魚類副產物之水解物中血管收縮素轉換酶 (ACE) 抑制胜肽之純化與分子對接分析

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報告日期：2023 年 03 月 08 日

內容	時間掌控	表達能力	投影片	書面資料
40%	10%	30%	10%	10%

指導教授簽名:＿＿＿＿＿＿＿＿＿＿＿＿

Purification of Angiotensin I-Converting Enzyme (ACE) Inhibitory Peptides and Their Molecular Docking Analysis from Fish Byproduct Hydrolysates

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2023/03/08

Outline

Abstract

One of the major causes of death around the world today is cardiovascular disease (CVD), its risk factors include hypertension and high blood pressure. When angiotensin I is exposed to angiotensin I-converting enzyme (ACE), resulting in the formation of angiotensin II causes blood pressure to rise. Synthetic ACE inhibitors have side effects, so the search for active peptides that inhibit ACE from natural sources has received attention. Through enzyme inhibition kinetics and molecular docking to discover the underlying mechanisms that allow peptides to serve as potential antihypertensive agents.

Atlantic salmon bones protein hydrolysates (SBPH) undergo ultrafiltration for further fractionation, it was found that the greatest ACE-inhibitory activity was achieved by a molecular weight lower than 0.65 kDa. The peptide in F7 fraction comprised eight amino acids FCLYELAR (IC_{50} on ACE = 0.033 mg/mL), it serve as an uncompetitive ACE inhibitor. In molecular docking process showed that FCLYELAR peptide bind to zinc ions, which lead to ACE inhibition.

Shortfin scad waste hydrolysate (SWH) ultra-filtered peptide fraction (< 3 kDa) possessed the highest ACE inhibitory activity, followed by the fraction 14 by gel filtration, the amino acid sequence of RGVGPVPAA (IC_{50} on ACE = 0.20 mg/mL) was identified, it obtained acted as a competitive ACE inhibitor. The molecular docking studies showed that the SWH peptide exhibit hydrogen bonds with ACE active site.

In conclusion, the purified peptides isolated from fish waste protein hydrolysates have potential antihypertensive properties which could potentially be used as functional food ingredients.

一、前言

　　心血管疾病 (Cardiovascular disease, CVD) 是全球主要的死亡原因之一，每年造成 1860 萬人死亡，其中高血壓 (Hypertension) 為主要原因 (Fisher and Curfman, 2018; WHO, 2022)。根據衛福部於 110 年國人十大死因調查中顯示，每十萬人口中有 33.6 人死於高血壓，顯示調節血壓對於國人健康的重要性 (衛生福利部統計處，2022)。

　　腎素-血管收縮素系統 (Renin-angiotensin system, RAS) 為調節血壓系統，腎素 (Renin) 將血管收縮素原 (Angiotensinogen) 水解為血管收縮素 I (Angiotensin I)，接著血管收縮素 I 轉換酶 (Angiotensin I converting enzyme, ACE) 將血管收縮素 I 催化成血管收縮素 II (Angiotensin II)，從而導致血管收縮，ACE 也會降解具有血管擴張特性的緩激肽 (Bradykinin) (Pacurari et al., 2014; Bhandari et al., 2020)，因此抑制體內 ACE 之活性不僅具有調節血壓的效果，且亦可降低罹患 CVD 之風險。常見的 ACE 抑制合成藥物長期食用可能產生咳嗽 (Yilmaz, 2019)、頭暈 (Leru and Anton, 2018)、高鉀血症 (Dixit et al., 2019) 等副作用，因此尋找天然來源具有抑制 ACE 之活性胜肽受到重視。

　　根據 2021 年聯合國糧食及農業組織 (Food and agriculture organization of the United Nations, FAO) 統計，漁業與水產養殖生產加工過程中，約有 30-70% 的加工魚成為副產物，而魚類副產物中約含有 49-58% 蛋白質，經酵素水解會產生具有生理活性之胜肽 (Zamora Sillero et al., 2018; Kandyliari et al., 2020)。先前有許多研究指出，魚類副產物已被作為萃取具 ACE 抑制能力之活性胜肽的原料，如海鱺魚皮 (Lin et al., 2019)、鮭魚碎骨 (Neves and Harnedy, 2017) 及鰹魚殘肉 (Zheng et al., 2022) 等。

　　酵素抑制動力學 (Enzyme inhibition kinetics) 可以了解胜肽作為抗高血壓藥物的潛在機制 (Nuchprapha et al., 2020)。以 Lineweaver-Burk 圖對不同胜肽濃度與反應速率之數據進行雙倒數分析，確定酵素與基質間親和力指標 (K_m)、最大速率 (V_{max}) 及 ACE 抑制型態 (Ma et al., 2019)。另外一方面，先前研究 ACE 的抑制機制大多無法明確了解其抑制劑鍵結的活性位點 (Active site)，然而現在可以利用分子對接 (Molecular docking) 模擬抑制劑之胜肽序列與 ACE 在分子上形成的鍵結，研究 ACE 與胜肽之間的相互作用，以了解胜肽之抑制機制 (Chamata et al., 2020; Tao et al., 2020)。

　　綜合上述，本實驗將探討鮭魚與長身圓鰺之副產物經酵素水解後，進一步透過逆相

1 高效液相層析法 (Reverse phase high performance liquid chromatography, RP-HPLC) 進行

2 水解物之純化,再利用質譜 (Mass spectra, MS) 鑑定出胜肽序列,測定其抑制 ACE 能力

3 之效果,並以酵素抑制動力學分析胜肽對 ACE 之抑制型態,最後以分子對接預測純化

4 後的胜肽序列對抑制 ACE 的結合位點與機制。

5 二、生物活性胜肽之純化與 ACE 抑制動力學之分析

6 2.1 鮭魚骨蛋白水解物

7 　　本研究目的為探討大西洋鮭魚骨經 0.4% (w/w) 之 Trypsin 水解 360 min 後,接著

8 將鮭魚骨水解物 (Salmon bone protein hydrolysates, SBPH) 經超過濾 (Ultrafiltration) 與

9 RP-HPLC 的純化後,利用液相層析串聯質譜儀 (Liquid chromatography-Mass

10 spectrometry/Mass spectrometry, LC-MS/MS) 鑑定出胜肽序列,並測量與獲得最高 ACE

11 抑制能力之胜肽序列後,進行抑制 ACE 動力學及分子對接之分析,以了解胜肽對 ACE

12 之抑制型態。

13 　　首先使用不同的截留分子量膜 (Molecular weight cut-off, MWCO) 將 SBPH 進行超

14 過濾,得到五種不同劃分物,分別為分子量 >10 kDa、10-5 kDa、5-3 kDa、3-0.65 kDa 及

15 <0.65 kDa,並測定各劃分物之 ACE 抑制能力 (Table 1)。結果顯示,<0.65 kDa 之組別

16 具有最好 ACE 抑制能力 (IC_{50}=0.093 μg/mL),較未經超過濾之組別 (IC_{50}=7.140 μg/mL)

