properties. Developing natural antihypertensive peptides from foods has the benefits of specificity, potency, and potential administration at high doses due to low toxicity [2]. Milk and egg protein hydrolysates are highly studied as antihypertensive activity has been observed in peptides produced [4]. Although plant proteins may not have received as much attention as animal proteins, there has been increasing interest as widely consumed plants of wheat, rice, pea, soybean, and corn have been shown to contain antihypertensive peptides [4].

There has been an increase in research on legume proteins and their biopeptides due to their potential antihypertensive properties [5]. Soybean is one of the most studied legumes as it is highly consumed, while other legumes such as chickpea and yellow pea proteins have also been widely studied and shown high ACE inhibition activity [5]. Biopeptides are isolated from legume proteins by applying a range of different methods such as enzymatic hydrolysis, fermentation, and germination [4]. Further analysis of these biopeptides through fractionation and purification methods helps to determine amino acid sequences and improve understanding of structure-

function properties that contribute to antihypertensive activity [2]. This review discusses the antihypertensive properties of selected legumes of horse gram, pigeon pea, kidney bean, and lentils, as well as processing methods of legumes. The most common factor of antihypertensive properties, which is ACE inhibition activity will be discussed, along with other antihypertensive factors that have been observed in the legumes such as renin inhibition activity and GABA production.

2. LEGUMES

Legumes comprise 18,000-19,000 species including grain, pasture, and agroforestry species that can be classified into 670-750 genera [6]. These plants have unusual flowering structures, podded fruit, and 88% of the species that have been examined can form nodules with rhizobia, a diazotrophic bacteria that fix nitrogen which aid in plant metabolism [6]. Legumes are also referred to as Fabaceae and a substantial number of species have been domesticated, for example, soybeans, fava bean, chickpea, lentil, peanut, pigeon pea, and mung bean [7]. These legume crops have a global production of 74.7 million tons and occupy 81.8 million hectares across the world; where dry beans constitute 34% of production, followed by chickpea (18%), field pea (14%), pigeon pea (6%), lentil (6%), and fava bean (5%) [8]. These grain legumes produce edible seeds which are how they are usually consumed and are a vital component of human nutrition as they supply high amounts of plant protein [9]; [7]. There are many uses of legumes as a food ingredient as they can be milled into flour and used to make baked goods; or they can be processed into liquid form which is then used to produce milk, yogurt, and infant formula [6].

Legume crops are grown across the world as they are a good source of protein, minerals, vitamins, and provide adequate amounts of lysine [9]. Commonly consumed examples of legumes such as peas, beans, peanuts, and soybeans provide high protein content ranging from 17% - 40%, which is comparable to meats (18% - 25%) [10]; [11]. Legumes also contain high carbohydrate content which ranges from 50% - 65%, along with high insoluble and soluble fiber contents [12]. Additionally, they are good sources of B-vitamins including niacin, thiamine, riboflavin, and minerals such as iron, zinc, and calcium [11]. Phytochemicals such as phenolic acids, anthocyanins, proanthocyanidins, and flavonols have also been reported in various legumes [8].

High carbohydrate and high fiber contents of legumes were found to help in weight management as resistant starch increases satiety, reduces glycaemic response, and improves insulin resistance [11]. Analysis of legume replacement of foods in the diet demonstrated that systolic and mean arterial blood pressure were reduced, which could be associated with the

high fiber content from legumes [12]. Further research on bioactive compounds of legumes showed potential health benefits [10]. For example, research on hyacinth bean (*Lablab purpureus*) showed potential antihypertensive properties; while Kudzu (*Pueraria Montana* var. lobata Willd.) is a source of isoflavone daidzein which is a potential antihypertensive, antimicrobial, and anti-inflammatory agent [10]. Antidiabetic properties were observed where black bean anthocyanins inhibited intestinal glucose transporters, α-glucosidase, α-amylase, and dipeptidyl peptidase IV (DPP-IV) [8].

Studies have also demonstrated the potential antihypertensive properties of legumes as dietary fiber, protein, and potassium contents are associated with blood pressure reduction, as well as a decrease in *in vivo* oxidation, reduce systemic inflammation and improve arterial compliance [13]. There has been an increase in research suggesting a reduction of *in vivo* oxidation helps to decrease cell damage and deregulated production of adipocytokines due to oxidative stress decreases obesity-associated insulin resistance and hypertension risk [14]. Minerals that are reported to be found in legumes such as potassium, calcium, and magnesium could facilitate the synthesis of vasodilator prostacyclin and nitric oxide (NO) to reduce peripheral vascular resistance; providing further evidence that increased legume intake is beneficial for blood pressure reduction [13].

