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Research Article

IN SILICO APPROACH OF COLLAGEN FROM TUNA FISH BY-PRODUCT AS ANGIOTENSIN-CONVERTING ENZYME INHIBITOR

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ABSTRACT

Objective: This study explores the sustainable valorization of by-products from tuna fish based on *in silico* approach.

Methods: *In silico* approaches (BIOPEP database, PeptideRanker database, peptide calculator [PepCalc] database, and toxin prediction [ToxinPred] database) were employed to evaluate the potential of collagens from tuna as a potential source of bioactive peptides. Furthermore, primary structure, biological potential, physicochemical, sensory, and toxicity characteristics of the theoretically released angiotensin-converting enzyme (ACE) inhibitor collagen peptides were predicted.

Results: Tuna collagen was selected as a potential precursor of bioactive peptides based on *in silico* analysis. Most notable among these are ACE inhibitory peptides. First, the potential of tuna collagen for the releasing bioactive peptides was evaluated by determining the frequency of occurrence of fragments with a given activity. Through the BIOPEP database analysis, there are many bioactive peptides in tuna collagen sequences. Then, an *in silico* proteolysis using selected enzymes (papain and pepsin) to obtained ACE inhibitory peptides was investigated and then analyzed using PeptideRanker and PepCalc. Cytotoxicity analysis using the online toxic prediction tool ToxinPred revealed that all *in silico* proteolysis-derived ACE inhibitory peptides are non-cytotoxic.

Conclusions: Overall, the present study highlights that the tuna collagens could be a promising precursor of bioactive peptides that have an antihypertensive effect (ACE inhibitory activities) for developing functional food or nutraceutical products.

Keywords: *In silico*, Valorization, Tuna, By-product, Angiotensin-converting enzyme inhibitor.

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INTRODUCTION

Fish skin gelatin is extensively employed as an ingredient to improve the elasticity, consistency, and stability of foods, but it can also be a source of collagen. Collagen has a triple helical molecule that contains three polypeptide α-chains that are coiled around each other and composed of (Glycine-X-Y)n, where X and Y are often proline and hydroxyproline (Hyp), respectively. At present, 29 different collagen types (type I–XXIX) have been identified. Type I is the most common form of collagen in the vertebrate connective tissues [1].

Nowadays, collagen and its derivatives have potential commercial value for a broad application range of pharmaceutical, cosmetic, food, and biomedical industry applications [1,2]. The skin and bone of land-based animals (bovine and porcine) have been the conventional sources of collagen [2,3]. Unfortunately, the use of bovine and porcine collagens has raised serious problems regarding consumers' health [2,3]. Therefore, there is a strong necessity nowadays to find the alternative sources of collagen. Aquatic species and their processing by-products have been recognized as potential alternative sources, owing to their availability, lack of disease risk, lack of dietary restriction, and high collagen yields. Incorporation of collagen-derived bioactive compounds can be an innovative approach to the production of functional food and nutraceutical products with potential health-promoting properties [1,4].

Marine-derived bioactive peptides from collagen are recently emerging nutraceutical field as supplements in health functional food formulations due to their diverse health-promoting benefits [1,3,5]. Interestingly, bioactive peptides are able to inhibit the ACE, involved in the regulation of human blood pressure and fluid homeostasis via the renin–angiotensin system. In fact, the ACE is responsible to convert the angiotensin I into angiotensin II that constricts the arteries and, as a consequence, increases the blood pressure [6,7]. Further, it is involved in the inactivation of the bradykinin, which is a known vasodilator [8].

Screening for ACE-I inhibitors from novel substrates using conventional methods is an expensive and time-consuming process. It involves using previously reported studies to select proteases that demonstrate the highest potential to liberate ACE-I inhibitory peptides and further experimentally testing each one of them for their *in vitro* activity. The process can be simplified using *in silico* analysis and tools such as BIOPEP (http://www.uwm.edu.pl/biochemia/index.php/pl/biopep) and Expssy Peptide Cutter (http://web.expasy.org/peptide_cutter/). These tools allow for the theoretical prediction of the potential of various substrates with known protein sequences to generate bioactive peptides, using enzymes with known cleavage specificities [9]. As well, this work aims to investigate the tuna collagens that could be a promising precursor of bioactive peptides that have an antihypertensive effect (ACE inhibitory activities) for developing functional food or nutraceutical products.