17 提升達 75 倍。先前有研究指出,較低分子量之胜肽於腸道中較容易被吸收,且易與 ACE

18 活性位點結合,從而增加抑制 ACE 活性 (Sonklin *et al.*, 2020)。

19 　　將最高 ACE 抑制能力之劃分物 (< 0.65 kDa) 經 RP-HPLC 進一步純化。首先使用

20 C_{18} 管柱將乙腈 (Acetonitrile, ACN) 以流速 0.7 mL/min 於 50 min 內由 0 提高至 45%

21 (v/v) 進行梯度沖提,分離出不同的主要波峰 (F_1-F_{16}) 並分別測定其 ACE 之抑制能力

22 (Fig. 1; Table 2)。結果顯示,F_7 具有最高 ACE 抑制能力,其 ACE 之 IC_{50} 值為 0.26

23 μg/mL,進一步透過 LC-MS/MS 分析 F_7 之胜肽序列並進行胜肽合成 (Fig. 2)。結果顯

24 示,F_7 之胜肽序列為 FCLYELAR (Phe-Cys-Leu-Tyr-Glu-Leu-Ala-Arg),其 ACE 之 IC_{50}

25 值為 0.033 mg/mL。目前 ACE 抑制胜肽主要由 2-12 個胺基酸組成,並且胜肽中具有帶

26 負電荷之胺基酸,如 Glutamate 與 Cysteine,能與 ACE 中的 Zn^{2+} 螯合,進而降低其催

27 化速率及活性 (Aluko, 2015; Andújar-Sánchez *et al.*, 2004; Mirzaei *et al.*, 2019)。

透過不同馬尿醯組胺醯亮胺酸 (Hippuryl-histidyl-leucine, HHL) 之濃度 (0.5、1、3、5 及 7 mM) 與 FCLYELAR 之濃度 (0.05、0.1 及 0.2 mM) 下，測定酵素抑制反應之 K_m 與 V_{max} 值，並將其繪製成 Lineweaver Burk 圖，以了解此胜肽對 ACE 之抑制模式 (Fig. 3; Table 3)。結果顯示，三條不同胜肽濃度之直線皆保持平行，且胜肽濃度從 0.05 mM 提升至 0.2 mM 時，其 K_m 值從 2.53 mM 降低至 0.91 mM，而 V_{max} 值從 0.11 降低至 0.04 mM/min，此型態為不競爭型抑制 (Uncompetitive inhibition)。不競爭型抑制型態之抑制劑僅能與受質與酵素的複合物結合，因此當受質濃度增加，有利於提升抑制劑之抑制能力 (Delaune and Alsayouri, 2019)。

ACE 之活性區域包含 S1、S1'、S2 及 Zn^{2+}，S1 活性區域由 ACE 殘基 Ala 354、Glu 384 及 Tyr 523 組成；S1' 活性區域由 ACE 殘基 Glu 162 組成；S2 活性區域由 ACE 殘基 Gln 281、His 353、Lys 511、His 513 及 Tyr 520 組成，而 Zn^{2+} 活性區域由 ACE 殘基 His 383、His 387 和 Glu 411 組成 (Andújar-Sánchez *et al*., 2004; Pan *et al*., 2012)。為了模擬 ACE 結構與胜肽之間的相互作用，使用 GOLD program V.5.7 軟體進行了分子對接模擬 (Table 4; Fig. 4)。結果顯示，$F_1C_2L_3Y_4E_5L_6A_7R_8$ 與 ACE 鍵結總共包含 13 個氫鍵 (Hydrogen bonds)、4 個離子鍵 (Electrostatic bonds)、3 個疏水鍵 (Hydrophobic bonds) 及 2 個金屬受體相互作用 (Metal acceptor)，皆沒有與 ACE 之活性區域 (S1、S1'、S2 及 Zn^{2+}) 鍵結，表示此胜肽不與基質競爭相同 ACE 之活性位點，與抑制動力學結果相符。先前有研究指出，合成藥物 Lisinopril 中羧基 (Carboxyl group) 會與 ACE 中的 Zn^{2+} 形成金屬與受體間相互作用，以達到降低 ACE 之能力 (Jimsheena and Gowda, 2011)，因此推測此胜肽主要透過 Y_4 和 E_5 與 ACE 中 Zn 576 形成金屬與受體的相互作用進而抑制 ACE 之活性。

本研究結果顯示，SBPH 經超過濾後分子量 <0.65 kDa 的劃分物具有最高 ACE 抑制能力 (IC_{50} = 0.093 μg/mL)，接著經 RP-HPLC 純化後 F_7 具有最高 ACE 抑制能力 (IC_{50} = 0.26 μg/mL)，並鑑定出其胜肽序列為 FCLYELAR (IC_{50} = 0.033 mg/ mL)。當 FCLYELAR 濃度提升時，其 K_m 與 V_{max} 值分別下降 64.0% 與 63.6%，表示此胜肽為不競爭型抑制劑，進一步以分子對接模擬推測，此胜肽主要透過 Glutamate 與 Tyrosine 和 ACE 中 Zn 576 形成金屬與受體間相互作用，進而降低 ACE 之活性。

2.2 長身圓鰺蛋白水解物

　　本研究目的探討長身圓鰺 (*Decapterus macrosoma*) 之副產物 (魚骨、魚皮及魚尾) 經 2.92% (w/w) 之 Alcalase (pH = 7.0-9.0, temperature = 30-65°C) 水解 60 min 後，利用超過濾、膠體過濾層析 (Gel filtration Chromatogram) 及 RP-HPLC 進行純化後分別測定胜肽之 ACE 抑制能力，並使用基質輔助雷射脫附游離飛行時間質譜技術 (Matrix-assisted laser desorption ionization time of flight mass spectrometry, MALDI-TOF MS) 鑑定胜肽序列，最後以抑制動力學及分子對接之分析，探討胜肽對 ACE 之抑制型態及結合位點。