3. FACTORS AFFECTING ANTIHYPERTENSIVE PROPERTIES

As legumes have been cited by many sources as potential antihypertensive food, further studies have been done to determine how legumes could contribute to this property. One of the most studied factors that provide legume antihypertensive property is the angiotensin I-converting enzyme (ACE) inhibition activity; however, there has also been growing research on other factors such as renin inhibition activity and GABA production, which will be discussed further.

3.1 ACE Inhibition Activity

Angiotensin I-converting enzyme (ACE) inhibition activity of legumes has been widely studied as it is one of the most common antihypertensive properties that legumes exhibit. ACE is a dipeptidyl carboxypeptidase that is a key component of the human Renin-Angiotensin-Aldosterone System (RAAS), where RAAS is responsible for blood pressure, volume, and electrolyte management which affect the heart, vasculature, and kidney [15]. This membrane-bound exopeptidase is localized on plasma membranes of various cell types such as vascular endothelial cells, microvillar brush-border epithelial cells, and neuroepithelial cells [15]. ACE hydrolyses the inactive decapeptide angiotensin I, causing the removal of the C-terminal dipeptide to form octapeptide angiotensin II, which is an active, potent vasoconstrictor

[16]. It is also involved in the degradation of peptides such as bradykinin and kallidin, which are vasodilator peptides to inactive products [15]. The disturbance of balance caused by increased ACE activity would lead to vasoconstrictive and salt-retentive angiotensin II, and decreased vasodilatory and natriuretic bradykinin, which could disrupt blood pressure regulation [17].

The inhibition of ACE is one of the treatments for hypertension, which led to the development of chemical synthetic drugs that are potent and specific ACE inhibitors [16]. ACE inhibitors restore the balance disrupted by increased ACE activity through the decreased formation of angiotensin II and bradykinin degradation [17]. Some synthetic ACE inhibitors that are currently used for hypertension treatment include captopril, enalapril, and lisinopril, but these synthetic inhibitors have also induced side effects such as hyperkalemia, dry cough, angioedema, and skin rashes [17]. This encouraged various research to determine biopeptides from food proteins that could produce similar effects to synthetic ACE inhibitors. Different processing methods such as enzymatic hydrolysis, gastrointestinal digestion, and food processing are used to activate biopeptides that are encrypted within parent proteins [18].

Most of the ACE inhibitory peptides that have been isolated and identified were short peptides with 2-20 amino acid residues that exhibit different activities [19]. These amino acid sequences suggested that the C-terminal tripeptide sequence strongly influences ACE binding, where potent ACE inhibitors were observed in peptides with hydrophobic amino acid residues such as Pro, Phe, and Tyr at the 3rd C-terminal position [19]. The ACE inhibition activity of the peptides is also affected by N-terminal branched-side aliphatic amino acids such as Gly, Ile, Leu, and Val, and whether the C-terminal contains a proline [20]. Studies have found that some ACE inhibitor peptides tend to show better activity in vitro compared to in vivo, which could be caused by intracellular peptidases or enzymes in the digestive tract that degrades the peptides [20]. The IC_{50} value is used to express the potency of the ACE inhibitory peptides, which measures the amount of peptide needed to inhibit 50% of ACE activity [21]. Other ways to express ACE inhibitory activity are the ACE-inhibition index or by defining the concentration of an inhibitor as percentage inhibition [18]. Before comparing ACE inhibitory activity between peptides, reaction parameters such as nature and concentration of substrate, volume, enzyme quantity, and detection methods of reaction products should be considered to prevent making biased conclusions [21].

