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In-silico identification and molecular docking analysis of ACE-inhibitory peptides derived from soybean glycinin

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ABSTRACT

This study explored the potential of soybean glycinin as a source of natural ACE-inhibitory peptides, given the limitations and side effects associated with conventional synthetic ACE inhibitors. Following *in silico* enzymatic hydrolysis by chymotrypsin and pepsin, the released peptides were identified and their potential ACE-inhibitory activity was assessed. Molecular docking analysis revealed that two peptides, PSY and VVF, exhibited the strongest binding affinity toward ACE (- 7 Kcal/mol). Among them, PSY formed a broader and more stable interaction network within the active site of ACE, suggesting a higher inhibitory potential compared to other peptides. Overall, the findings indicate that soybean glycinin is a promising source of natural ACE inhibitors and can be further explored for the development of antihypertensive functional foods and supplements.

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1. Introduction

Hypertension is a major public health concern worldwide, particularly in developing countries, where it represents one of the leading risk factors for arteriosclerosis, stroke, myocardial infarction, and end-stage renal disease. Effective prevention and management of hypertension are therefore essential (1).

The angiotensin I-converting enzyme (ACE, EC 3.4.15.1) plays a central role in the renin–angiotensin system, which regulates blood pressure and circulatory function. ACE facilitates the conversion of angiotensin I to angiotensin II, a potent vasoconstrictor, thereby increasing blood pressure (2).

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Conventional hypertension treatment relies on pharmacological agents such as ACE inhibitors (e.g., lisinopril, captopril) and vasodilators. However, the long-term use of synthetic ACE inhibitors is often associated with adverse side effects, including taste disturbances, persistent cough, and angioneurotic edema (swelling beneath the skin) (3). As a result, research has increasingly focused on natural ACE inhibitors derived from food sources. These natural compounds include alkaloids, flavonoids, phenylpropanes, proanthocyanidins, terpenoids, oligosaccharides, xanthones, fatty acids, and peptides/amino acids (4). Among them, bioactive peptides (BPs) from plant and animal origins have drawn significant attention due to their multifunctional properties, such as ACE inhibitory, antioxidant, anti-inflammatory, dipeptidyl peptidase IV inhibitory, and memory-enhancing activities. BPs are naturally occurring compounds that have garnered interest from researchers, healthcare professionals, and consumers for their health-promoting potential (5).

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In particular, soybean has been recognized as a promising source of such peptides because of its high protein content and rich phytochemical composition. Soybean protein is not only an economical but also a high-quality source of vegetable protein (6). Recent studies have shown that hydrolysates of soybean protein contain ACE inhibitory peptides that are resistant to gastrointestinal proteases *in vitro* and capable of exerting antihypertensive effects in vascular tissues *in vivo* (7). Many potential antihypertensive peptide sequences have been identified within glycinin, the major storage protein (11S globulin) of soybean (8). Several methods have been developed for the production of BPs from proteins sources. Among these, enzymatic hydrolysis plays a crucial role in releasing these BPs from soybean proteins, making it a key step in the isolation and development of natural antihypertensive agents (9).

However, despite the growing interest in food-derived ACE inhibitors, limited information is available

regarding the identification of antihypertensive peptides derived from soybean glycinin through computational strategies. Therefore, the present study aimed to identify and characterize potential ACE inhibitory peptides generated from soybean glycinin using *in silico* enzymatic hydrolysis, followed by molecular docking analysis to evaluate their binding affinity and interactions with the ACE enzyme.

2. Materials and Methods

2. Protein digestion, molecular docking and virtual screening

The primary structure of soybean protein (glycinin), was obtained from the UniProtKB database (accession ID 1OD5; see 10.1093/nar/gkae1010). The sequence is provided below:

MGKPFFTLSLSSLCLLLLSSACFAITSSKFNECQLNN
LNALEPDHRVESEGGLIETWNSQHPELQCAGVTVS
KRTLNRNGSHLPSYLPYPQMIIVVQGKGAIGFAFPG
CPETFEKPQQQSSRRGSRSQQQLQDSHQKIRHFNEG
DVLVIPLGVPYWTYNTGDEPVVAISPLDTSNFNNQ
LDQNPRVFYLAGNPDIEHPETMQQQQQQKSHGGR
KQGQHRQQEEEGGSVLSGFSKHFLAQSFNTNEDTA
EKLRSPDDERKQIVTVEGGLSVISPKWQEQEDEDED
EDEEYGRTPSYPPRRPSHGKHEDDEDEDEEEDQPRP
DHPPQRPSRPEQQEPRGRGCQTRNGVEENICTMKL
HENIARPSRADFYNPKAGRISTLNSLTLPALRQFGLS
AQYVVLYRNGIYSPDWNLNANSVTMTRGKGRVRV
VNCQGNAVFDGELRRGQLLVVPQNPAVAEQGGE
QGLEYVVFKTHHNAVSSYIKDVFRVIPSEVLSNSYN
LGQSQVRQLKYQGNSGPLVNP.

These proteins were digested *in silico* using the BIOPEP database (10.3390/ijms20235978). Digestion using pepsin, and chymotrypsin occurred individually. The three-dimensional structure was produced using ChimeraX (USA, UCSF, Version 1.8), and energy optimization of the structures was performed with Avogadro software (USA, Version 1.2.0) using MMFF94 force field, and the energy optimization result was saved. The structures were converted from PDB to PDBQT using MGL-Tools software (USA, The Scripps Research Institute, Version 1.5.6). PyRx software (USA, The Scripps Research Institute, Version 0.8) was used for molecular docking and virtual screening of peptides obtained from digestion. In this software, molecular docking is performed using Auto-Dock Vina. The ACE protein (PDB ID: 1O8A) was used for molecular docking against peptides. Exhaustiveness was set to 8, and Grid Box was set to maximum. Based on the binding affinity value, the best docking conformation was selected, and the binding site of the target peptides on the ACE enzyme were identified. figures related to the binding site were saved using MGL-Tools software.

3. Results

3.1. Hydrolysis of soy protein by chymotrypsin and pepsin

The enzymatic hydrolysis of soybean protein using chymotrypsin and pepsin was analyzed with the BIOPEP tool to evaluate the potential for generating antihypertensive peptides. Two parameters were calculated: Ae and W. The parameter Ae (frequency of BPs occurrence) represents the number of bioactive fragments released per amino acid residue in the protein sequence, indicating the potential of the enzyme to produce BPs. The parameter W (potential biological activity) reflects the overall theoretical activity of the hydrolysate, combining both the

frequency and the activity intensity of the released peptides.

For chymotrypsin, the obtained values (ACE inhibitory) were Ae = 0.0188 and W = 0.0441, while for pepsin, the corresponding values were Ae = 0.0075 and W = 0.0176 which can be seen in Table 1.

Table 1. Bioactive peptide profiles of soybean glycinin after *in silico* hydrolysis by chymotrypsin and pepsin (A_e and W values).

		Chymotrypsin		Pepsin	
NO	**Activity**	**A_e**	**W**	**A_e**	**W**
1	ACE inhibitor	0.0188	0.0441	0.0075	0.0176
2	antioxidative	0.0113	0.1227	0.0019	0.0206
3	dipeptidyl peptidase III inhibitor	-	-	0.0019	0.0235
4	dipeptidyl peptidase IV inhibitor	0.032	0.0537	0.0113	0.019
5	phospholipase A2 inhibitor	0.0019	0.5		
6	neuropeptide	0.0019	0.044	0.0019	0.044
7	anti inflammatory	0.0019	0.3393	-	-
8	regulating	0.0038	0.4043	-	-
9	tubulin-tyrosine ligase inhibitor	0.0019	0.3393	-	-
10	inhibitor of tripeptidyl peptidase II	0.0019	0.0674	0.0019	0.0674
11	hypouricemic	0.0038	0.1836	0.0038	0.1836
12	hypotensive	0.0019	0.1267	-	-

$$A_E = \frac{d}{N}$$

$$W = \frac{A_E}{A}$$

- d is the number of fragments with function released after *in-silico* digestion.
- N is the number of AA resides in the protein.
- A is the occurrence frequency.