METHODS

Profiles of the potential biological activity of tuna collagen

The assessment of the biological potential of tuna collagen was carried out using the BIOPEP analysis (http://www.uwm.edu.pl/biochemia/index.php/en/biopep). The collagen type I protein sequences obtained from the NCBI database were analyzed for the profiles of potential biological activity. The potential of type I fish collagen-derived ACE inhibitor peptide sequences was also screened. In addition, a frequency of the occurrence of bioactive fragments in the type I collagen chains was

also calculated. Furthermore, collagen type I-1 protein sequences were also subjected to *in silico* proteolysis to predict the theoretical peptide sequences cleaved by enzyme mixture (pepsin + papain) [1]. Finally, a list of potential ACE peptides was generated for further analysis.

Peptide ranking
The potential of the fish collagen-derived ACE inhibitor peptides was predicted using PeptideRanker (http://bioware.ucd.ie/compass/biowareweb/) tool. The PeptideRanker tool was used to calculate the peptide score of the selected fish collagen-derived ACE inhibitor peptides. PeptideRanker gave the peptide score in the range of 0–1. The maximum score [1] showed the most active peptides, and on the other hand, the least score (0) showed the least active peptides.

Sensory characteristic prediction
Peptides and amino acids are belonging to compounds that have the capacity to altering the taste of food commodities. The occurrence frequency of sensory characteristics in the hydrolyze collagen α-chains was predicted using the BIOPEP analysis. The occurrence frequencies of the various sensory characteristics were also predicted for the hydrolyzing α-chains of the collagen using papain, pepsin, and their mixtures. Furthermore, the sensory characteristics of the selected collagen-derived ACE inhibitor peptides were also predicted.

The physicochemical characteristics of the collagen derived
The potential ACE inhibitor peptides were evaluated using online peptide calculators. The theoretical molecular weight, isoelectric point, the peptide charge at pH 7, estimated solubility, and extinction coefficient of the screened antioxidative collagen peptides were estimated with the online PepCalc software (http://pepcalc.com/).

Toxicity prediction
The toxicity of the peptides is one of the major concerns toward the development of collagen-based functional food ingredients. Therefore, the *in silico* toxicity prediction of the identified collagen-derived ACE inhibitor peptides was investigated using ToxinPred (http://www.imtech.res.in/raghava/toxinpred/index.html).

RESULTS AND DISCUSSION

Profile of potential biological activity
The several potential biological activities of both collagen chains were reported in Table 1. Hence, the time-saving and more economical computer-simulated method or *in silico* methods can be used to predict the generation of the bioactive collagen peptides. The biological activities of the collagen type I-1 were evaluated using the BIOPEP analysis tool. It was predicted by the tool that collagen has several potential biological activities (Table 1).

Table 1: Profile of biological activity of collagen type 1 from tuna fish

No.	Activity	Frequency (A)
1.	ACE inhibitor	0.7458
2.	Activating ubiquitin-mediated proteolysis	0.0026
3.	Alpha-glucosidase inhibitor	0.0013
4.	Anorectic	0.0222
5.	Antiamnestic	0.1636
6.	Antioxidative	0.0411
7.	Antithrombotic	0.1799
8.	Bacterial permease ligand	0.0013
9.	Chemotactic	0.0235
10.	Dipeptidyl peptidase IV inhibitor	0.7555
11.	Embryotoxic	0.0007
12.	Hypotensive	0.0072
13.	Immunomodulating	0.0007
14.	Immunostimulating	0.0007
15.	Inhibitor	0.0130
16.	Neuropeptide	0.0072
17.	Opioid	0.0007
18.	Regulating	0.1643
19.	Stimulating	0.0111

ACE: Angiotensin-converting enzyme

The predicted major potential biological activities of the collagen were ACE inhibitory activity and dipeptidyl peptidase IV inhibitory activity. The frequency of the ACE inhibitory and dipeptidyl peptidase IV inhibitory activity in the collagen I-1 was approximately 0.7458 and 0.7555, respectively. In addition, the frequency of the antioxidative peptides in collagen type I was 0.0411. The results of this study are in agreement with the report previously who reported the fish collagen peptides to have the various potential biological activities. The previous study of tuna frame collagen hydrolysate also has been investigated to have ACE inhibitor activity based on *in vitro* assay. From the result of the study, it is suggested that the ACE inhibitory peptide from tuna frame protein hydrolysate could be potential candidates to develop nutraceuticals and pharmaceuticals against hypertension and its related disease. In addition, it is expected that this study will contribute to developing an interest in basic research and potential application of bioactive peptides [10].

