Angiotensin-I-converting enzyme

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The Angiotensin-I-converting enzyme (ACE) is a peptidase with a significant role in the regulation of blood pressure.

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1. Introduction

Hypertension is a high prevalence disease and is considered one of the major health problems globally [1]. Lim et al. [2] reported that cardiovascular diseases due to complications of hypertension account for 9.4 million deaths every year. It is therefore of importance to take the appropriate mitigations to reduce the mortality rate due to hypertension. Otherwise known as high blood pressure, hypertension is a medical condition where the arterial blood pressure (BP) is abnormally high. According to the 2019 ACC/AHA Guideline on the Primary Prevention of Cardiovascular Disease [3], a normal BP is described as having a systolic and diastolic pressure of less than 120 and 80 mmHg, respectively (BP < 120/80 mmHg). There are two stages of hypertension. Stage 1 is defined with BP 130–139/80–89 mmHg, while stage 2 hypertension is for BP ≥ 140/90 mm Hg [3]. As mentioned above, hypertension could lead to cardiovascular diseases and stroke. Hypertension is usually treated with blood pressure regulating drugs such as angiotensin-I-converting enzyme (ACE) inhibitors (e.g., lisinopril, captopril), vasodilator, etc. Given the side effects of synthetic ACE inhibitors (e.g., taste disturbances, cough, and swelling of the lower layer of human skin or angioneurotic edema) [4], various investigations have been afforded to find potent ACE inhibitors from natural products, especially from food proteins. As a result of the increasing interest regarding functional foods in the past few years, it has been reported that food proteins-derived bioactive peptides have several benevolent effects on human health, including inhibitory activity against ACE [5][6]. Therefore, bioactive peptides can be considered as an alternative for managing hypertension.

A bioactive peptide is defined as an organic compound with a positive impact on human health (e.g., inhibitory activity against ACE, antioxidant capacity, antimicrobial activity, anti-thrombotic, immunomodulatory, etc.) which consists of 2–20 amino acids joined by covalent bonds called peptide bonds [7]. In the digestive system of the human body, bioactive peptides are liberated by digestive proteases, such as pepsin or microbial enzymes. Additionally, processing food and ripening can release bioactive peptides.

Nature is an abundant source of bioactive peptides produced by organisms such as plants and animals. Although animal products remain the greatest source of bioactive peptides, this work will mainly discuss plant source bioactive peptides derived specifically from soybean and velvet beans. Soybean nutritional content consists of 35–40% protein, 20% lipids, and 9% dietary fiber based on dry-weight soybean [8][9]. Because of its high protein content, soybean is mostly utilized as a source of bioactive peptides among other plants. Meanwhile, a less well-known type of legumes called velvet bean has a nutritional content of approximately 25% protein and 14% crude fat based on its dry weight [10]. As both beans are considered as potent protein sources in the human diet, their utilization as sources of parent proteins for producing bioactive peptides is promising. However, in the case of velvet bean, studies related to its utilization as a parent protein source are scarce. Thus, it is important to elucidate the technological approach of producing velvet bean-derived peptides especially for inhibiting ACE activity.

2. Substrate Preparation as Source of ACEi Peptides from Soybean and Velvet Bean

The preparation of substrates from soybeans is rarely discussed in the literature. Substrates from soybeans as sources of parent proteins can be soy protein concentrate or isolate, soybean flour-rich in protein, and principal soybean storage proteins (i.e., glycinin or β-conglycinin). Gouda et al. [11] prepared the soy protein substrate, glycinin. This method follows a previously described method developed in a study by Rao and Rao [12] with the use of $(NH_4)_2SO_4$ precipitation and centrifugation. Water containing β-mercaptoethanol (0.1% *v*/*v*) is used to extract defatted soybean flour for 4–6 h under

constant agitation. The solution is then centrifuged at 6000–8000 rpm for 45 min at 25 °C, followed by the addition of dry $MgCl_2$ until the final $MgCl_2$ concentration in the solution reaches 5 mM. Glycinin is collected by centrifugation, and the precipitate is dried with a freeze drier. Freeze drying is used as a preferred water removal method because it has the advantage to cause less damage to the structure of the protein substrate. Nevertheless, the fractionation of glycinin in most studies involves the precipitation of the alkaline soy protein extract at pH 6.3–7.0 [13][14][15].

For the preparation of the velvet bean substrate, wet fractionation is the method that is commonly used [16][17][18]. Initially, velvet bean flour is prepared by grounding the grains with a disk mill followed by sieving. The prepared bean flour then undergoes suspension in 3% sodium bisulfite with a 1:6 ratio (*w*:*v*) and left to soak for an hour with a constant agitation under alkaline pH (pH = 8). The role of sodium bisulfite is to increase the solubility of the velvet bean protein. Abtahi and Aminlari [19] stated that the modification of protein with a chemical treatment, such as sodium bisulfite, increases the protein dispersibility index (PDI). After fiber solid separation and washing with 3% sodium bisulfite, the protein-starch suspension is then left to sediment for 30 min. The purpose of sedimentation is to recover starch. The pH of protein solution pH is adjusted to an isoelectric point (i.e., pH 4.2) using 1.0 M HCl solution. The precipitate is obtained by centrifuging the solution at 1317× *g* for 20 min and further dried using a freeze-drier at −47 °C and pressure of 13×10^{-3} mbar [16][17][18]. In another study by Mugendi et al. [20] who characterized the nutritional properties of velvet bean protein isolate, the extraction was conducted with distilled water at pH 9 followed by centrifugation. The pH of the extract was then adjusted to 4.5 to precipitate the protein.