　　首先使用不同的截留分子量膜將長身圓鰺蛋白水解物 (Shortfin scad waste protein hydrolysate, SWH) 進行超過濾，劃分為 < 3 kDa、3-5 kDa、5-10 kDa、> 10 kDa 四種分子量，並分別測定各劃分物之 ACE 抑制活性 (Table 5)。結果顯示，分子量 < 3 kDa 之組別具有最高 ACE 抑制能力 (IC_{50} = 2.2 mg/mL)，較分子量 > 10 kDa 之組別提升 73.2%，故選用分子量 < 3 kDa 之組別進行後續膠體過濾層析。

　　將 SWH (< 3 kDa) 以 Sephadex G-25 進行純化分析，總共劃分出 28 個主要劃分物並分別將其命名為 Fraction 7-34，進一步收集並測定各 Fraction 抑制 ACE 之能力 (Fig. 5; Fig. 6)。結果顯示，Fraction 14 具有最高 ACE 抑制率 (81.5%)，相較於最低 ACE 抑制率之 Fraction 22 (49.0%) 高約 66.3%。接著將 Fraction 14 利用 RP-HPLC 進行純化與分離，使用 C_{18} 管柱將 ACN 以流速 1.0 mL/min 於 30 min 內由 0 提高至 70% (v/v) 進行線性沖提，測定出主要波峰及其 ACE 抑制能力 (Table 6; Fig. 7)。結果顯示，沖提時間約 17 min 時觀察到主要波峰 (Peptide P)，其 ACE 之 IC_{50} 值為 0.20 mg/mL，與 SWH 相比 (IC_{50} = 3.98 mg/mL) 提升約 19 倍。將 Peptide P 加入基質 α-氰基-4-羥基肉桂酸 (α-cyano-4-hydroxycinnamic acid) 以增加胜肽之電離效果，接著經 MALDI-TOF MS 鑑定其胜肽序列 (Fig. 8)。結果顯示，Peptide P 之胺基酸序列為 RGVGPVPAA (Arg-Gly-Val-Gly-Pro-Val-Pro-Ala-Ala)，主要由疏水性與芳香族胺基酸組成。先前有研究指出，當胜肽中疏水性胺基酸數量越多，有利於提高胜肽對抑制 ACE 之能力 (Aslam *et al.*, 2019)。

　　透過不同 RGVGPVPAA 之濃度 (0.25、0.5 及 1.0 mg/mL) 與不同 HHL 之濃度 (0.5、1.0、1.5、2.0 及 2.5 mM) 下繪製出 Lineweaver Burk 圖，並評估 RGVGPVPAA 對 ACE 抑制機制 (Fig. 9)。結果顯示，三條不同胜肽濃度之直線皆交於 y 軸，表示隨著胜

肽濃度增加，其 V_{max} 值不變，且 K_m 值約從 1.33 提升至 2.22 mM，此型態為競爭型抑制 (Competitive inhibition)。競爭型抑制型態之抑制劑會直接與 ACE 活性位點相互作用，阻止基質結合，從而抑制 ACE 活性 (Ring *et al.*, 2021)。

接著以 Discovery Studio 軟體進行分子對接，模擬胜肽 $R_1G_2V_3G_4P_5V_6P_7A_8A_9$ 與 ACE 活性區域 (S1、S1'、S2 及 Zn^{2+}) 的結合型態 (Table 7; Fig. 10)。結果顯示，此胜肽主要與 ACE 活性區域 S2 與 Zn^{2+} 鍵結，總共含 2 個氫鍵與 4 個 π-π 鍵，胜肽中 A_8 與 ACE 活性區域 S2 (His 353) 與 Zn^{2+} (His 383) 總共形成 3 個 π-π 鍵並與 Zn^{2+} (His 383) 形成 1 個氫鍵，另外胜肽中 A_9 與 ACE 活性區域 S2 (His 353) 形成 1 個氫鍵並與 S2 (His 513) 形成 1 個 π-π 鍵，表示此胜肽直接與 ACE 之活性位點鍵結，與抑制動力學結果相符。先前有研究指出，合成藥物 Captopril 中的羰基 (Carbonyl group) 會與 ACE 活性區域 S2 之 His 353 形成氫鍵進而降低 ACE 之活性 (Ji *et al.*, 2020)，因此推測此胜肽主要藉由疏水性胺基酸 Alanine 直接與 ACE 活性區域 S2 (His353) 形成氫鍵，以達到抑制 ACE 之能力。

本研究結果顯示，SWH 經超過濾後分子量 < 3 kDa 組別具有最高 ACE 抑制能力 (IC_{50} = 2.2 mg/mL)，再經膠體過濾層析後發現 Fraction 14 具有最高 ACE 抑制率為 81.5%，接著將其經 RP-HPLC 分離出 Peptide P，其 ACE 之 IC_{50} 值為 0.20 mg/mL，並鑑定出其胜肽序列為 RGVGPVPAA。當此胜肽濃度提升時，其 V_{max} 值不變且 K_m 值提升約 66.9%，表示此胜肽為競爭型抑制劑，透過分子對接模擬發現，此胜肽主要以 Alanine 與 ACE 活性區域 S2 的 His 353 形成氫鍵，以達到抑制 ACE 之能力。

大西洋鮭魚骨水解胜肽，其序列為 FCLYELAR (IC_{50} = 0.033 mg/mL)，其對 ACE 之抑制型態為不競爭型抑制，主要與 ACE 中 Zn 576 形成金屬與受體相互作用，進而降低 ACE 活性。長身圓鯵之副產物水解物鑑定出的胜肽序列為 RGVGPVPAA (IC_{50} = 0.20 mg/mL)，其對 ACE 之抑制型態為競爭型抑制，主要與 ACE 活性區域 S2 的 His 353 形成氫鍵，使此胜肽具有抑制 ACE 之能力。