3.2 Renin Inhibition Activity

RAAS is an important structure in the homeostatic control of arterial pressure and functions as an endocrine axis that allows the formation of

vasoconstrictor angiotensin II in the extracellular space [15]. The first and rate-limiting step of the RAAS pathway is catalyzed by renin, an enzyme that is part of the aspartate protease family that includes pepsin, chymosin, and cathepsin D [22]. The formation of the decapeptide angiotensin I by renin, which cleaves the N-terminal portion of the globulin, Angiotensinogen, helps to regulate the RAAS. Renin secretion into the bloodstream through biosynthesis by the juxtaglomerular cells occurs when there is a decrease in perfusion pressure or NaCl delivery, and an increase in sympathetic activity [15]. Prorenin is produced by renal cells, and later converted to mature and active renin through proteolytic removal of a 43-amino-acid pro-segment peptide from prorenin N-terminus by enzymes such as proconvertase 1 and cathepsin B [15];[22]. In human circulation about 70% to 90% of immunoreactive renin is prorenin, and it can exhibit a part of the proteolytic activity of renin to convert angiotensinogen to angiotensin-I when bound to the respective receptor. The measurement of plasma renin activity to represent physiological total renin activity is a good indicator of hypertension risk [22].

As renin is important for catalysis of the first step of the RAAS pathway, the inhibition of renin suppresses the upstream angiotensin I production which will reduce angiotensin II concentration [22]. A commonly used renin inhibitor for high blood pressure treatment is aliskiren, which binds to the proteolytically active site of renin with high specificity [23]. Administration of aliskiren was shown to produce comparable reductions of systolic and diastolic blood pressure to ACE inhibitors. However, some adverse effects that would be expected of aliskiren due to its mechanism of action include hyperkalemia and hypotension. Aliskiren is the only renin inhibitor that has been approved for blood pressure control, as the development of renin inhibitors proved challenging due to ineffective *in vivo* activity [24].

While synthetic inhibitors have been approved for hypertension treatment, there are still questions surrounding their side effects which have led to research on renin inhibition through natural products such as food proteins. Food protein-derived peptides which contain N-terminal aliphatic residues such as leucine, isoleucine, valine, and C-terminal bulky amino acid residues such as phenylalanine or tryptophan were hypothesized to provide higher renin inhibition activity [25]. Renin inhibition activity has been reported in hydrolysates of seed protein, microalgae protein, and animal protein [24].

3.3 γ-Aminobutyric Acid (GABA) Production

γ-Aminobutyric acid (GABA) is an inhibitory neurotransmitter found in the mammalian central nervous system, and present in brain regions in high concentrations. GABA production requires a precursor which is typically glucose, however, pyruvate and other amino acids could also be used [26].

GABA is also produced in plants as a result of stress because the increase of cytosolic Ca^{2+} levels causes glutamate decarboxylase or glutamic acid decarboxylase to catalyze irreversible decarboxylation of L-Glu to GABA [27]. Studies have shown that GABA exhibits antihypertensive properties as it secretes excess salt to reduce blood pressure [28]. The antihypertensive property is suggested to be due to GABA receptors on the cell membrane being activated by GABA, which opens chlorine ion channels for an influx of chloride ions into nerve cells leading to cell membrane hyperpolarization and excitability reduction. GABA by itself can decrease blood pressure by regulating antidiuretic hormone vasopressin levels which cause vasodilation [28].

Although synthetic GABA drugs were observed to have potential antihypertensive properties, clinical studies have found that synthetic GABA is not as effective as natural GABA and contains serious side effects as opposed to natural GABA which has minimal side effects [29]. Various research has been done to develop GABA from natural sources of animals, plants, and microorganisms as a functional food ingredient that can provide the same effect as synthetic drugs but with lesser to no adverse side effects [27]. Some natural sources that have been studied include cereals, barley, beans, and vegetables [27]. Determining a natural way to synthesize GABA has led to the development of different techniques that can optimize and enhance GABA production. For example, GABA content in soybeans was increased by 20-40-fold through mechanical and cold stimulation [27]. Another study found that GABA was accumulated in green tea through anaerobic fermentation and showed that the GABA-enhanced tea was able to reduce blood pressure [29].

4. PROCESSING METHODS

Different methods were used by different studies to process legumes before analysis was done to determine the antihypertensive properties of legumes, and some legumes had a combination of processing methods depending on the aim of the study. The results of antihypertensive properties that were obtained from the methods reviewed were briefly discussed.

4.1 Enzymatic Hydrolysis

The use of the enzyme hydrolysis method to generate protein hydrolysates is efficient and dependable due to its productivity, scalability, and reaction time. Protein hydrolysates can exhibit various biological functions which are associated with biopeptides present in hydrolysates that are 3-50 amino acid residues long [19]. Based on the enzymatic hydrolysis process the molecular weight, amino acid composition, and sequence of peptides can vary which then affects their biological activities [19].

