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BIOACTIVE PEPTIDE PREDICTION OF SKIPJACK TUNA (*KATSUWONUS PELAMIS) HYDROLYSATES* USING IN SILICO

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ABSTRACT

Katsuwonus pelamis or locally called skipjack tuna has been used as food for centuries by different populations. It is one of the important sources of protein for human health. Hydrolysate is one of the ways to utilize the primary function of protein as a natural protein source. The hydrolysate is known to have many bioactivates that may benefit human health such as immunodulators, anticancer, antihypertensive, antioxidant and anti-inflammatory. The current study aimed to explore the potential of *Katsuwonus pelamis* hydrolysate as a potential bioactivity agent. Gene bank of *Katsuwonus pelamis* obtained from NCBI then analyzed using BIOPEP. The software can predict the potential biopeptides as a bioactive agent. Furthermore, the BIOPEP also capable of predicting the contributing sensory taste based on peptides from *Katsuwonus pelamis.* The toxicity of promising peptides is then analyzed using ProTox II to determine the exact LD50 of each potential peptide. Results show that the most dominant bioactivities of *Katsuwonus pelamis* is ACE inhibitor followed by DPP IV inhibitor. Sensory analysis shows that the most contributing taste on *Katsuwonus pelamis* peptides is bitter followed by sour taste. The potential peptides are dominated by class V toxicity levels with LD50 value of 2000<LD50≤5000 mg/kg. The research suggests that *Katsuwonus pelamis* hydrolysates are proven to be a potential bioactive agent that can be utilized as a functional food for human health benefits.

Keywords: *katsuwonus pelamis*, hydrolysate, peptide, in silico.

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INTRODUCTION

The immune response plays an important role in maintaining human health by identifying and killing pathogens, aging cells, or tumor cells. Its function can be affected by many factors, including the presence of pathogens, tissue injury, and cardiac infarction [1]. Immunomodulation refers to the ability of the immune system to control various life-threatening diseases, such as cancer, human immunodeficiency virus, multiple sclerosis, and aging [2, 3, 4]. Clinically, immunomodulators can be classified as immunoadjuvants, immunostimulants and immunosuppressants. Immunotherapy is the treatment of disease by modulating the host's immune system. Currently, many drugs are used clinically to control human immune function. However, most of the synthesized immunomodulatory drugs exhibit toxicity and side effects, limiting their use in cases such as chronic diseases [5]. In contrast, most of the naturally derived immunomodulatory proteins or peptides show no side effects and are less expensive, indicating their potential for use in immunotherapy.

The exploration of bioactive compounds from proteins and their derivatives, namely peptides or amino acids produced from food, has developed rapidly nowadays; this is because the bioactive compounds contained in them have special functional properties for human health [6]. Peptide compounds or amino acids resulting from protein hydrolysis have a lower molecular weight so that they are easier to digest and use for their physiological functions for the body. Protein hydrolysis can be produced from foods that have high protein such as skipjack fish (58.87%) [7], from protein hydrolysis, simpler compounds are obtained in the form of peptides and amino acids that have bioactive properties as immunomodulators, anticancer, antihypertensive, antioxidant, anti-inflammatory, anti-cholesterol, antidiabetic, osteoprotective and antimicrobial [8-14].

Fish protein hydrolysis (HPI) can be produced from various types of fish (skip fish) and their wastes in the form of heads, gills, entrails, fins and fish bones. Waste in the form of offal and or stomach contents (viscera), head, skin, skeleton, and bones contain components such as fatty acids, phospholipids, dissolved vitamins and bioactive components [15]. The protein content resulting from the hydrolysis of yellowfin tuna (*Thunnus albacores*) waste offal (fish viscera) is 72.34% [16], so the waste that is classified as unutilized material can be processed into a material that has added value. Protein hydrolysis can be produced in several ways, one of which is enzymatically, enzymatic hydrolysis (protease enzymes) is the most widely used to obtain bioactive compounds from a substrate. Some of the advantages include the process being easier to control, more effective in breaking down peptide compounds, and not showing side effects on decreasing nutritional value [17].

Immunomodulators are substances that are able to increase, decrease or modify the immune response by changing parts of the immune system. Some allopathic drugs have side effects and relatively high costs make their use unsuitable for prevention and chronic use [18, 19] so

that modulation of the immune system through food components is considered as an effective and efficient measure. Recently, protein hydrolysis as an immunomodulator source was produced from protein sources derived from fish [20, 10, 21-23], shellfish [24], Oyster [19, 25], and clam [26,27].