四、結論

魚類副產物經酵素水解後具有萃取出 ACE 抑制胜肽的潛力，兩者胜肽序列皆主要由疏水性胺基酸組成，並且發現分子對接模型皆與酵素抑制動力學結果相符。

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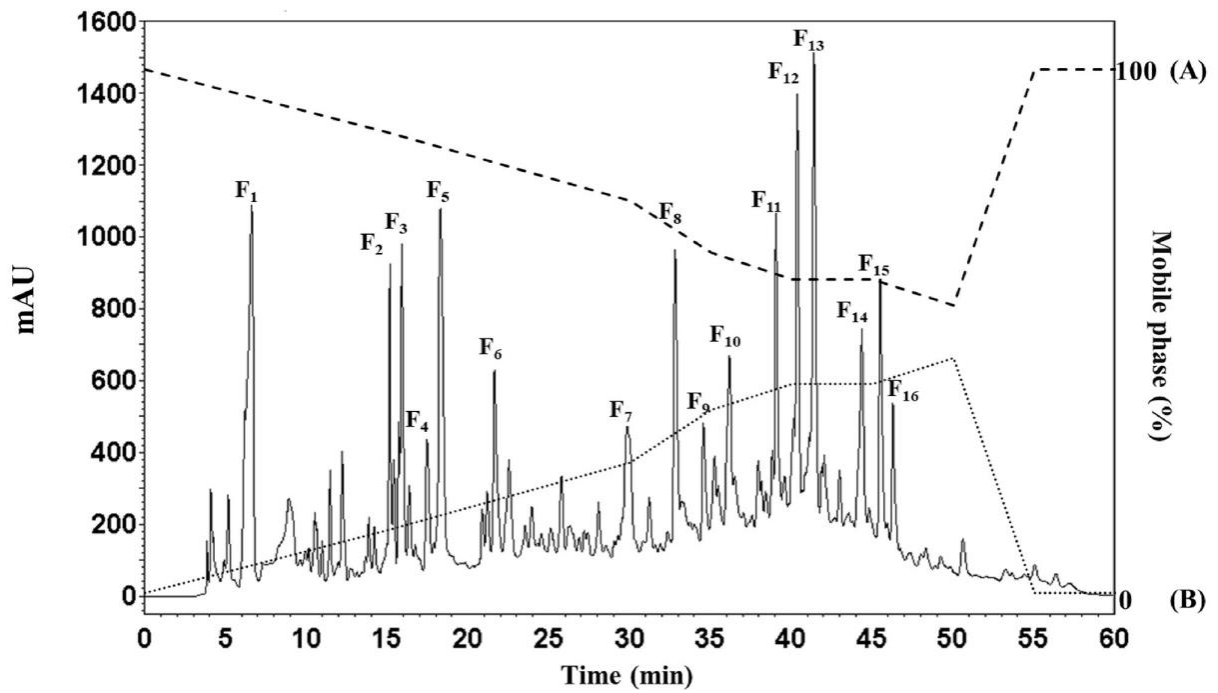
Fig. 1. The RP-HPLC chromatogram of the molecular weight cut-off < 0.65 kDa fraction of the trypsin hydrolysate obtained from salmon bones.
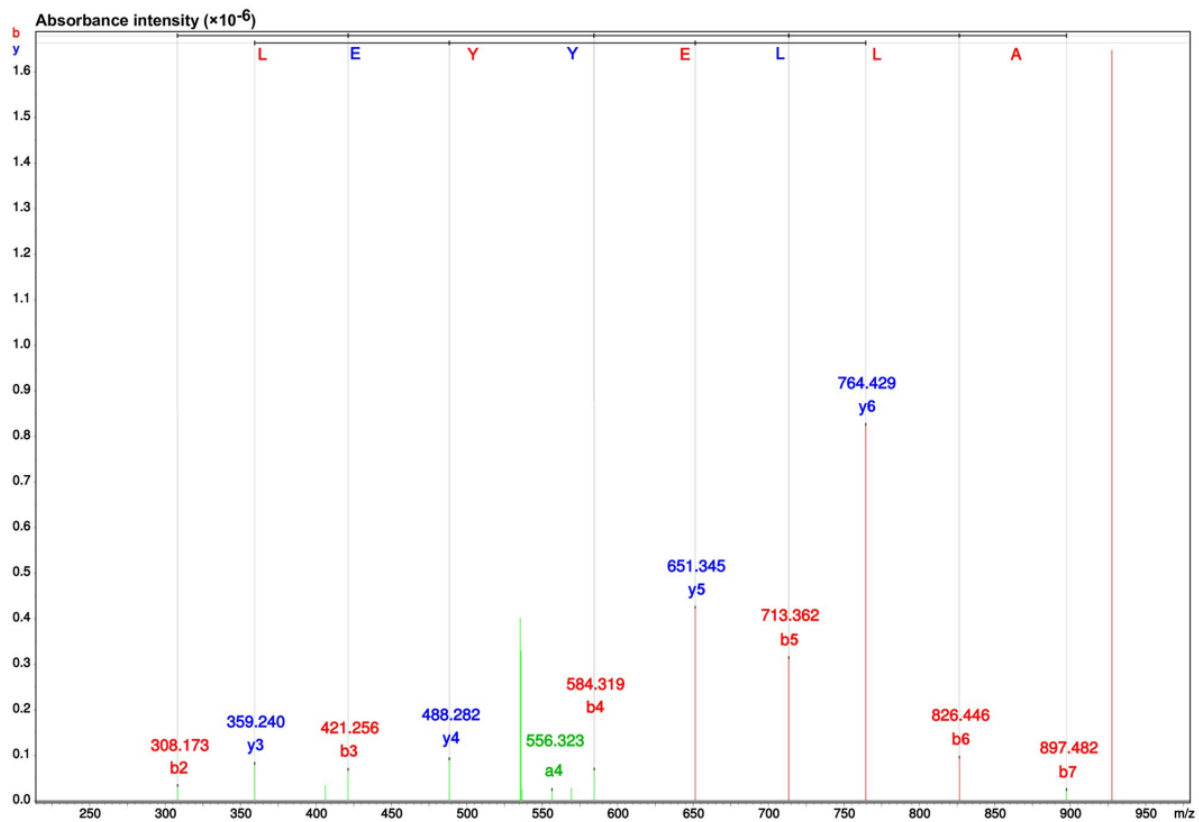
(Kaewsahnguan *et al.*, 2021)