Biopeptides can be produced by adding enzymes sequentially or simultaneously during hydrolysis depending on the conditions in which hydrolysis is conducted. Some commonly used enzymes in the study of biological properties of legumes include neutrase, flavourzyme, alcalase, trypsin, and chymosin [30]. Endo- and exopeptidase pepsin and pancreatin are especially good in improving ACE inhibition activity as it hydrolyses peptide bonds with specificity to liberate higher hydrophobic amino acids which are useful ACE inhibitory peptides [30].

A study on the legume tarwi (*Lupinus mutabilis* Sweet) used different enzymes such as alcalase, neutrase, and flavourzyme separately and in combination to observe their effects on antihypertensive properties [31]. The study found that in general, the use of two enzymes increased hydrolysis degree, but also that using flavourzyme as a secondary enzyme after alcalase or neutrase increased the breakdown of peptide bonds which contributed to the increased hydrolysis degree. Results also showed that antihypertensive activity was higher for hydrolysates that used two enzymes and that a combination of alcalase and neutrase showed the highest ACE inhibitory activity [31].

The extraction of lima bean (*Phaseolus lunatus*) hydrolysates used a mixture of pepsin and pancreatin, another flavourzyme and alcalase [32]. The combination of flavourzyme and alcalase had a higher hydrolysis degree than pepsin and pancreatin; while the renin inhibition activity of hydrolysates produced from the flavourzyme and alcalase was also higher. However, hydrolysates of pepsin and pancreatin enzyme had the highest ACE inhibitory activity when compared to flavourzyme and alcalase, even though it was lower than pepsin hydrolysates of other legumes from separate studies due to process conditions [32].

Analysis of the stink bean (*Parkia speciosa*) and horse gram (*Macrotyloma uniflorum*) applied the use of alcalase enzyme to extract the biopeptides [33];[34]. Both studies observed that the alcalase enzyme, which has a broad specificity for protein hydrolysis would only be effective to a certain degree of hydrolysis as both studies recorded a decrease in ACE inhibition activity which suggests the production of inactive peptides. Another study obtained Bambara groundnut (*Vigna subterranean*) hydrolysates with the use of alcalase, trypsin and pepsin enzymes [35]. Comparing the hydrolysate yield of each enzyme it was observed that alcalase was the most effective to release peptides from Bambara proteins, and the hydrolysate produced with alcalase also showed the highest ACE inhibition activity [35].

4.2 Fermentation

Fermentation is a common and low-cost method that is used for legume processing and the main factors that affect the fermentation process

are microbial culture, enzyme activity, and environmental conditions of time, temperature, and pH [36]. The kind of microbial culture used affects peptides with ACE inhibitory potency, and numerous studies have found that proteolytic bacteria *Bacillus subtillus* spp., lactic acid bacteria (LAB) such as *Lactococcus* (L.) *lactis, Lactobacillus* (Lb.) *helveticus,* and moulds could produce ACE-inhibitory peptides and γ-Aminobutyric acid (GABA). These factors affect the increase of protein digestibility and the level of free amino acids, degradation of phytic acids, and decrease of ACE inhibition activity [36].

A study was done on lentils carried out both solid-state fermentation (SSF) and liquid-state fermentation (LSF) where both fermentations were run for 96 hours but SSF at 30°C and LSF at 37°C [37]. The study also compared results between two LSF of lentils where one was carried out spontaneously or naturally fermented (NF), and the other was inoculated with L. *Plantarum* (LP). GABA production observed that NF had the highest content (10.42mg/g extract) which was followed by LP (7.16mg/g extract) and BS (6.54mg/g extract); there was not much difference in total phenolic content (TPC) between both LSF conditions, but BS showed a rise in TPC, and both NF and LP showed high ACE inhibition activity, but BS ACE inhibitory activity was lower [37].