Table 2. The characteristics of peptides produced from the digestion of soy protein by chymotrypsin

Enzyme ID: Chymotrypsin (EC 3.4.21.1)			
NO	**Sequence**	**Location**	**Function**
1	PSY	[86-88]	ACE inhibitor
2	AF	[107-108]	ACE inhibitors
3	GL	[402-403]	ACE inhibitor
4	SY	[509-510]	ACE inhibitor
5	KY	[521-522]	ACE inhibitor
6	KL	[364-365]	ACE inhibitor
7	EY	[477-478]	ACE inhibitor
8	PEL	[64-66]	Antioxidative peptide
9	TY	[161-162]	Antioxidative peptide
10	AL	[40-41]	DPP IV inhibitor
11	SL	[9-10]	DPP IV inhibitor
12	SL	[392-393]	DPP IV inhibitor
13	GL	[402-403]	DPP IV inhibitor
14	AF	[107-108]	DPP IV inhibitor
15	EY	[477-478]	DPP IV inhibitor
16	KY	[521-522]	DPP IV inhibitor
17	PY	[90-91]	DPP IV inhibitor
18	QL	[183-184]	DPP IV inhibitor
19	RN	[80-81]	DPP IV inhibitor
20	RN	[412-413]	DPP IV inhibitor
21	SY	[509-510]	DPP IV inhibitor
22	TL	[7-8]	DPP IV inhibitor
23	TL	[394-395]	DPP IV inhibitor
24	TN	[251-252]	DPP IV inhibitor
25	TY	[161-162]	DPP IV inhibitor
26	VN	[530-531]	DPP IV inhibitor
27	GSH	[82-84]	ACE inhibitor
28	VVF	[479-481]	ACE inhibitor
29	PY	[90-91]	Phospholipase A2 inhibitor
30	PY	[90-91]	Ligand of neurotensin receptor
31	VVL	[408-410]	ACE inhibitor

32	PY	[90-91]	Anti-inflammatory peptide
33	GSH	[82-84]	Antioxidative peptide
34	SL	[9-10]	Regulator of phosphoglycerate kinase activity
35	SL	[392-393]	Regulator of phosphoglycerate kinase activity
36	KY	[521-522]	Antioxidative peptide
37	EY	[477-478]	Tubulin-tyrosine ligase inhibitor
38	AF	[107-108]	Inhibitor of tripeptidyl peptidase II
39	TL	[7-8]	Hypouricemic peptide
40	TL	[394-395]	Hypouricemic peptide
41	GVPY	[156-159]	Antioxidative peptide
42	PY	[90-91]	Antioxidative peptide
43	KY	[521-522]	Hypotensive peptide

ACE: Angiotensin-Converting Enzyme; DPP IV: Dipeptidyl peptidase 4.

Table 3. The characteristics of peptides produced from the digestion of soy protein by pepsin

Enzyme ID: Pepsin (EC 3.4.23.1)			
NO	**Sequence**	**Location**	**Function**
1	YL	[194-195]	ACE inhibitor
2	AF	[107-108]	ACE inhibitor
3	GL	[402-403]	ACE inhibitor
4	YL	[194-195]	Anxiolytic peptide
5	VNP	[530-532]	ACE inhibitor
6	SL	[9-10]	DPP IV inhibitor
7	GL	[402-403]	DPP IV inhibitor
8	AF	[107-108]	DPP IV inhibitor
9	TL	[7-8]	DPP IV inhibitor
10	TL	[394-395]	DPP IV inhibitor
11	YL	[194-195]	DPP IV inhibitor
12	YL	[194-195]	DPP IV inhibitor
13	SL	[9-10]	Regulator of phosphoglycerate kinase activity
14	AF	[107-108]	Inhibitor of tripeptidyl peptidase II
15	YL	[194-195]	Antioxidative peptide
16	TL	[7-8]	Hypouricemic peptide
17	TL	[394-395]	Hypouricemic peptide

ACE: Angiotensin-Converting Enzyme; DPP IV: Dipeptidyl peptidase 4.