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Fig. 2. MS/MS spectrum analysis and amino acid sequence details for the ACE-inhibitory FCLYELAR peptide from the F7 fraction obtained via RP-HPLC.
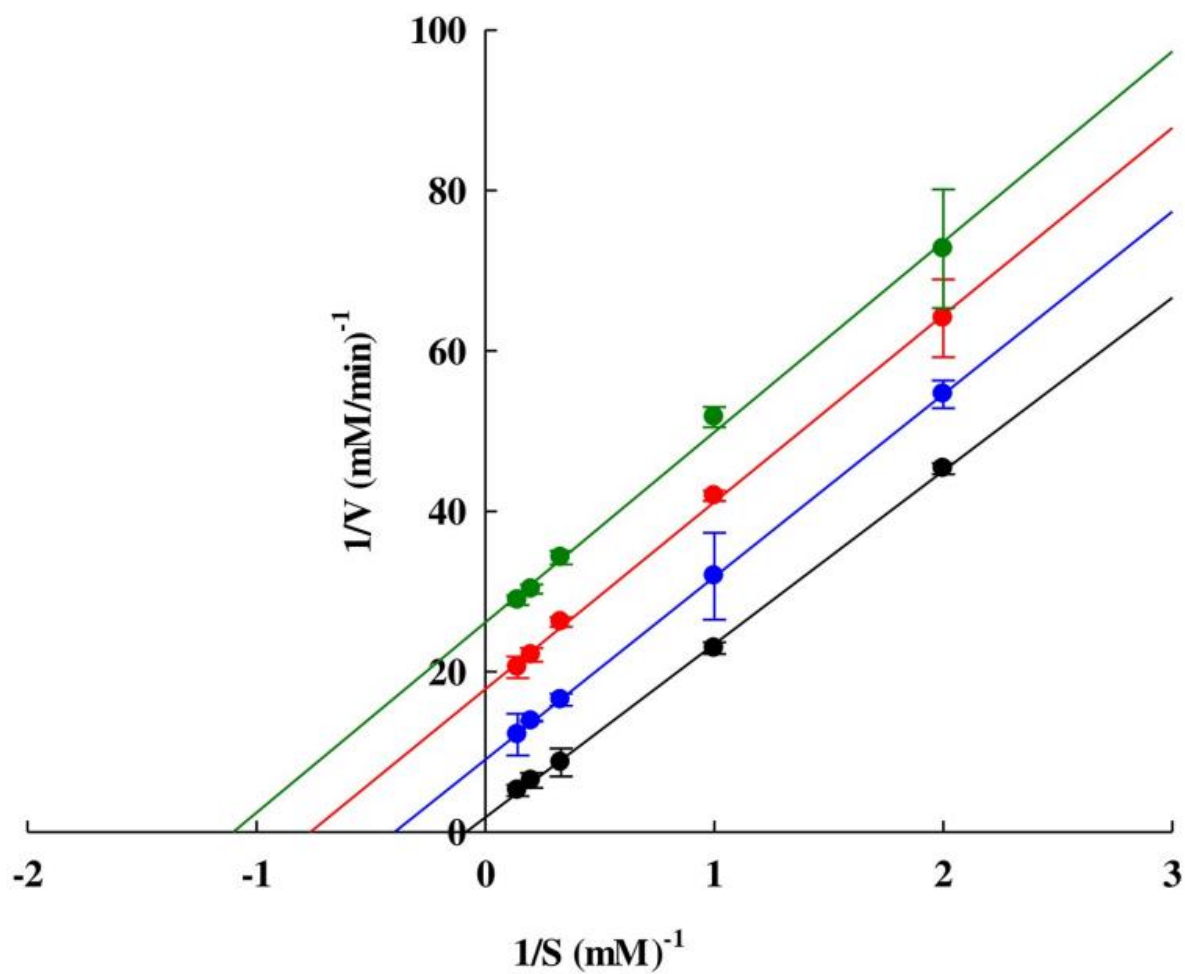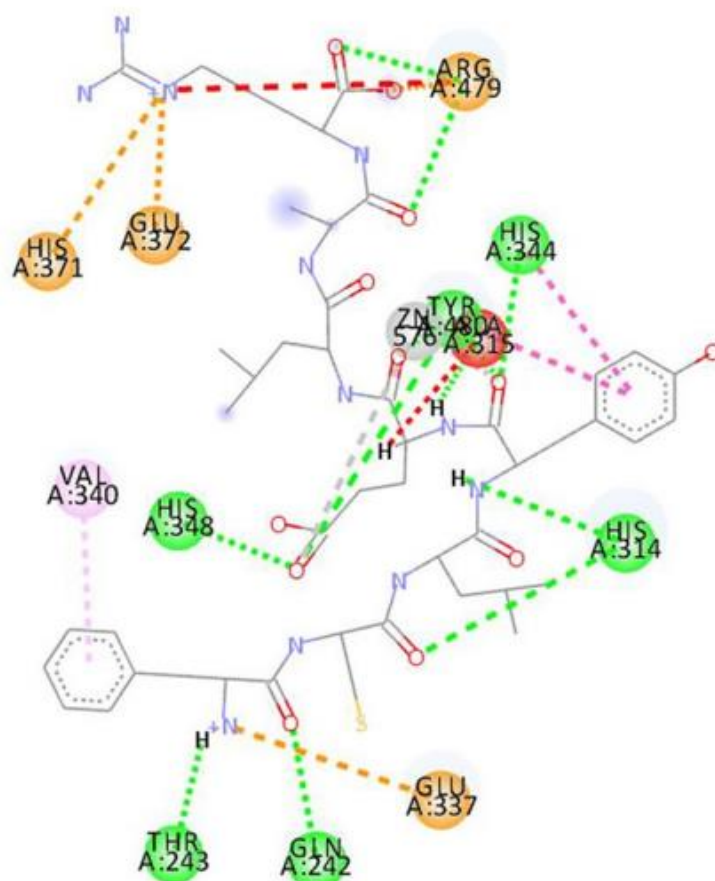
(Kaewsahnguan *et al*., 2021)

Fig. 3. The Lineweaver-Burk plot illustrating the influence of FCLYELAR uncompetitive inhibition upon ACE. The ACE-inhibitory activities were assessed when various concentrations of FCLYELAR peptide were present or absent (control; 0.05 mM; 0.1 mM; 0.2 mM). 1/V and 1/S are the respective reciprocals of the velocity and substrate.

