Antioxidant Potential of Mung Bean (*Vigna radiata*) Albumin Peptides Produced by Enzymatic Hydrolysis Analyzed by Biochemical and *In Silico* Methods

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INTRODUCTION

- Mung bean (*Vigna radiata*) is a popular legume in countries such as Indonesia and China where its consumption is associated with positive health outcomes¹
- Mung bean has three storage proteins: globulin, albumin and prolamin, out of which globulin has been the most studied, while there is a lack of research for albumin and prolamin²
- Legume albumins and their peptides have been found to have antioxidant potential, however, the antioxidant potential of mung bean albumin peptides have yet to be studied²

OBJECTIVE

To investigate the antioxidant potential of mung bean albumin peptides derived from enzymatic hydrolysis using thermolysin and gastrointestinal enzymes (pepsin-pancreatin), and to sequence the peptides

HYPOTHESIS

Mung bean albumin peptides will have antioxidant potential since derivatives of other legume albumins, such as lunasin, have been found to have antioxidant potential

MATERIALS AND METHODS

Extraction

Peeled raw mung bean

Coffee grinder

MB flour mixed with water, pH adjusted to 3.0 and 7.5 before being centrifuged, and the supernatant was collected, then precipitated at pH 4.6, before being centrifuged again. Pellets were collected.

MB flour mixed with sodium borate buffer. Before being centrifuged with 3 kDa MWCO and precipitated at pH 4.6, then centrifuged again with 30 kDa MWCO

Antioxidant Assays

Iron-ion chelating assay

ABTS assay

ORAC assay

SDS-PAGE to confirm the presence of albumin

BIOPEP database to evaluate the antioxidant potential of the peptides

MassLynx software for peptide sequencing

RESULTS

Figure 1. SDS-PAGE of mung bean albumin extracts and 30 kDa MWCO filtrates. MWL = molecular weight ladder. (1) Water pH = 7.5 extract, (2) Albumin isolate from water pH = 7.5 extract, (3) Water pH = 3.0 extract, (4) Albumin isolate from water pH = 3.0 extract, (5) Borate buffer pH = 8.3 extract, (6) Albumin isolate from borate buffer pH 8.3 extract. Samples on lanes (2), (4), and (6) were filtered using a 30 kDa MWCO centrifugal filter to produce the permeates on (7), (8), and (9) respectively.

Table 1. Possible Small Antioxidant Peptides Produced from the *In Silico* Hydrolysis of Mung Bean Albumin Sequences by Various FDA-approved proteases

Protease	Protein Sequence	Potentially Antioxidant Peptides Expected to be Produced	Number of Potentially Broken Antioxidant Fragments
Papain	Q9FRT8	FC	0
	Q43680	VY, AY, LK, KP	6
Thermolysin	Q9FRT8	FC, LK	1
	Q43680	VY, AY, IR, LK, FC, EL, KP, HL	9

*other FDA-approved proteases such as alcalase, stem bromelain, and ficin were found out during the *in silico* hydrolysis to not produce potentially antioxidant peptides. Thermolysin was then chosen for the *in vitro* hydrolysis due to its potential to produce the most antioxidant peptides

Table 2. Antioxidant Potential of Mung Bean Hydrolysates From Two Different Enzymatic Hydrolysis Procedure

Enzymatic Hydrolysis Procedure	ABTS Radical Scavenging Activity (%)	Ferrous Ion Chelating Activity (%)	ORAC value (Trolox Equivalent (μM))
Thermolysin	9.3 ± 0.08	93.3 ± 0.14	127.3 ± 17
Pepsin-Pancreatin	8.1 ± 0.08	87.4 ± 0.07	127.0 ± 15

*ABTS = 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid

**Trolox was used as standard curve and control

Table 3. Sequences and Functional Properties of Mung Bean Albumin Peptides Derived From Simulated Gastrointestinal Enzymatic Hydrolysis

Peptide Sequence	Molecular Mass (Da)	Hydrophobicity (Kcal/mol)	Isoelectric Point	Charge
MD	264	10.87	2.95	-1
QSA	304	9.63	5.49	0
EW	333	9.44	3.27	-1
LGW	374	5.71	5.69	0
KK	274	13.5	10.57	2
SVP	301	8.04	5.18	0
DVAF	450	9.87	3.05	-1

Table 4. Sequences and Properties of Mung Bean Albumin Peptides Derived From Thermolysin Enzymatic Hydrolysis

Peptide Sequence	Molecular Mass (Da)	Hydrophobicity (Kcal/mol)	Isoelectric Point	Charge
KK	274	14.5	10.57	2
DM	264	10.87	3.02	-1
SY	268	7.65	5.38	0
W	204	5.81	5.64	0

CONCLUSIONS

- Sodium borate buffer at pH 8.3 was more effective method to extract mung bean albumin protein compared to water due to the higher solubility of mung bean albumin at pH 8.0 compared to the solubility of other mung bean proteins.
- Mung bean albumin peptides showed antioxidant capacity in term of ABTS radical scavenging, ferrous ion chelating and ORAC values.
- Mung bean albumin peptides were effective ferrous ion chelators.
- Mung bean albumin peptides derived from thermolysin enzymatic hydrolysis had higher antioxidant capacity overall due to their high hydrophobicity and low molecular mass.

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