The conventional method commonly used in research on bioactive peptides begins by hydrolyzing proteins with various enzymes and then testing them in vitro to determine the activities of various bioactive peptides. Such method takes a lot of time and costs, so other methods that are more efficient and practical in determining bioactive peptides should be developed. The in-silico method is a computer-based method with the advantage of its economic efficiency and the results can be used as a reference before conducting actual research. According to Mekenyan [28], the advantage of the in-silico method is that it can be used as a reference before conducting actual research. This study aims to find the content of bioactive peptides in skipjack tuna (*Katsuwonus pelamis*) hydrolysate with different enzymes so as to find the highest and best bioactive peptide compounds using the in-silico method.

MATERIALS AND METHODS

Preparation of *Katsuwonus Pelamis* Database

The materials used in this research are the gen bank of *Katsuwonus pelamis* acquired from NCBI (National Center for Biotechnology) with the code of database BAA95128.1 Gen Bank. The sequences obtained from NCBI are MAPKKAKRRQQQGEGGSSNVFSMFEQSQIQEYKEA FTIIDQNRDGIISKDDLRDVLATMGQLNVKNEELEA MVKEASGPINFTVFLTMFGEKLKGADPEDVIVSAFK VLDPEGTGAIKKEFLEELLTTQCDRFTAEEMTNLWA AFPPDVAGNVDYKNICYVITHGEDKEE

Computational Analysis

The tools used in this research are laptops with Acer Aspire 5 specifications, Windows 10 operating system and software (software) BIOPEP and Peptide ranker. Prediction of bioactive activity and proteolytic analysis of *Katsuwonus pelamis* is conducted using BIOPEP software (http://www.unm.ude.pl/biochemia/index.php/en/biop ep) [29]. Examination of sensory characteristic Katsuwonus pelamis hydrolysate by analyzing sequence that obtained from NCBI. Sensory characteristic Katsuwonus pelamis hydrolysate by analyzing sequence that obtained from NCBI. Sensory characteristic collagen from done by BIOPEP software. Peptide rank analysis is using peptide cut material from collagen sequence with the highest bioactivity and analyzed with Peptide Ranker (http://bioware.ucd.ie/compass/biowareweb/). Peptide ranker is a server that capable to ranking peptide according to the level of peptide activity. The score of Peptide ranker is based on predictions of peptide bioactivity. Peptide ranker can predict the probability (between 0 and 1) of a peptide being bioactive [30]. ProTox II (https://tox-new.charite.de/protox II/index.php?site=compound input) is used to determine toxicity [31].

RESULTS AND DISCUSSIONS

Bioactive Activity Prediction of *Katsuwonus Pelamis* Hydrolysate

Biological activity in *Katsuwonus pelamis* hydrolysate can be analyzed using BIOPEP software. This software was developed by the Mazury University Poland biochemistry laboratory. This software provides database information in the form of proteins, bioactive peptides, allergenic proteins with their epitopes and sensory peptides and amino acids. One of the uses of this software is for the evaluation of proteins as bioactive peptide precursors [32].

The BIOPEP-UWM database contains sequences of bioactive biological peptides which have been available since 2003 and are constantly being updated. The database has a function to provide information for working in the health sector. Databases have recently been widely used in food science and nutrition as a source of information on peptides as functional foods involved in chronic disease prevention. The Peptide Rank of *Katsuwonus pelamis* can be seen in Table-1.

Table-1. Pep Rank of *Katsuwonus pelamis*.

PepRank	Bioactive sequence
0.996643	MF
0.973259	AF
0.943972	MG
0.815398	VF
0.546994	AG
0.487442	HG
0.38751	QG
0.312686	KG
0.2924	QL
0.234552	KL
0.145789	PE
0.132683	AD
0.130521	QI
0.126025	VL
0.124869	AS
0.080954	QS
0.0710058	AT

G-Glycine, P-Proline, Q-Glutamine, W-Tryptophan, L-Leucine, D-Aspartic acid, F-Phenylalanine,
T-Threonine, M-Methionine, A-Alanine, R-Arginine, S-Serine, I-Isoleucine, K-Lysine.

Based on Table 1, it can be seen that there are 24 ranks of bioactive peptide found on *Katsuwonus pelamis*

hydrolysate. The lowest value obtained by AT sequence with 0.071 while the highest value obtained by MF sequence with 0.996. The value of PepRank shows the probability of bioactivity of each peptide.

