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Lima Bean (*Phaseolus lunatus*) Protein Hydrolysates with ACE-I Inhibitory Activity

Luis Chel-Guerrero[1], Mario Domínguez-Magaña[2], Alma Martínez-Ayala[3], Gloria Dávila-Ortiz[2], David Betancur-Ancona[1]

[1]Facultad de Ingeniería Química, Universidad Autónoma de Yucatán, Mérida, México; [2]Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, Miguel Hidalgo, México; [3]Centro de Investigación en Biotecnología Aplicada, Instituto Politécnico Nacional, Tlaxcala, México.
Email: bancona@uady.mx

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ABSTRACT

Several protein sources can be used to produce bioactive peptides with angiotensin I-converting enzyme (ACE) inhibitory activity. Protein concentrates from ungerminated and germinated lima bean *Phaseolus lunatus* seed flours were hydrolyzed with Alcalase 2.4 L or pepsin-pancreatin sequential hydrolysis, and ACE inhibitory activity measured in the different hydrolysis treatments. Protein hydrolysate production was analyzed with a 2^3 factorial design with four replicates of the central treatment. Evaluated factors were protein concentrate source (ungerminated seeds, PC_1; germinated seeds, PC_2), enzyme/substrate ratio E/S (1/50 or 1/10) and hydrolysis time (0.5 or 2.0 h for Alcalase; 1 or 3 h for pepsin-pancreatin). Degree of hydrolysis (DH) was high for the Alcalase hydrolysates (24.12% - 58.94%), but the pepsin-pancreatin hydrolysates exhibited the highest ACE inhibitory activity (IC_{50} = 0.250 - 0.692 mg/mL). Under the tested conditions, the hydrolysates with the highest ACE inhibitory activity were produced with sequential pepsin-pancreatin using either PC_1 at 1 h hydrolysis time and a 1/10 E/S ratio or PC_2 at 1 h hydrolysis time and a 1/50 E/S ratio. Lima bean protein hydrolysates prepared with Alcalase or pepsin-pancreatin are a potential ingredient in the production of physiologically functional foods with antihypertensive activity.

Keywords: Lima Bean; Degree of Hydrolysis; ACE Inhibition; Protein Hydrolysates; IC_{50}

1. Introduction

Hypertension is a major risk factor for stroke and other cardiovascular diseases. Among other strategies, angiotensin I-converting enzyme inhibitors (ACE-I) are used in the prevention of hypertension and congestive heart failure [1]. Studies have also been done of ACE-I in the treatment of chronic heart failure and myocardial infarction [2], as well as cancer [3,4]. ACE (peptidyldipeptide hydrolase, EC 3.4.15.1) catalyzes various reactions, two of which play key physiological roles in regulating blood pressure: conversion of inactive decapeptide angiotensin I into angiotensin II, a powerful vasoconstrictor and salt-retaining octapeptide; and inactivation of the vasodilator nonapeptide bradykinin, which is conducive to lowering blood pressure [5]. ACE-I have been shown to exhibit antihypertensive activity in spontaneously hypertensive rats and hypertensive patients [6].

Recent research has focused on ACE-I hydrolysates and peptides from animal and vegetal sources. The primary animal sources are proteins from casein, whey protein, fish protein, pig and chicken muscle, hemoglobin, blood plasma protein, gelatin [7] and egg yolks [8,9]. The primary plant sources are proteins from legume seeds such as mung bean [10], soybean [11,12], pea [13], and chickpea [14]; oilseeds such as rapeseed [15,16]; and cereal crop derivatives such as maize gluten, wheat germ and buckwheat [7]. These studies involve hydrolysate and peptide extraction with commercial enzymes (Alcalase, Flavourzyme, pepsin, pancreatin, trypsin, chymotrypsin, neutrace, etc.) at different hydrolysis times and enzyme/substrate ratios. An important variable in these studies is the concentration (mg protein/mL) of hydrolysate or peptide required to inhibit 50% of ACE-I activity under assayed conditions (IC_{50}); most enzymatic hydrolysates and peptides have values between 0.20 and 246.7 mg/mL [10]. Protein hydrolysis can be done chemically using acids or alkalis, although this oxidizes, destroys or modifies some amino acids, thus reducing protein quality [17]. Enzymatic hydrolysis is therefore preferred since it can improve the physicochemical, functional and sensory properties of native proteins