(Kaewsahnguan *et al*., 2021)

Fig. 4. Simulations of molecular docking for FCLYELAR binding to ACE (PDB: 1O8A). 2D representation of the anticipated interactions involving the synthesized FCLYELAR peptides and ACE molecules (Images produced using Discovery Studio Visualizer v19.1.0 software)
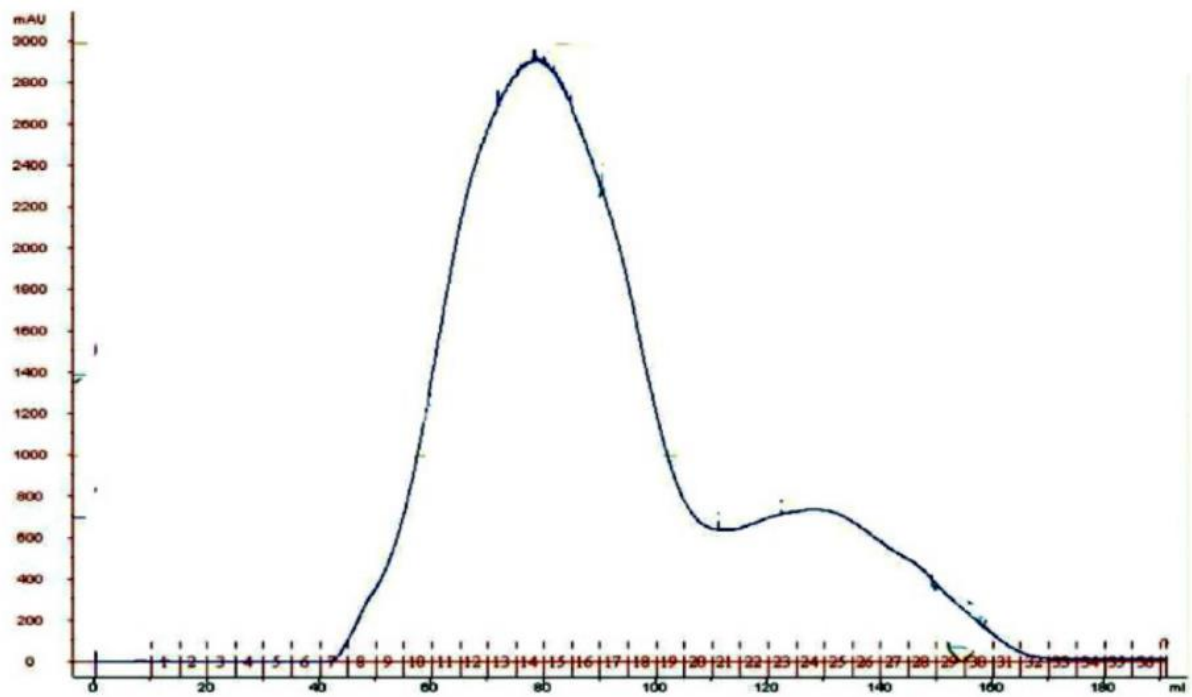
(Kaewsahnguan *et al*., 2021)

Fig. 5. Gel filtration chromatogram of the <3 KDa peptide fraction on a Sephadex G-25 column and detected at 215 nm.
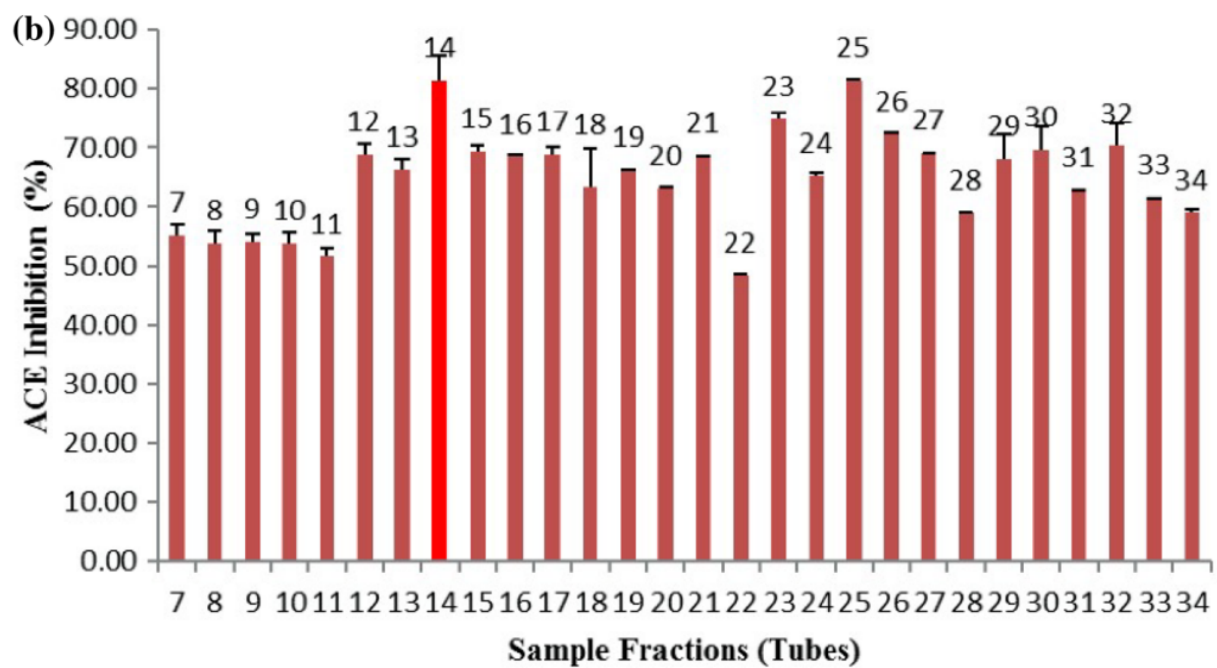
(Ishak *et al*., 2021)

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Fig. 6. ACE inhibition activity of shortfin scad waste hydrolysate (< 3 kDa) separated on gel filtration Sephadex G-25 column.

(Ishak *et al*., 2021)
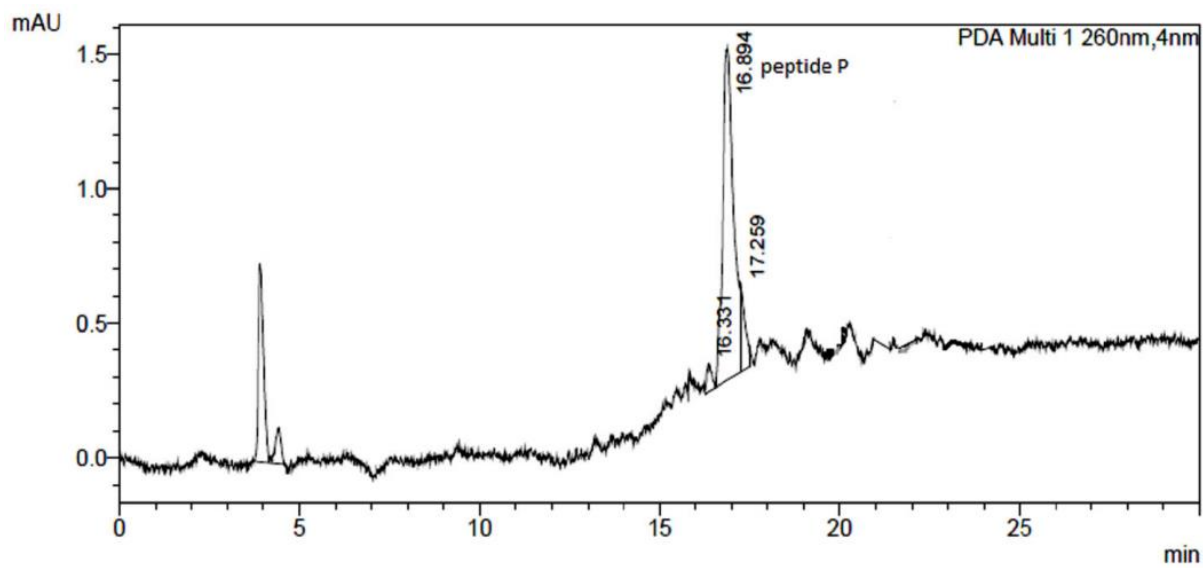
Fig. 7. Chromatography of GF 14 peptide separated by reverse phase high performance liquid chromatography (RP-HPLC).