Table-2. Profile of potential enzymatic activity of *Katsuwonus pelamis.*

Peptide ID	Bioactive sequence	Location	Activity	Monoisotopic mass	Chemical mass
3384	VF	[20-21]	ACE inhibitor	264.1465	264.3252
3384	VF	[85-86]	ACE inhibitor	264.1465	264.3252
3385	MF	[23-24]	ACE inhibitor	296.1189	296.3902
3385	MF	[89-90]	ACE inhibitor	296.1189	296.3902
7583	AF	[35-36]	ACE inhibitor	236.1155	236.2712
7583	AF	[106-107]	ACE inhibitor	236.1155	236.2712
7583	AF	[146-147]	ACE inhibitor	236.1155	236.2712
7600	AG	[152-153]	ACE inhibitor	146.0685	146.1462
7604	KG	[95-96]	ACE inhibitor	203.1255	203.2412
7609	MG	[59-60]	ACE inhibitor	206.0719	206.2652
7614	HG	[166-167]	ACE inhibitor	212.0895	212.2092
7617	QG	[12-13]	ACE inhibitor	203.0895	203.1982
7693	KL	[93-94]	ACE inhibitor from wakame	259.1885	259.3492
7841	KE	[33-34]	ACE inhibitor	275.1465	275.3052
7841	KE	[170-171]	ACE inhibitor	275.1465	275.3052
8320	VL	[55-56]	Glucose uptake stimulating peptide	230.1625	230.3082
8320	VL	[109-110]	Glucose uptake stimulating peptide	230.1625	230.3082
8134	KD	[49-50]	peptide derived from dried bonito	261.1314	261.2782
8757	AD	[97-98]	dipeptidyl peptidase IV inhibitor (DPP IV inhibitor)	204.0744	204.1832
8758	AE	[136-137]	dipeptidyl peptidase IV inhibitor (DPP IV inhibitor)	218.0895	218.2102
8759	AF	[35-36]	dipeptidyl peptidase IV inhibitor (DPP IV inhibitor)	236.1155	236.2712
8759	AF	[106-107]	dipeptidyl peptidase IV inhibitor (DPP IV inhibitor)	236.1155	236.2712
8759	AF	[146-147]	dipeptidyl peptidase IV inhibitor (DPP IV inhibitor)	236.1155	236.2712
8760	AG	[152-153]	dipeptidyl peptidase IV inhibitor (DPP IV inhibitor)	146.0685	146.1462
8762	AS	[77-78]	dipeptidyl peptidase IV inhibitor (DPP IV inhibitor)	176.0795	176.1742
8763	AT	[57-58]	dipeptidyl peptidase IV inhibitor (DPP IV inhibitor)	190.0945	190.1992
8808	KE	[33-34]	dipeptidyl peptidase IV inhibitor (DPP IV inhibitor)	275.1465	275.3052
8808	KE	[170-171]	dipeptidyl peptidase IV inhibitor (DPP IV inhibitor)	275.1465	275.3052
8810	KG	[95-96]	dipeptidyl peptidase IV inhibitor (DPP IV inhibitor)	203.1255	203.2412
8827	MF	[23-24]	dipeptidyl peptidase IV inhibitor (DPP IV inhibitor)	296.1189	296.3902
8827	MF	[89-90]	dipeptidyl peptidase IV inhibitor (DPP IV inhibitor)	296.1189	296.3902
8828	MG	[59-60]	dipeptidyl peptidase IV inhibitor (DPP IV inhibitor)	206.0719	206.2652
8869	QE	[30-31]	dipeptidyl peptidase IV inhibitor (DPP IV inhibitor)	275.1105	275.2622
8871	QG	[12-13]	dipeptidyl peptidase IV inhibitor (DPP IV inhibitor)	203.0895	203.1982
8873	QI	[28-29]	dipeptidyl peptidase IV inhibitor (DPP IV inhibitor)	259.1525	259.3062
8874	QL	[61-62]	dipeptidyl peptidase IV inhibitor (DPP IV inhibitor)	259.1525	259.3062
8877	QS	[26-27]	dipeptidyl peptidase IV inhibitor (DPP IV inhibitor)	233.1005	233.2262
8915	VD	[155-156]	dipeptidyl peptidase IV inhibitor (DPP IV inhibitor)	232.1054	232.2372
8917	VF	[20-21]	dipeptidyl peptidase IV inhibitor (DPP IV inhibitor)	264.1465	264.3252
8917	VF	[85-86]	dipeptidyl peptidase IV inhibitor (DPP IV inhibitor)	264.1465	264.3252
8920	VI	[102-103]	dipeptidyl peptidase IV inhibitor (DPP IV inhibitor)	230.1625	230.3082
8922	VL	[55-56]	dipeptidyl peptidase IV inhibitor (DPP IV inhibitor)	230.1625	230.3082
8922	VL	[109-110]	dipeptidyl peptidase IV inhibitor (DPP IV inhibitor)	230.1625	230.3082
8926	VS	[104-105]	dipeptidyl peptidase IV inhibitor (DPP IV inhibitor)	204.1105	204.2282
9694	PE	[99-100]	Alpha-glucosidase inhibitor	244.1045	244.2482
9694	PE	[112-113]	Alpha-glucosidase inhibitor	244.1045	244.2482
9695	AD	[97-98]	Alpha-glucosidase inhibitor	204.0744	204.1832
9504	PE	[99-100]	DPP-III inhibitor	244.1045	244.2482
9504	PE	[112-113]	DPP-III inhibitor	244.1045	244.2482

G-Glycine, P-Proline, Q-Glutamine, W-Tryptophan, L-Leucine, D-Aspartic acid, F-Phenylalanine, T-Threonine, M-Methionine, A-Alanine, R-Arginine, S-Serine, I-Isoleucine, K-Lysine.