Chickpeas were also fermented by *Bacillus subtilis* which was screened from sourdough and had the highest protease activity. The sample was incubated for 72 hours at 37°C. The soluble protein content of chickpea increased as fermentation time went on, and it was found that the maximum value of soluble protein was 15.4mg/g at 48 hours, and the protease activity measured was also found that remained high after 48 hours at 5494.1U/g [38]. Another study on fermented chickpeas used *Cordyceps militaris* (L.), a medicinal and edible mushroom as the inoculation culture [39]. The study analyzed chickpeas that were fermented with C. *militaris* (CFC) at 25°C for 7 days, and non-fermented chickpeas (NFC). It was observed that hippuric acid (HA), which is released from Hippuryl-Histidyl-Leucine due to ACE action, was released in lower amounts in CFC extract presence compared to the control, while NFC extract released HA amounts that indicated no ACE inhibition activity [39].

4.3 Germination

Legume germination is common practice to prepare it for human consumption as it is a low-cost method that produces simpler compounds from storage proteins [36]. This results in significant differences in legumes before and after germination as the process changes the biochemical, nutritional, and sensory characteristics of legume seeds [36]. Germination increases protein and carbohydrate digestibility, the bioavailability of

vitamins, antioxidants, and ACE inhibition activity; as well as reduces antinutritional factors [40]. The conditions in which germination is conducted affect these changes such as temperature, soaking, culture media, germination time, humidity, light, elicitor, and most importantly, the type of legume used [40];[36].

A study was done on kidney bean germination using elicitor solutions of ascorbic acid, folic acid, and glutamic acid, and two other solutions of chitosan in glutamic acid and chitosan in lactic acid [41]. The study found that during the first 4 days ascorbic acid and chitosan in glutamic acid had the highest GABA content (0.70 and 0.65 mg/g d.m.). At the end of germination, the highest GABA content was found in beans with a glutamic acid solution (0.95mg/g d.m.); as glutamic acid is a substrate of glutamate decarboxylase to produce GABA. Based on the high ACE inhibition activity of all sprouts (85%-91%), elicitor solutions did not affect ACE inhibition during germination. The effectiveness of ACE inhibitory activity of bean extracts was determined through *in-vitro* gastrointestinal digestion; and results showed that IC_{50} after digestion was 0.18 mg/mL, indicating ACE inhibition potency had increased 89-fold [41].

Germination of lentil sprouts with water as the control and elicitor solutions of ascorbic acid, folic acid, glutamic acid, and chitosan in glutamic acid was done for 8 days at 20°C [42]. Determination of GABA content showed that chitosan in the glutamic acid elicitor was the most effective (2.02 mg/g dw), as the other elicitors produced GABA content similar to the control. Simulated gastrointestinal digestion was carried out and results showed that all elicitors could inhibit ACE activity [42]. Another study germinated mung beans with only water and no use of elicitors at 30°C-40°C in the dark for 5 days and found that the maximum total flavonoid content of mung beans was reached at a germination temperature of 30°C [40]. Measurement of ACE inhibition activity found that mung bean had 82% ACE inhibition at 30°C during germination, however, at 40°C the percentage of ACE inhibition decreased which suggests ACE inhibition peptides were continuously hydrolyzed [40].

5. ANTIHYPERTENSIVE PROPERTIES OF SELECTED LEGUMES

Research has been carried out on various legumes to determine their potential as an antihypertensive and substitute for synthetic drugs. The most common antihypertensive property that is present in legumes is ACE inhibition activity, however, there has been an increase in studies looking at renin inhibition activity, GABA production, and polyphenol content as it has been observed to contribute to antihypertension.

Table I: List of the scientific name, common name, and antihypertensive properties of some legumes.

Legume **Scientific Name**	**Common Name**	**Type of antihypertensive property**
Parkia speciosa	Petai, Stink Bean, Bitter Bean	ACE inhibition
Lens culinaris var. *castellana*	Lentil	GABA production / ACE inhibition
Macrotyloma uniflorum	Horse gram	ACE inhibition
Lupinus albus, cv ares	Lupins	ACE inhibition
Pisum sativum	Pea	ACE inhibition
Cicer arietinum	Chickpea	ACE inhibition
Phaseolus vulgaris L., var Nayarit Black	Black bean	ACE inhibition
Glycine max	Soybean	ACE inhibition
Vigna unguiculata L.	Cowpea	ACE inhibition
Cajanus cajan	Pigeon pea	ACE inhibition/renin inhibition
Phaseolus vulgaris var. *Pinto*	Kidney bean	GABA production / ACE inhibition
Lupinus mutabilis Sweet	Tarwi	ACE inhibition
Phaseolus lunatus L.	Lima bean	ACE inhibition/renin inhibition
Vigna subterranea	Bambara groundnut	ACE inhibition/renin inhibition
Phaseolus vulgaris Pinto Group	Pinto beans	ACE inhibition
Vigna radiata var. *humilis*	Mung bean	ACE inhibition