Table 4. Affinity binding of ACE inhibitory peptides

Peptides	Affinity binding (Kcal/mol)
AF	-6.3
EY	-6.6
GL	-5.4
GSH	-5.7
KL	-5.9
KY	-6.2
PSY	-7.0
SY	-6.4
VNP	-6.3
VVF	-7.0
VVL	-6.2
YL	-6.2

ACE: Angiotensin-Converting Enzyme

3.2. Identification of peptides

Following the enzymatic hydrolysis of soybean protein, the resulting peptide sequences were analyzed using the BIOPEP database to identify fragments with potential antihypertensive activity.

The hydrolysis by chymotrypsin resulted in the release of several peptide sequences with predicted antihypertensive properties (Table 2), including PSY, AF, GL, SY, KY, KL, EY, GSH, VVF, and VVL.

Similarly, hydrolysis by pepsin generated YL, AF, GL, and VNP, which were also identified as peptides associated with antihypertensive (ACE-inhibitory) activity (Table 3). These identified peptides correspond to sequences known to possess bioactive potential based on the BIOPEP database and previous reports of ACE-inhibitory peptides derived from soybean protein.

3.3. Affinity binding analysis of peptides

In silico digestion of soybean glycinin using chymotrypsin and pepsin produced multiple peptide fragments. Docking analysis against the ACE target protein indicated that peptides PSY and VVF, derived from chymotrypsin hydrolysis, showed the strongest binding interactions, with binding affinity values of approximately -7 Kcal/mol (Table 4).

3.4. Visualization of peptide-target interactions

According to the conducted analyses, the PSY peptide interacts with the amino acid residues Leu139, Arg124, Leu140, Lys522, Glu143, Phe512, His353, Asn70, Asn66, Ser516, Tyr523, and Val518 within the ACE enzyme. In addition, the VVF peptide exhibits interactions with Ala170, Leu375, Thr302, Thr301, Lys449, Met299, and Glu376.

4. Discussion

4.1. Effect of enzyme type on peptide production

These results indicate that chymotrypsin exhibits a higher potential for generating antihypertensive peptides from soybean protein compared to pepsin. This can be attributed to the substrate specificity of chymotrypsin, which preferentially cleaves peptide bonds on the carboxyl side of aromatic and large hydrophobic amino acids such as tryptophan, phenylalanine, and tyrosine. Lee SY et al., 2017 (10) importantly noted that these cleavage characteristics are significant for producing BPs, particularly those with ACE-inhibitory and antihypertensive properties. The specificity allows for targeted enzymatic hydrolysis of proteins to generate potentially therapeutic peptide fragments.

In contrast, pepsin, although also favoring hydrophobic residues, has a broader and less specific cleavage pattern, resulting in a lower release rate of targeted BPs. Therefore, the higher Ae and W values obtained for chymotrypsin demonstrate its superior efficiency in producing antihypertensive peptides during soybean protein hydrolysis.

4.2. Molecular docking analysis of interactions

ACE features a Zn^{2+} ion at its catalytic center and contains three principal binding pockets: S1, S2, and S1′. The S1 pocket is formed by the residues Ala354, Glu384, and Tyr523, while the S2 pocket includes Gln281, His353, Lys511, His513, and Tyr520. The S1′ pocket is primarily defined by Glu162. In general, lower binding energies correspond to stronger peptide–receptor affinities (11). For ACE inhibition, PSY, and VVF exhibited the lowest binding energy (-7.0 kcal/mol), suggesting the strongest binding affinity.

Among the peptides generated through in silico digestion, PSY and VVF were selected for detailed docking analysis because both exhibited a comparable binding affinity of –7 kcal/mol toward ACE. Despite having identical predicted binding energies, the two peptides displayed markedly different interaction profiles within the ACE binding pocket, indicating distinct binding modes that may influence their functional behavior.

Peptide PSY formed interactions with a diverse set of residues, including LEU139, ARG124, LEU140, LYS522, GLU143, PHE512, HIS353, ASN70, ASN66, SER516, TYR523, and VAL518. Notably, several of these residues, such as HIS353, PHE512, TYR523, and LYS522 are closely associated with catalytically important or substrate-recognition regions of ACE (Fig. 1). This interaction pattern suggests that PSY occupies a deeper and more central position within the active site, potentially achieving stabilization through hydrogen bonds, electrostatic contacts, and aromatic interactions. Such a binding orientation may enable PSY to exert inhibitory effects more effectively despite having the same binding affinity value as VVF.