The threshold value for peptide bioactivity is 0.5. The higher the value means that the peptides have a strong bioactivity while the lower the value (especially below threshold 0.5) indicating that there is low or non-existing bioactivity of a peptide [33]. Each of the peptide analysed and ranked may have varying activity. The potential activity of peptides can be seen on Table-2.

Table-2 shows the profile of the potential enzymatic activity of *Katsuwonus pelamis* hydrolysate. There are total of 49 different types of bioactive sequence and location of analysed peptides. Based on the tables, there are total of 7 big groups of activity dominated by 2 big groups of activity which is ACE inhibitor and dipeptidyl peptidase IV inhibitor (DPP IV inhibitor). The minority of peptide activity are glucose uptake stimulating peptides, peptides derived from dried bonito, alpha-glucosidase inhibitor and DPP-II inhibitor. All of the activity obtained are originated from the dipeptides class. The highest monoisotopic mass obtained by MF peptides with the value of 296.12 that can act as ACE inhibitor and DPP IV inhibitor. While the lowest obtained by AG peptides with the value 146.06 that can act as ACE inhibitor and DPP IV inhibitor. There is a little bit different between monoisotopic mass and chemical mass of active peptides that has the same biological activity. BIOPEP also capable of analyzing sensory peptides. The sensory peptides of *Katsuwonus pelamis* hydrolysates can be seen on Table-3.

Table-3. Sensory peptide of *Katsuwonus pelamis*

PEPTIDE/AMINO ACID ID	Bioactive sequence	Location	Name	Activity	Monoisotopic mass	Chemical mass
1	R	[9-9]	bitter amino acid	bitter	174.1010	174.1880
1	R	[53-53]	bitter amino acid	bitter	174.1010	174.1880
1	R	[133-133]	bitter amino acid	bitter	174.1010	174.1880
7	F	[122-122]	bitter amino acid	bitter	165.0680	165.1770
7	F	[134-134]	bitter amino acid	bitter	165.0680	165.1770
50	AF	[35-36]	bitter peptide	bitter	236.1050	236.2560
50	AF	[106-107]	bitter peptide	bitter	236.1050	236.2560
50	AF	[146-147]	bitter peptide	bitter	236.1050	236.2560
51	VF	[20-21]	bitter peptide	bitter	264.1360	264.3100
51	VF	[85-86]	bitter peptide	bitter	264.1360	264.3100
105	V	[151-151]	bitter amino acid	bitter	117.0680	117.1330
110	VL	[55-56]	bitter peptide	bitter	230.1520	230.2930
110	VL	[109-110]	bitter peptide	bitter	230.1520	230.2930
111	VI	[102-103]	bitter peptide	bitter	230.1520	230.2930
134	VD	[155-156]	bitter peptide	bitter	232.0949	232.2220
145	AD	[97-98]	bitter peptide	bitter	204.0639	204.1680
172	L	[52-52]	bitter amino acid	bitter	131.0840	131.1600
172	L	[69-69]	bitter amino acid	bitter	131.0840	131.1600
172	L	[87-87]	bitter amino acid	bitter	131.0840	131.1600
172	L	[123-123]	bitter amino acid	bitter	131.0840	131.1600
172	L	[126-126]	bitter amino acid	bitter	131.0840	131.1600
172	L	[127-127]	bitter amino acid	bitter	131.0840	131.1600
225	D	[44-44]	Sour amino acid	sour	133.0269	133.0890
225	D	[51-51]	Sour amino acid	sour	133.0269	133.0890
225	D	[54-54]	Sour amino acid	sour	133.0269	133.0890
225	D	[101-101]	Sour amino acid	sour	133.0269	133.0890
225	D	[111-111]	Sour amino acid	sour	133.0269	133.0890