The biological activities of peptides isolated from marine collagen and also gelatin have been reported in different studies. In most studies in the field of food science and technology, the focus has been on antioxidant and antihypertensive/ACE inhibitory activities. The antioxidant activities of bioactive peptides are mainly due to the presence of some aromatic amino acids and histidine. Gelatin peptides are rich in hydrophobic amino acids which result in higher emulsifying ability and, marine gelatin peptides possess higher antioxidant effects than peptides derived from other sources [11].

Furthermore, collagen and also gelatin-derived peptides represent numerous other bioactivities such as antimicrobial activity, mineral-binding capacity, lipid-lowering effect, immunomodulatory activity, and beneficial effects on the skin, bone, or joint health. Due to the dominant presence of hydrophobic amino acids in gelatin, it exhibits high emulsifying ability for hydrophilic-hydrophobic partitioning. Furthermore, specific amino acid arrangements such as Gly, Pro, and Hyp merit special consideration, as the content of Pro is able to scavenge free radicals [12].

***In silico* proteolysis**
The potential fish collagen type I-derived peptide sequences displaying the ACE inhibitor were predicted using the BIOPEP analysis tool. The lists of the theoretically released ACE inhibitor peptide from tuna collagen are summarized in Table 2. The tuna collagen-1 and protein sequences were also subjected to *in silico* proteolysis using the BIOPEP tool. The supposed ACE inhibitory peptide derived from the hydrolysis of tuna collagen-1 and by pepsin and papain proteases is summarized and presented (Table 2). Previously yet researched showed the mix of pepsin and also papain can generate several bioactive peptides [1]. Hence, we also try to use these two enzymes to screen several bioactive peptides that can act as an ACE inhibitor.

Based on Table 2, mostly peptides released by hydrolysis using papain and pepsin are dipeptides. This result aligned with the previous research using these two different enzymes but different raw materials which are carp collagens [1]. All of the ACE inhibitor peptides generated from the mixture of the enzymes were dipeptides with ACE inhibitor activity, as predicted by BIOPEP tool. The pepsin and papain mixture or alone could be able to generate several ACE inhibitor dipeptides. This fact suggests that these enzymes could not be able to hydrolyze the tuna collagen effectively for releasing the numerous ACE inhibitor peptide sequences. Therefore, the specific enzyme such as collagenase may be able to cleave the type I collagen at the various sites for the generation of the ACE inhibitor peptides [1].

Peptide ranking of ACE inhibitor collagen peptides
The identified ACE inhibitor collagen-derived peptide profile was subjected to activity prediction by *in silico* methods. The potential of the ACE inhibitor peptides has been calculated using PeptideRanker. PeptideRanker server can rank the peptide sets according to

Table 2: *In silico* assay using pepsin and papain release peptides with ACE inhibitor from tuna collagen as precursor