(Ishak *et al*., 2021)
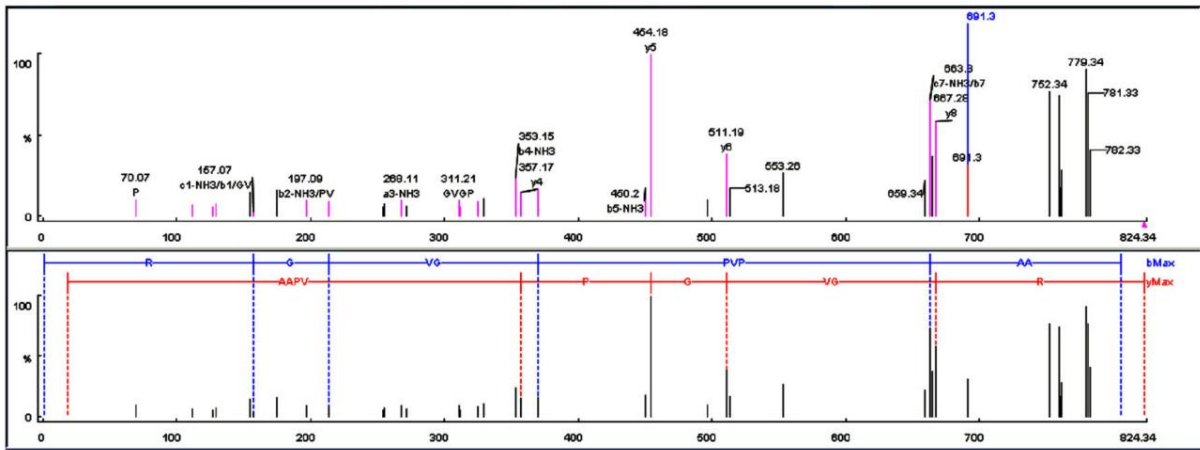
Fig. 8. MS/MS spectra of purified peptides from shortfin scad waste using MALDI-TOF/TOF with a 5800 Proteomics Analyzer over the m/z 823.3366

(Ishak *et al*., 2021)
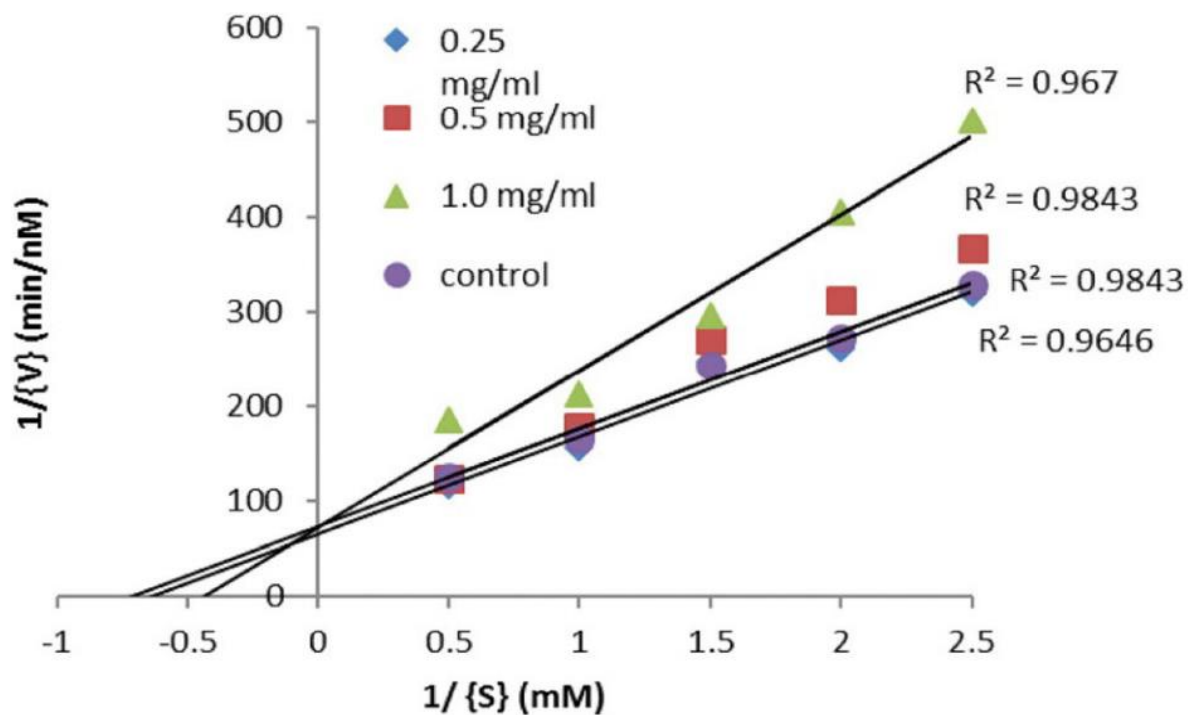
Fig. 9. Lineweaver - Burk plots of SWH ACE inhibitor activity in the presence of the purified peptide. [V] and [S] represent reaction velocity and substrate concentration, respectively.

(Ishak *et al*., 2021)

Fig. 10. Inter molecular interactions between amino acids in both ACE and peptide inhibitor.