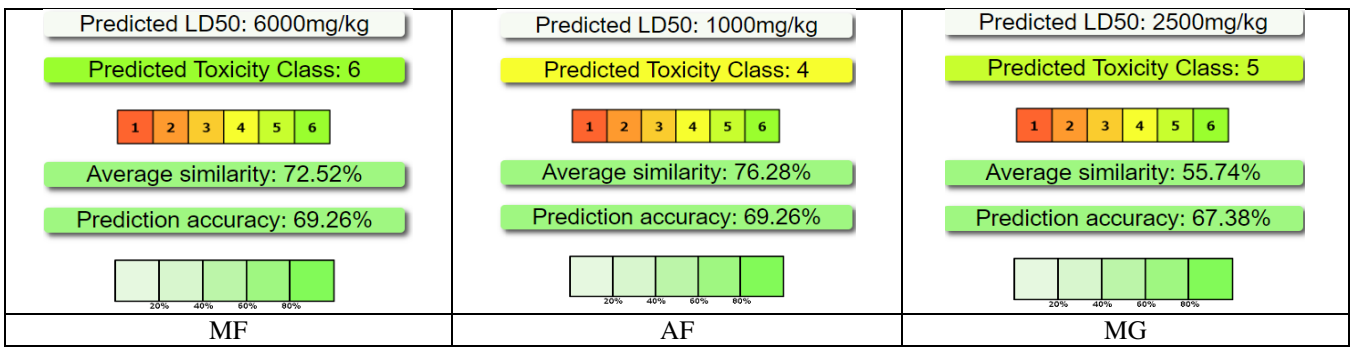
Table-3. *Cont.*

PEPTIDE/AMINO ACID ID	Bioactive sequence	Location	Name	Activity	Monoisotopic mass	Chemical mass
226	D	[169-169]	Umami amino acid	umami	133.0269	133.0890
227	D	[44-44]	Salty amino acid	salty	133.0269	133.0890
227	D	[51-51]	Salty amino acid	salty	133.0269	133.0890
227	D	[54-54]	Salty amino acid	salty	133.0269	133.0890
227	D	[101-101]	Salty amino acid	salty	133.0269	133.0890
227	D	[111-111]	Salty amino acid	salty	133.0269	133.0890
227	D	[169-169]	Salty amino acid	salty	133.0269	133.0890
228	E	[14-14]	Sour amino acid	sour	147.0420	147.1160
228	E	[25-25]	Sour amino acid	sour	147.0420	147.1160
228	E	[68-68]	Sour amino acid	sour	147.0420	147.1160
228	E	[70-70]	Sour amino acid	sour	147.0420	147.1160
228	E	[92-92]	Sour amino acid	sour	147.0420	147.1160
228	E	[124-124]	Sour amino acid	sour	147.0420	147.1160
228	E	[125-125]	Sour amino acid	sour	147.0420	147.1160
228	E	[138-138]	Sour amino acid	sour	147.0420	147.1160
228	E	[168-168]	Sour amino acid	sour	147.0420	147.1160
228	E	[172-172]	Sour amino acid	sour	147.0420	147.1160
229	E	[14-14]	Umami amino acid	umami	147.0420	147.1160
229	E	[25-25]	Umami amino acid	umami	147.0420	147.1160
229	E	[68-68]	Umami amino acid	umami	147.0420	147.1160
229	E	[70-70]	Umami amino acid	umami	147.0420	147.1160
229	E	[92-92]	Umami amino acid	umami	147.0420	147.1160
229	E	[124-124]	Umami amino acid	umami	147.0420	147.1160
229	E	[125-125]	Umami amino acid	umami	147.0420	147.1160
229	E	[138-138]	Umami amino acid	umami	147.0420	147.1160
229	E	[168-168]	Umami amino acid	umami	147.0420	147.1160
229	E	[172-172]	Umami amino acid	umami	147.0420	147.1160
234	K	[108-108]	Sour amino acid	sour	146.0940	146.1740
235	K	[108-108]	Sweet amino acid	sweet	146.0940	146.1740
236	K	[108-108]	Bitter amino acid	bitter	146.0940	146.1740
237	K	[108-108]	Astringent amino acid	astringent	146.0940	146.1740
257	V	[151-151]	sweet amino acid	sweet	117.0680	117.1330
264	VD	[155-156]	Umami peptide	umami	232.0949	232.2220
265	VD	[155-156]	Sour peptide	sour	232.0949	232.2220
270	G	[15-15]	Sweet amino acid	sweet	75.0210	75.0520
270	G	[16-16]	Sweet amino acid	sweet	75.0210	75.0520
270	G	[45-45]	Sweet amino acid	sweet	75.0210	75.0520
270	G	[79-79]	Sweet amino acid	sweet	75.0210	75.0520