Phaseolus vulgaris L. cv. Dermason	Common dry bean	ACE inhibition
Lens culinaris L. cv. Sultani	Green lentils	ACE inhibition
Phaseolus vulgaris L., var Azufrado Higuera	Common bean	ACE inhibition
Mucuna pruriens Linn.	Velvet bean	ACE inhibition

5.1 Horse Gram

Horse gram (*Macrotyloma uniflorum* (Lam.) Verdc.) is an underutilized legume, which is a cheap source of protein for those living in poorer conditions. It is a hardy pulse crop that is typically grown in dry and rain-fed land zones such as Australia, Burma, India, Sri Lanka, and Africa [43]. Horse gram has traditionally been used to treat jaundice, hyperglycemia, edema, rheumatism, and to help maintain body temperature during winters [43]. Horse gram being a rich source of protein, carbohydrate, micronutrient, bioactive compounds, and low lipid content has increased interest in developing it as a functional food product. Recent studies have found that bioactive compounds present in horse gram can provide ACE inhibition activity and antioxidant properties which can be released during gastrointestinal digestion or processing [34].

Jamdar [44] studied the bioactive potential of legumes and found that horse gram showed good ACE inhibition activity, as IC_{50} values that were recorded ranged between 1-5mg/ml after undergoing different processing conditions such as drying, soaking, and germination. In vitro protein digestion of horse gram further increased ACE inhibition activity as the IC_{50} values recorded after enzymatic digestion had decreased to 0.2-0.7 mg/ml [44]. Another study comparing ACE inhibitory properties of horse gram and cowpea flour to chickpea flour found that extracts of horse gram flour exhibited the highest inhibitory activity of 67.3% inhibition, whereas chickpea and cowpea extracts were observed to be 33.2% and 17.3% inhibition at the same concentration [45]. The recorded IC50 values showed that horse gram extract had IC50 of 32.8 µg/ml, which was 1.9 and 2.7 times lower than the IC_{50} of chickpea and cowpea extracts. The phenolic extract of horse gram had the highest total flavonoid content and better metal chelating ability compared to chickpea and cowpea, which was hypothesized to contribute to the good inhibitory activity of horse gram [45].

Several studies observed that ACE inhibitory peptides are typically peptides of low molecular weights as it improves bioavailability, and retentate fractions of HGH with molecular weights of < 3 KDa showed high ACE inhibitory activity [34]. Analysis of horse gram hydrolysate showed that peptides that had the highest ACE inhibitory activity were Thr-Val-Gly-Met-Thr-Ala-Lys-Phe and Gln-Leu-Leu-Leu-Gln-Gln. The peptide Thr-Val-Gly-Met-Thr-Ala-Lys-Phe, contains aromatic amino acid phenylalanine, positively charged amino acid lysine, and aliphatic amino acid alanine, which was suggested to improve the potency of ACE inhibitory peptide. The potency of the ACE inhibitory peptide Gln-Leu-Leu-Leu-Gln-Gln could be affected by glutamine at the C-terminal position, which has been shown to improve peptide potency [34].

5.2 Pigeon Pea

Pigeon pea (*Cajanus cajan* (L.) Millspaugh) is a grain legume crop that is capable of tolerating drought conditions and mainly grown in semi-arid tropical regions [46]. Compared to soybean, peas, and field beans, antinutritional factors such as protease inhibitors, amylase inhibitors, and polyphenols are less of a problem in pigeon pea [46]. Studies found that pigeon pea contains high nutritive value as its protein content reportedly ranges between 18-26%, while closely related Cajanus spp. has reported 30% protein content. This supports the observation that a significant amount of hydrophobic amino acid in the seed protein contributes to peptide bioactivity [24]. This has led to pigeon pea being identified as a potential source of ACE inhibitors due to the present peptide bioactivity [47].