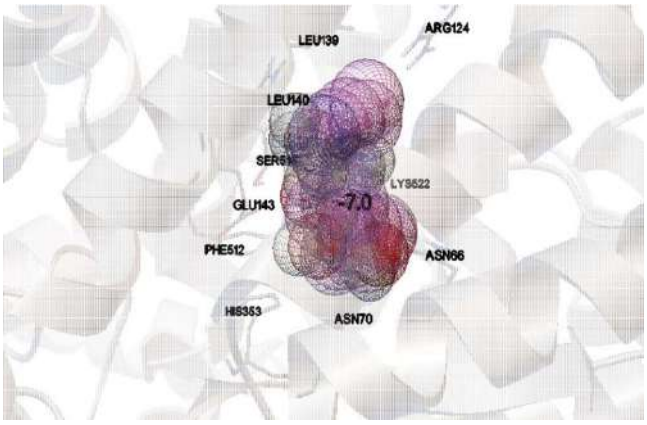

Figure 1. Molecular docking and interactions of PSY and ACE inhibitory enzyme

In contrast, VVF interacted with a more limited and spatially distinct group of residues, including ALA170, LEU375, THR301, THR302, LYS449, MET299, and GLU376 (Fig. 2). These residues are generally located in peripheral or auxiliary regions of the binding pocket rather than in direct proximity to the catalytic core. The narrower interaction network suggests that VVF occupies a different sub-pocket and may rely on fewer specific interactions to achieve its overall binding energy.

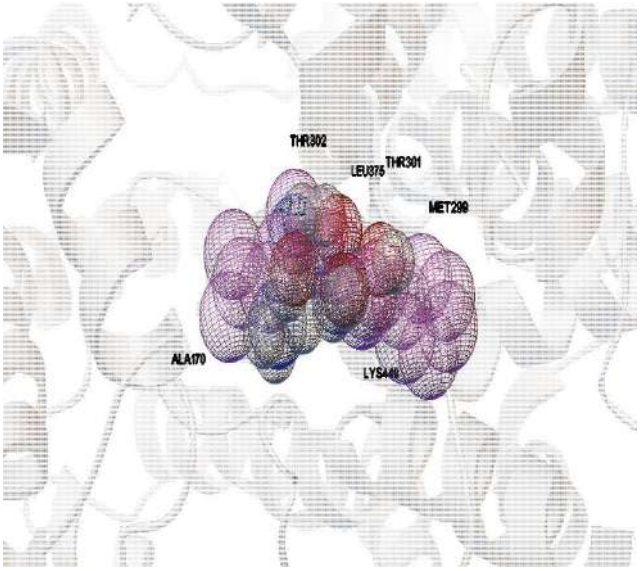

Figure 2. Molecular docking and interactions of VVF and ACE inhibitory enzyme

Overall, although PSY and VVF share the same docking-derived binding affinity, their distinct residue-level interactions highlight different structural strategies for ACE engagement. PSY appears to form a more extensive and catalytically relevant interaction network, whereas VVF binds through a more localized set of contacts, which may lead to differences in their inhibitory efficiency, stability within the active site, or physiological relevance.

5. Conclusion

Based on the results obtained from *in silico* hydrolysis and molecular docking, it can be concluded that soybean glycinin has strong potential for generating ACE-inhibitory antihypertensive peptides. Among the identified sequences, PSY and VVF demonstrated the highest binding affinity to ACE, with PSY showing a more extensive network of interactions within the enzyme's active site. Considering the adverse effects associated with synthetic ACE inhibitors, these peptides could serve as promising candidates for the development of functional foods, nutraceuticals, or natural supplements aimed at blood pressure management. Nonetheless, future *in vitro* and *in vivo* studies are required to validate their physiological efficacy.

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Authorship contribution

Meisam Barati: Conceptualization; data curation, writing, review and editing. Mahshad Davoudi: Conceptualization; data curation; writing. Yasaman Eshraghi Nejad, Kiyanoush Jafari, and Masoumeh Jabbari: Writing.

Declaration of competing interest

The authors declare that they have no conflicts of interest.

Data availability

Data are available upon request.

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