Peptide ID	Sequence	Location	Name	Function	Activity	Monoisotopic mass	Chemical mass
3537	PR	(178–179)	ACE inhibitor	Inhibitor of ACE (EC 3.4.15.1) (MEROPS ID: XM02-001)	ACE inhibitor	271.1530	271.3050
3751	KK	(1411–1412)			Bacterial permease ligand	274.1880	274.3480
7513	PL	(909–910)	ACE inhibitor from Alaska pollock fish skin	Inhibitor of ACE (EC 3.4.15.1) (MEROPS ID: XM02-001)	ACE inhibitor	228.1360	228.2770
7558	VK	(1493–1494)	ACE inhibitor from buckwheat		ACE inhibitor	245.1620	245.3070
7586	KR	(468–469)	ACE inhibitor		ACE inhibitor	302.1950	302.3620
7594	VG	(895–896)	ACE inhibitor	Inhibitor of ACE (EC 3.4.15.1) (MEROPS ID: XM02-001)	ACE inhibitor	174.0890	174.1850
7595	IG	(460–461)	ACE inhibitor	Inhibitor of ACE (EC 3.4.15.1) (MEROPS ID: XM02-001)	ACE inhibitor	188.1050	188.2120
7600	AG	(229–230)	ACE inhibitor	Inhibitor of ACE (EC 3.4.15.1) (MEROPS ID: XM02-001)	ACE inhibitor	146.0580	146.1310
7604	KG	(507–508)	ACE inhibitor	Inhibitor of ACE (EC 3.4.15.1; MEROPS ID: XM02-001)	ACE inhibitor	203.1150	203.2260
7613	WG	(1450–1451)	ACE inhibitor	Inhibitor of ACE (EC 3.4.15.1) (MEROPS ID: M02-001)	ACE inhibitor	261.1000	261.2650
7617	QG	(23–24)	ACE inhibitor		ACE inhibitor	203.0790	203.1830
7618	SG	(265–266)	ACE inhibitor		ACE inhibitor	162.0530	162.1320
7622	EG	(36–37)	ACE inhibitor	Inhibitor of ACE (EC 3.4.15.1) (MEROPS ID: XM02-001)	ACE inhibitor	204.0630	204.1680
7624	NG	(1191–1192)	ACE inhibitor		ACE inhibitor	189.0639	189.1560
7625	PG	(135–136)	ACE inhibitor	Inhibitor of ACE (EC 3.4.15.1) (MEROPS ID: M02-001)	ACE inhibitor	172.0730	172.1690
7628	VR	(20–21)	ACE inhibitor from k-Casein (fr. 67–68)	Inhibitor of ACE (EC 3.4.15.1) (MEROPS ID: XM02-001)	ACE inhibitor	273.1690	273.3210
7681	DG	(94–95)	ACE inhibitor from soy	Inhibitor of ACE (EC 3.4.15.1) (MEROPS ID: XM02-001)	ACE inhibitor	190.0479	190.1410
7683	NF	(151–152)	ACE inhibitor from garlic		ACE inhibitor	279.1109	279.2810
7685	SF	(3–4)	ACE inhibitor from garlic	Inhibitor of ACE (EC 3.4.15.1) (MEROPS ID: M02-001)	ACE inhibitor	252.1000	252.2570
7742	AR	(246–247)	ACE inhibitor	Inhibitor of ACE (EC 3.4.15.1) (MEROPS ID: XM02-001)	ACE inhibitor	245.1380	245.2670
7833	PT	(572–573)	ACE inhibitor		ACE inhibitor	216.0990	216.2220

ACE: Angiotensin-converting enzyme

structure–function patterns [13]. The peptide generated from the pepsin hydrolysis showed the maximum peptide ranking scores. The ACE inhibitor dipeptide PR and KK showed the maximum peptide rank score of 0.99 and 0.94, respectively (Table 3). Papain and pepsin were generated as the most active ACE inhibitor peptides [2,14].

Sensory characteristic profile of tuna collagens hydrolysates and peptides

As we know that the sensory characteristics of the potential ACE inhibitor peptides were also evaluated (Table 3). Taste is the major factor that makes organisms to choose what to ingest and also protects from ingesting toxic compounds. Taste is the key factor responsible for the determination of any food commodity quality.

Humans recognize five basic taste sensations (bitter, salty, sour, sweet, and umami). The taste of foods is affected by some molecules with specific chemical nature such as peptides derived from food proteins. In this study, it was predicted that all of the ACE inhibitor peptides have a bitter taste. In addition, the collagen hydrolysate prepared from the hydrolysis of the papain and pepsin was also subjected to the prediction of overall sensory characteristics. The sensory characteristic occurrence frequency was also predicted in the hydrolyze collagen chains. *In silico* conditions, it was observed that the occurrence frequency of sweet characteristics in collagen chains hydrolysate is lower as compared to the bitter hydrolysate (Table 3). Therefore, this study provided theoretical feasibility for application of *in silico* analysis to predict and release of the ACE inhibitory peptides from tuna collagen.

Table 3: Peptide ranker and sensory evaluation and toxicity prediction of peptides from collagen precursor as ACE inhibitor

No.	Peptide	Peptide rank score	Sensory evaluation
1.	PR	0.992384	Bitter
2.	KK	0.948796	Bitter
3.	PL	0.941145	Bitter
4.	VK	0.877086	Bitter
5.	KR	0.811148	Bitter
6.	VG	0.787626	Umami
7.	IG	0.546994	Bitter
8.	AG	0.501816	Sweet
9.	KG	0.407386	Umami
10.	WG	0.394584	Bitter
11.	QG	0.391148	Sweet
12.	SG	0.387797	Sweet
13.	EG	0.38751	Sweet
14.	NG	0.312686	Bitter
15.	PG	0.249461	Bitter
16.	VR	0.213072	Salt
17.	DG	0.168013	Umami
18.	NF	0.114691	Bitter
19.	SF	0.100261	Bitter
20.	AR	0.0591457	Salty
21.	PT	0.03329	Bitter