A study fermenting pigeon pea identified the potential ACE inhibition peptide Val-Val-Ser-Leu-Ser-Ile-Pro-Arg [47]. This sequence was identified as a potent ACE inhibitor as it contained a high content of hydrophobic amino acids, the N-terminal has a branched amino acid valine, and the third position from the C-terminal contains a hydrophobic amino acid isoleucine [47]. Pigeon pea hydrolyzed with pepsin and pancreatin (PPHPp) and pigeon pea hydrolyzed with pepsin (PPHPe) especially showed high levels of hydrophobic amino acid (HAA) and aromatic amino acid (AAA) [24]. The presence of AAA potentially contributes to ACE inhibition activity as it interacts with the active site of ACE between the three subsites. Results showed that PPHPp had superior ACE activity inhibition (61.82%) which was suggested to be due to its higher proline content, while pancreatin hydrolyzed pigeon pea had the highest renin inhibition activity of 14.28%. The lower renin inhibition activity compared to ACE inhibition activity was hypothesized to be renin having active sites that are less accessible due to its folded protein conformation, while the active sites of ACE are easily accessible with its open conformation [24].

The antihypertensive effects of pigeon pea protein isolate (PPI) and hydrolysate in vivo were conducted using spontaneously hypertensive rats (SHR), where results showed that hydrolysates managed to reduce systolic blood pressure (SBP) by 25mmHg, but PPI had a delayed lowering effect of SBP [24]. In comparison to the synthetic ACE inhibitor captopril which had a lowering effect of -50mmHg, results suggest that the protein hydrolysates affected blood pressure reduction. The delayed lowering effect observed in PPI was hypothesized that the isolate required digestion by intestinal enzymes of the rat to utilize the peptides as it was in its intact form [24]. Another study fermented pigeon pea with *Bacillus subtilis* which produces a serine fibrinolytic enzyme called nattokinase, that helps to reduce blood clotting and prevent CVDs [48]. The effect of single oral administration of water extracts of pigeon pea decreased SBP and DBP by 9mmHg and 17mmHg, while water extracts of fermented pigeon pea decreased SBP and DBP by 21mmHg and 30mmHg. Comparison of the results to that of SHR that had oral administration of captopril and decreased SBP and DBP by 29mmHg and 36mmHg, fermented pigeon pea had exhibited antihypertensive potential [48].

5.3 Kidney Bean

Kidney beans (*Phaseolus vulgaris* L.) or also called common beans are a type of legume that is inexpensive, available, and a good source of nutrients as it contains proteins (20%-30%), carbohydrates (50%-60%), and adequate amounts of minerals and vitamins [30]. This legume is typically cultivated in Africa, India, and Latin America, with a low glycemic index that could reduce diabetes and CVD risk [49]. Although kidney bean protein globulin has a poor amount of sulfur-containing amino acids, it contains high arginine and branched-chain amino acids which are useful compounds to treat CVDs [30].

Mundi and Aluko [50] analyzed renin and ACE inhibition of kidney beans by obtaining protein hydrolysate using the alcalase enzyme. Amino acid analysis of the peptide fractions showed that high content of hydrophobic amino acids and aromatic amino acids was present in fractions containing a molecular mass of <1kDa and 5-10kDa, and these fractions also showed good renin inhibition activity. ACE inhibition activity analysis found that all peptide fractions and kidney bean hydrolysate had a high percentage (> 77%) of activity. The hydrolysate had the highest ACE inhibition activity (80%), however, the difference between inhibition activities of all samples was not significant [50]. Another study used solid-state fermentation with *B. subtilis* (SSF), liquid state fermentation naturally (NF), and *L. Plantarum* (LPF) to observe GABA production in kidney beans [51]. Results showed that SSF had a decrease in production, while NF and LPF had good GABA

content with NF having a significantly higher content than LPF. Low GABA production suggests that thermal treatment used during SSF caused inactivation of endogenous GAD activity which reduced GABA production; while *L. plantarum* had been found to contain the gene encoding GAD enzyme which improved GABA production. NF producing the highest GABA activity was hypothesized to be due to it containing a high number of GAD producing LAB strains and differing pH conditions during fermentation [51].