Table-3. *Cont.*

PEPTIDE/AMINO ACID ID	Bioactive sequence	Location	Name	Activity	Monoisotopic mass	Chemical mass
270	G	[91-91]	Sweet amino acid	sweet	75.0210	75.0520
270	G	[114-114]	Sweet amino acid	sweet	75.0210	75.0520
270	G	[116-116]	Sweet amino acid	sweet	75.0210	75.0520
307	A	[145-145]	sweet amino acid	sweet	89.0370	89.0790
348	W	[144-144]	Bitter amino acid	bitter	204.0790	204.2130
349	AE	[136-137]	Umami peptide	umami	218.0790	218.1950
365	KG	[95-96]	Umami peptide	umami	203.1150	203.2260
366	KG	[95-96]	Bitter peptide	bitter	203.1150	203.2260
371	R	[9-9]	Bitterness suppressing amino acid	bitterness suppressing	174.1010	174.1880
371	R	[53-53]	Bitterness suppressing amino acid	bitterness suppressing	174.1010	174.1880
371	R	[133-133]	Bitterness suppressing amino acid	bitterness suppressing	174.1010	174.1880
372	K	[108-108]	Bitterness suppressing amino acid	bitterness suppressing	146.0940	146.1740
414	PE	[99-100]	Umami enhancing peptide	umami enhancing	244.0940	244.2330
414	PE	[112-113]	Umami enhancing peptide	umami enhancing	244.0940	244.2330
449	AD	[97-98]	Umami peptide	umami	204.0639	204.1680

Based on Table-3, There are 86 types of peptides and amino acids analysed using BIOPEP that may contribute to the sensory properties of *Katsuwonus pelamis* meat. The identification numbers of peptides and amino acids vary from 1 to 449, showing that there are a lot of peptides and amino acids that contribute in the sensory profile of the meat. The sensory flavor is classified into four groups that consist of bitter taste, sour taste, umami taste, salty taste, sweet taste and astringent taste. Each of the groups has different total taste. The taste primarily dominated by bitter taste with 29 types of peptides and amino acid that contributes, followed by sour taste with 18 contributing amino acid and peptides. Umami taste have 22 contributing amino acid and peptides while salty taste has 6 contributing amino acid and peptides. Sweet and astringent taste are the least taste in the sensory profile of *Katsuwonus pelamis* with only 10 and 1 contributing amino acid and peptides respectively. The highest monoisotopic mass is 264.136 which contributes in a bitter taste and the lowest is 75.021 which contributes in a sweet taste. There are little bit differences in chemical mass if compared to monoisotopic mass. The highest chemical mass is 264.31 (bitter taste) while the lowest chemical mass is the same as monoisotopic mass with sweet taste.