ACE: Angiotensin-converting enzyme

Physicochemical characteristics of collagen peptides

The identified ACE inhibitor collagen-derived peptides were subjected to predict the various physicochemical characteristics and primary

Table 4: Toxicity prediction of peptides from collagen precursor as ACE inhibitor

No.	Peptide sequence	Support vector machine score	Prediction	Hydrophobicity	Hydropathicity	Hydrophilicity	Charge	Mol. wt.
1.	PR	−0.8	Non-toxin	−0.92	−3.05	1.5	1	271.33
2.	KK	−1	Non-toxin	−1.1	−3.9	3	2	274.38
3.	PL	−0.8	Non-toxin	0.23	1.1	−0.9	0	228.31
4.	VK	−0.8	Non-toxin	−0.28	0.15	0.75	1	245.34
5.	KR	−0.8	Non-toxin	−1.43	−4.2	3	2	302.39
6.	VG	−0.8	Non-toxin	0.35	1.9	−0.75	0	174.22
7.	IG	−0.8	Non-toxin	0.45	2.05	−0.9	0	188.25
8.	AG	−0.8	Non-toxin	0.21	0.7	−0.25	0	146.16
9.	KG	−0.8	Non-toxin	−0.47	−2.15	1.5	1	203.26
10.	WG	−0.8	Non-toxin	0.27	−0.65	−1.7	0	261.3
11.	QG	−0.8	Non-toxin	−0.26	−1.95	0.1	0	203.22
12.	SG	−0.79	Non-toxin	−0.05	−0.6	0.15	0	162.16
13.	EG	−0.8	Non-toxin	−0.23	−1.95	1.5	−1	204.2
14.	NG	−0.79	Non-toxin	−0.24	−1.95	0.1	0	189.19
15.	PG	−0.8	Non-toxin	0.04	−1	0	0	172.2
16.	VR	−0.8	Non-toxin	−0.61	−0.15	0.75	1	273.35
17.	DG	−0.8	Non-toxin	−0.28	−1.95	1.5	−1	190.17
18.	NF	−0.8	Non-toxin	−0.02	−0.35	−1.15	0	279.31
19.	SF	−0.8	Non-toxin	0.17	1	−1.1	0	252.28
20.	AR	−0.8	Non-toxin	−0.76	−1.35	1.25	1	245.29
21.	PT	−0.8	Non-toxin	−0.12	−1.15	−0.2	0	216.25

*Mol. wt.: Molecular weight. ACE: Angiotensin-converting enzyme

structure (http://pepcalc.com/). The result of this study indicates that most of the ACE inhibitor peptides have low-molecular-weight profile (Table 4). The isoelectric point of the predicted ACE inhibitor collagen peptides was found at the range of 0.68–11.39 pH. Most of the collagen-derived ACE inhibitor peptides showed that most of them are good water solubility (Table 4).

Toxicity prediction of collagen-derived ACE inhibitor peptides

Toxicity of the ACE inhibitor peptides from the tuna collagen type I chains may hamper the development of bioactive peptides in the food industry. Valine, threonine, arginine, glutamine, methionine, leucine, lysine, isoleucine, phenylalanine, and alanine are primary components of the non-toxic peptides, while Pro, histidine, cysteine, and asparagine amino acid residues are predominant in toxic peptides. In the present study, tuna collagen-derived ACE inhibitor peptides majority do not contain the amino acid residues that are present in the toxic peptides. It was predicted from the ToxinPred analysis that all *in silico*-derived tuna collagen ACE inhibitor peptides most of them are non-toxic (Support Vector Machine scores <0) (Table 4). Therefore, these peptides can be used as potential functional ingredients.

CONCLUSIONS

Using *in silico* approach, we can conclude that several bioactive peptides that have ACE inhibitory activity can be derived from collagen. The present study highlighted that *in silico* digestions of tuna collagen chains using papain and pepsin effectively generated the ACE inhibitor activities that can provide a basis for the development of tuna collagen as a precursor of antihypertensive peptides in the food industries. Therefore, the utilization of fish processing by-products for the extraction of collagen and the application concept of valorization can be assessed as a waste management approach.

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AUTHOR CONTRIBUTION

AN: Design *in silico* analysis research, performed *in silico* experiment and preparation manuscript. HSHM: Design *in silico* analysis research, performed *in silico* experiment.

CONFLICT OF INTEREST

The authors declare that we have no conflict of interests. The author wishes to thank the Toray Science Foundation for supporting this research. The funder had no role in study design, data collection, and analysis, decision to publish, or preparation of the manuscript.

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