3. Hydrolytic Conditions for Producing ACEi Peptides from Soybean and Velvet Bean Protein Substrates

Enzymes for proteolysis are classified as endopeptidases and exopeptidases, based on the site of action on the substrate. Exopeptidases hydrolyze at the N- or C-terminal ends of the peptide, while endopeptidases cleave peptide bonds within and distant from the ends of a polypeptide chain or at the non-terminals of the sequence [21]. The most common enzymes used for producing soybean-based bioactive peptides are pepsin [22][23], papain [6][24], alcalase [25][26][27], proteinase from M. purpureus [28], trypsin, chymotrypsin, ginger protease, and Amano Protease from *Aspergillus* sp. [11], and protease D3 from *E. coli* strain JM109 [29]. All of these enzymes are endopeptidases. Endopeptidases, such as alcalase and proteinase K produce short-chain hydrophobic amino acids which are preferred in enhancing ACEi activity [21]. Additionally, prolyl endopeptidases such as Protease P from *Aspergillus niger* are often used as it can yield in proline-containing bioactive peptides which are favored for their strong affinity to ACE [30]. Hydrolytic conditions of soybean proteins for producing ACEi peptides are shown in **Table 1**.

Table 1. Enzymatic hydrolysis conditions of soybean proteins using endopeptidases to produce ACEi peptides.

Enzyme	Substrate	Temp. (°C)	Time (h)	pH	Enzyme-to-Substrate Ratio E/S	Peptide Sequence	Ref.
Pepsin	Protein concentrate	37	24	2	6%	IA	Chen et al. [22]
						TLAGAG	
						PPL	
						ITLL	
						VMALPG	
Pepsin	Protein isolate	39	12	2	3%	-	Chen et al. [23]
	Acid-precipitated protein						
Alcalase	Protein concentrate	50	12	9	4%	-	Wu & Ding [25]
M. purpureus acid proteinase	β-conglycinin	37	10	3.3	-	LAIPVNKP	Kuba et al. [28]
						LPHF	
	Glycinin					SPYP	
						WL	

Enzyme	Substrate	Temp. (°C)	Time (h)	pH	Enzyme-to-Substrate Ratio E/S	Peptide Sequence	Ref.
Bovine trypsin	Glycinin	37	18	8.2	2%	VLIVP	Gouda et al. [11]
Bovine chymotrypsin		37	18	8.2			
Ginger protease		50	16	6			
Protease P (Amano-P from *Aspergillus* sp.)		37	18	8.2			
Protease D3 from *E. coli* strain JM109	Protein isolate	37–40	24–48	4.5	0.2%	YVVFK	Kodera & Nio [29]
						PNNKPFQ	
						NWGPLV	
						IPPGVPYWT	
Pepsin	Protein isolate	37	1	5.3	4%	-	Lo & Li-Chan [31]
Pancreatin		37	2	7.5			
Alcalase	Protein isolate	55	1	8	-	-	Rayaprolu et al. [26]
Alcalase	Protein isolate	30	0.25	9	6%	-	Li et al. [27]

For velvet bean, the proteolytic enzymes reported limitedly in the literature are a combination of pepsin-pancreatin [16][17][18] and alcalase–flavourzyme [16][17]. In contrast to soybean-derived peptides, for velvet bean sourced peptides, the hydrolysis is conducted with a combination of both endopeptidase and exopeptidase. The application of both endo- and exo-peptidase allows it to have a broad cleavage action and produce a shorter chain of peptides. **Table 2** shows the enzymatic hydrolysis conditions of velvet bean-derived proteins.

Table 2. Enzymatic hydrolysis conditions of velvet bean protein concentrate to produce ACEi peptides.

Enzyme	Enzyme Type	Hydrolysis Conditions				Ref.
		Temp. (°C)	Time (h)	pH	Enzyme-to-Substrate Ratio E/S	
Pepsin	Endopeptidase	37	0.75	2	10%	Herrera-Chale et al. [17]
Pancreatin	Exopeptidase	37	0.75	7.5		
Alcalase	Endopeptidase	50	0.75	8		
Flavourzyme	Exopeptidase	50	0.75	7		
Pepsin	Endopeptidase	37	0.75	2	10%	Tuz & Campos [18]
Pancreatin	Exopeptidase	37	0.75	7		
Pepsin	Endopeptidase	37	0.75	2	10%	Segura-Campos et al. [16]
Pancreatin	Exopeptidase	37	0.75	7		
Alcalase	Endopeptidase	50	0.75	8		
Flavourzyme	Exopeptidase	50	0.75	7		

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