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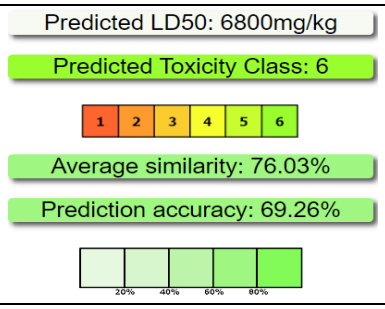	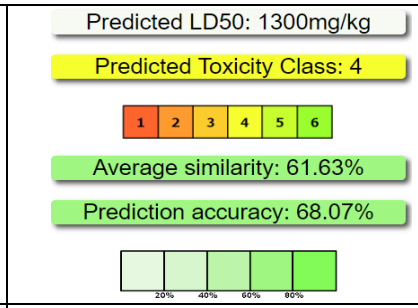	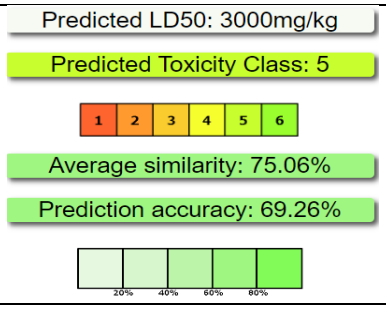
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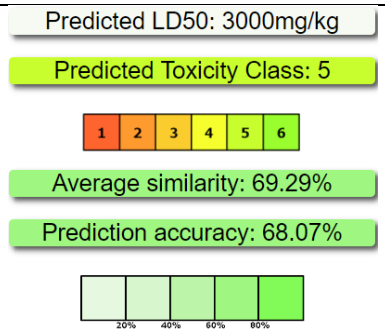	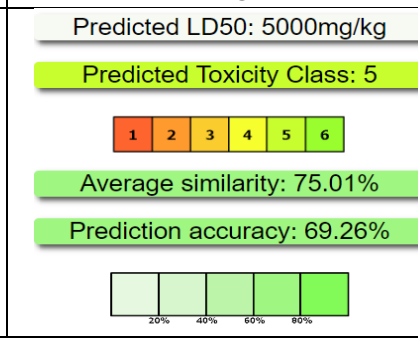	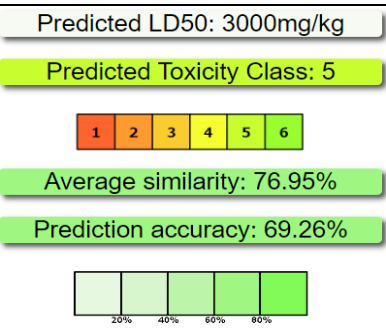
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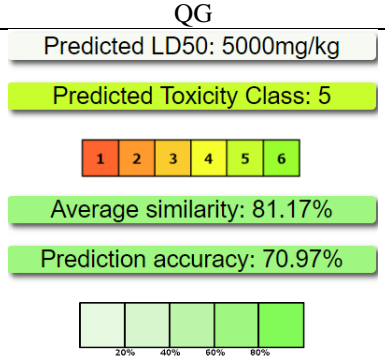	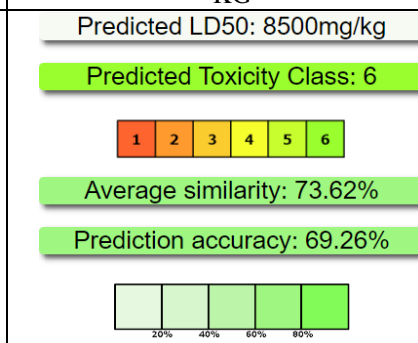	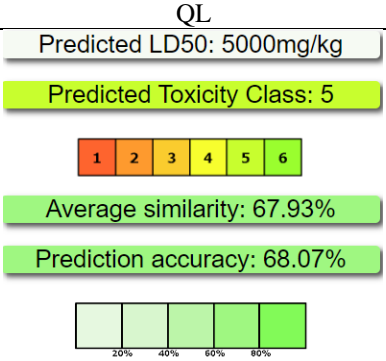
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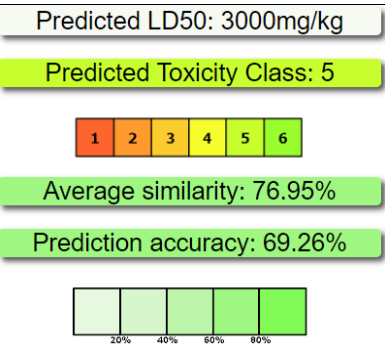	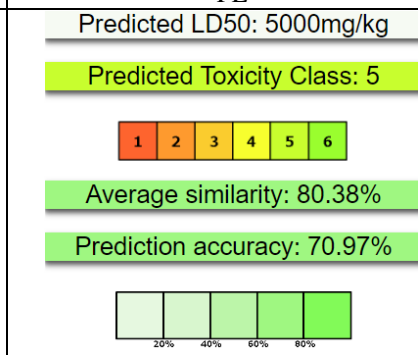	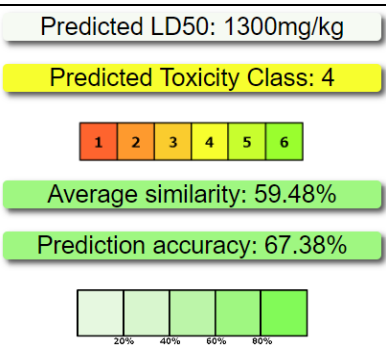
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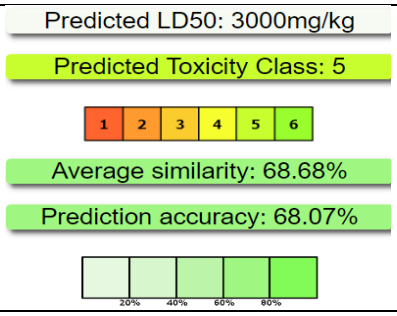	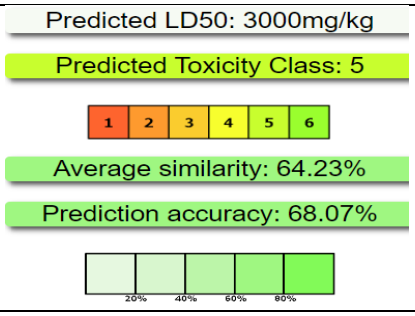	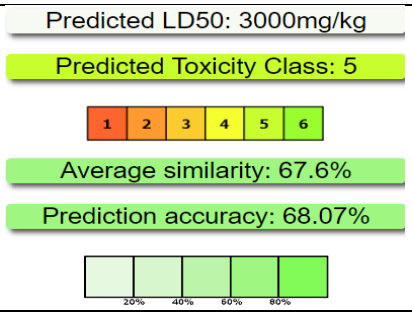
QS	AT	KD