The antihypertensive effect of kidney beans *in vivo* was determined using SHRs, where total hydrolysates were obtained from varieties of FRI064-2109993 (PB) and FRI-001-220995 (AH) [52]. The peptidic fraction of AH F3-10 was shown to decrease SBP of the SHR by 27.13 ± 11.17 mmHg at 2 hours after an administered dose of 4mg/Kg. This lowering of SHR SBP by the peptidic fraction showed a significant difference and produced similar antihypertensive activity to that of Captopril which had a lowering of 29.67 ± 18.89 mmHg at 2 hours. The study also reported that the peptidic fraction of AH F3-10 in their study produced similar intervals of reduction of SBP that were shown in SHR after vegetable protein hydrolysate administration [52].

5.4 Lentil

Lentils (*Lens culinaris* Medikus) are important pulse crops that aid in agricultural sustainability and nutritional food security as they contain 35-53% starch, 2-4% fiber, and are also good sources of antioxidants [53]. The high protein content of lentils which depends on the species ranges from 24.3-to 30.2%, making it a good substitute for meat in developing countries as it is also affordable. Lysine, an essential amino acid is present in lentil proteins in high amounts (63-73g/kg protein), so consumption of lentils with rice or wheat can ensure the intake of adequate amounts of essential amino acids for a balanced diet [53]. Lentil proteins can also provide peptides that contribute to antihypertensive properties and help protect against CVDs [37].

Liquid state fermentation with L. *Plantarum* (LP) and solid-state fermentation with B. subtilis of lentils (BS) was analyzed for the production of antihypertensive properties [37]. Results showed that lentil GABA content increased significantly during the first 96 hours of fermentation regardless of the fermentation process, and natural fermentation (NF) produced the highest GABA content (10.42mg/g extract), followed by LP (7.16mg/g extract) and BS (6.54mg/g extract). ACE inhibitory activity of NF increased up to 92% after 96 hours, while LP had an even higher activity of 93%; however, BS inhibition activity was significantly lower with 39% after 96 hours. Based on the results, it was suggested that liquid fermentation would

be a more effective method to produce functional food proteins from lentils [37].

Analysis of lentil hydrolysates to determine peptides present in 3kDA permeates found that fragments from vicilin, convicilin, and legumin had the highest content [54]. Peptide sequences identified that showed the highest ACE inhibition activity was Asn-Ser-Leu-Thr-Leu-Pro-Ile-Leu-Arg-Tyr-Leu, followed by Leu-Leu-Ser-Gly-Thr-Gln-Asn-Gln-Pro-Ser-Phe-Leu-Ser-Gly-Phe, and Thr-Leu-Glu-Pro-Asn-Ser-Val-Phe-Leu-Pro-Val-Leu-Leu-His. The ACE inhibition activity that these peptides exhibit is in line with the results of previous studies that observed hydrophobic or aromatic amino acids or C-terminal with Pro help to improve ACE inhibition activity [54].

A study compared different types of pulses to determine their effects in vivo using SHR and Wistar-Kyoto rats (WKY) [55]. Results showed that SHR that were fed lentils had even lower mean arterial pressure (144mmHg) than control group SHR (171mmHg), and SHR that were fed mixed pulses (177mmHg). The SBP measured showed SHR fed lentils had the lowest SBP of 174mmHg compared to the control group of 201mmHg, and the lowest DBP of 131mmHg compared to SHR fed mixed pulses of 163mmHg; suggesting that lentils had the best antihypertensive effect [55]. Yao *et al* [56] used Morton lentil polyphenol extract (MLPE) to determine its effect on angiotensin II-induced hypertension in male Sprague-Dawley rats. Results showed that MLPE was able to attenuate mean arterial pressure (MAP) in angiotensin II-induced rats but did not affect angiotensin II-induced heart rate increases. However, the administration of MLPE was able to reduce angiotensin II-induced peripheral vascular remodeling and perivascular fibrosis, suggesting that lentil extracts can help reduce angiotensin II-induced hypertension [56].

6. CONCLUSION

Legumes have been proven to be a good source of nutrients that provide various health benefits, which has encouraged research to be done on methods that can improve health benefits when ingested and the types of health benefits present. Research on the antihypertensive properties of legumes is relatively new, so the information is not as extensive as compared to other types of foods with antihypertensive properties. However, available studies have shown a huge amount of effort to determine the structure and function of antihypertensive properties that are obtained especially from protein hydrolysates of legumes. Some studies have even observed the antihypertensive properties of legumes in animal models to hypothesize possible outcomes in the human body. With the knowledge gained from research on legume antihypertensive properties, further studies on developing functional food products with specific health benefits can follow.

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