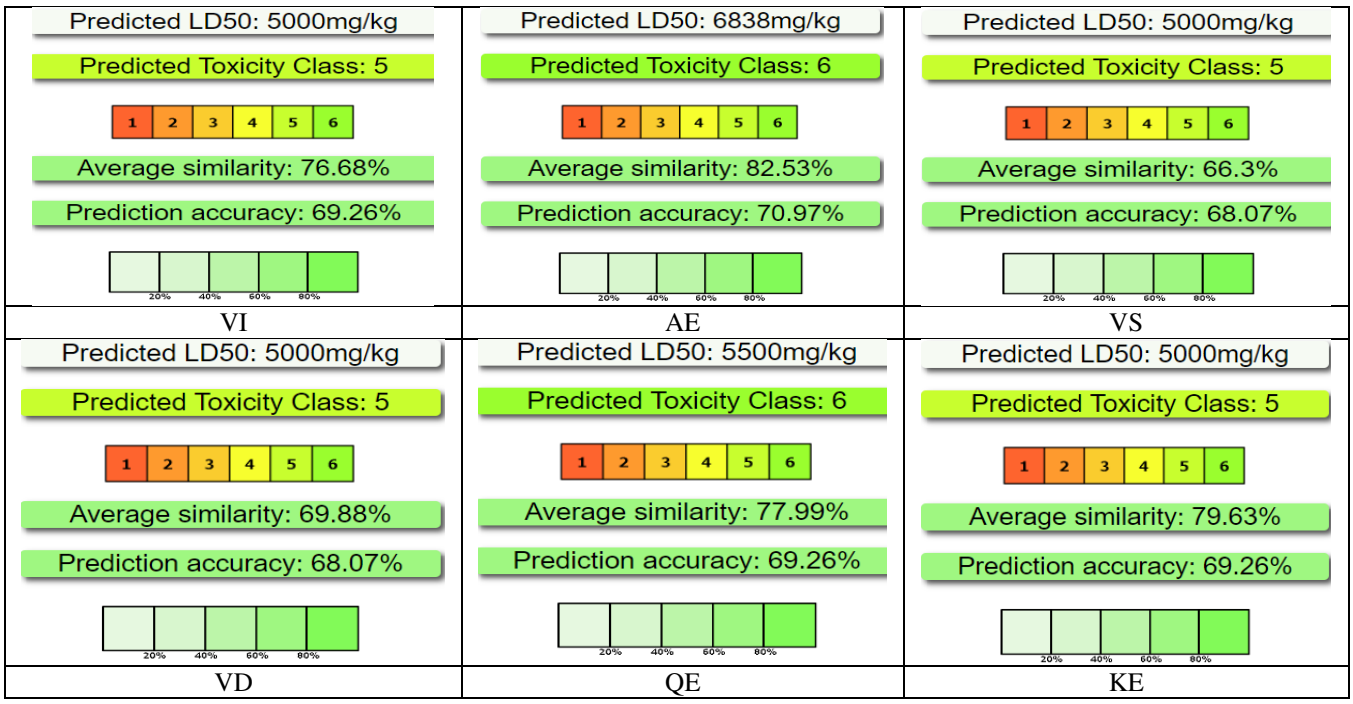

Figure-1. *Katsuwonus pelamis* peptide toxicity.

Figure-1 shows the predicted toxicity of 24 amino acids and peptides analyzed using ProTox II. Based on the results, the amino acids and peptides are divided into 3 groups which is based on toxicity class that consists of class IV,V and VI. The majority of peptides and amino acids, as many as 16 of it are analyzed and classifed into class V (maybe harmful if swallowed) with the LD50 value of 2000<LD50≤5000 mg/kg. There are 5 peptides and amino acids that are classfied as class VI (non-toxic) with the LD50 value of LD50 > 5000 mg/kg. The last 3 peptides and amino acids are classifed as class IV (harmful if swallowed) with the LD50 value of 300 < LD50 ≤ 2000 mg/kg. The higher-class numbers of toxicity indicate the substance is more likely categorized as less toxic or nontoxic compared to the substance categorized with lower class numbers. Class I is categorized as fatal if swallowed (LD50≤5 mg/kg while class VI is categorized as non-toxic (LD50>5000 mg/kg).

CONCLUSIONS

The current study suggested that hydrolysate of *Katsuwonus pelamis* may have potential as an ACE inhibitor and DPP IV inhibitor. Based on sensory analysis on *Katsuwonus pelamis,* the most dominant sensory attributes are bitter and sour tastes. The hydrolysate of *Katsuwonus pelamis* also shown to be categorized as less harmful with toxicity level primarily on class V and VI. Further research should be done in vitro and in vivo to elucidate the hydrolysate of *Katsuwonus pelamis*, which might be used as a bioactive agent for functional food.

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