(Ishak *et al*., 2021)

Table 1. ACE-inhibitory activity (IC_{50}) of the unfractionated trypsin hydrolysate and the five fractions obtained via ultrafiltration

Molecular weight (kDa)	ACE-inhibitory activity (IC_{50}; µg/mL)
Unfractionated	7.140 ± 0.051^b
>10	21.556 ± 0.891^c
5–10	42.640 ± 3.358^d
3–5	2.078 ± 0.424^a
0.65–3	0.303 ± 0.025^a
< 0.65	0.093 ± 0.004^a

(Kaewsahnguan *et al.*, 2021)

Table 2. ACE-inhibitory activity for each of the sixteen fractions obtained via RP-HPLC in terms of the IC_{50} value (µg/mL).

Fractions	ACE-inhibitory activity (IC_{50}; µg/mL)
F_1	N.D.
F_2	> 0.28
F_3	> 0.25
F_4	> 0.29
F_5	> 0.25
F_6	> 0.16
F_7	0.26 ± 0.02[a]
F_8	> 1.09
F_9	1.60 ± 0.15[d]
F_{10}	1.12 ± 0.05 [b, c]
F_{11}	0.34 ± 0.01[a]
F_{12}	0.88 ± 0.01[b]
F_{13}	0.97 ± 0.02[b, c]
F_{14}	1.22 ± 0.11[c]
F_{15}	1.70 ± 0.34[d]
F_{16}	1.22 ± 0.20[c]

(Kaewsahnguan *et al.*, 2021)

Table 3. The kinetics parameters for reactions undergoing catalysis via ACE at differing peptide concentrations.

Kinetics Parameters	control	FCLYELAR (mM)		
		0.05	0.1	0.2
K_m (mM)	12.0219	2.5293	1.3130	0.9099
V_{max} (mM/min)	0.5558	0.1109	0.0562	0.0383
K_i (mM)			0.0248	

(Kaewsahnguan *et al*., 2021)

Table 4. Interactions between the ACE residues and the FCLYELAR peptide.

FCLYELAR	ACE residues	Category (Types)	From Chemistry	To Chemistry	Distance (Å)
F: Phe1	Glu337	Electrostatic (Attractive Charge)	Phe1:N (Positive)	Glu337:OE2 (Negative)	4.2136
	Thr243	Conventional Hydrogen Bond	Phe1:HT3 (H-Donor)	Thr243:OG1 (H-Acceptor)	1.9759
	Gln242	Conventional Hydrogen Bond	Gln242:HE21 (H-Donor)	Phe1:O (H-Acceptor)	2.6485
	Val340	Hydrophobic (Pi-Alkyl)	Phe1 (Pi-Orbitals)	Val340 (Alkyl)	4.9222
C: Cys2	His314	Conventional Hydrogen Bond	His314:HD1 (H-Donor)	Cys2:O (H-Acceptor)	2.6499
Y: Tyr4	His314	Conventional Hydrogen Bond	Tyr4:HN (H-Donor)	His314:NE2 (H-Acceptor)	2.0228
	His344	Conventional Hydrogen Bond	His344:HE2 (H-Donor)	Tyr4:O (H-Acceptor)	2.5424
	His344	Hydrophobic (Pi-Pi T-shaped)	His344 (Pi-Orbitals)	Tyr4 (Pi-Orbitals)	5.7379
	Tyr480	Conventional Hydrogen Bond	Tyr480:HH (H-Donor)	Tyr4:O (H-Acceptor)	2.2704
	Tyr480	Hydrophobic (Pi-Pi Stacked)	Tyr4 (Pi-Orbitals)	Tyr480 (Pi-Orbitals)	4.8833
	Zn576	Metal-Acceptor	Zn576:Zn (Metal)	Tyr4:O (H-Acceptor)	2.5894
E: Glu5	Ala315	Conventional Hydrogen Bond	Glu5:HN (H-Donor)	Ala315:O (H-Acceptor)	1.6987
	His348	Conventional Hydrogen Bond	His348:HE2 (H-Donor)	Glu5:OE1 (H-Acceptor)	2.7819
	Tyr480	Conventional Hydrogen Bond	Tyr480:HH (H-Donor)	Glu5:O (H-Acceptor)	2.8036
	Tyr480	Conventional Hydrogen Bond	Tyr480:HH (H-Donor)	Glu5:OE1 (H-Acceptor)	2.6922
	Zn576	Metal-Acceptor	Zn576:Zn (Metal)	Glu5:OE1 (H-Acceptor)	2.5731
	Ala315	Unfavorable Bump;Carbon Hydrogen Bond	Glu5:HA (Steric;H-Donor)	Ala315:O (Steric;H-Acceptor)	1.5621
A: Ala7	Arg479	Conventional Hydrogen Bond	Arg479:HH11 (H-Donor)	Ala7:O (H-Acceptor)	2.2827
R: Arg8	Glu372	Electrostatic (Attractive Charge)	Arg8:NE (Positive)	Glu372:OE2 (Negative)	5.2081
	His371	Electrostatic (Pi-Cation)	Arg8:NE (Positive)	His371 (Pi-Orbitals)	3.9520
	Arg479	Electrostatic (Attractive Charge)	Arg479:NH2 (Positive)	Arg8:O (Negative)	4.3193
	Arg479	Conventional Hydrogen Bond	Arg479:HH11 (H-Donor)	Arg8:OXT (H-Acceptor)	2.0674
	Arg479	Conventional Hydrogen Bond	Arg479:HH21 (H-Donor)	Arg8:OXT (H-Acceptor)	2.1053
	Arg479	Unfavorable Positive-Positive	Arg8:NE (Positive)	Arg479:NH2 (Positive)	5.4535

(Kaewsahnguan *et al*., 2021)

Table 5. ACE inhibitory activity of fractionated shortfin scad waste hydrolysate (SWH).

Fractionate sample	ACE inhibitory activity (%)	IC_{50} (mg/ml)
MW < 3 kDa	81.50 ± 0.26	2.20
MW 3–5 kDa	69.53 ± 0.36	3.08
MW 5–10 kDa	57.11 ± 0.58	3.76
MW > 10 kDa	57.23 ± 0.52	3.81

(Ishak *et al*., 2021)

Table 6. ACE inhibitory activity of purified shortfin scad waste hydrolysate (SWH).

Fractionate sample	ACE inhibitory activity (%)	IC$_{50}$ (mg/ml)
Hydrolysate	79.34 $\pm$ 0.79	3.98
Ultrafiltration (< 3 kDa)	81.50 $\pm$ 0.26	2.20
Gel filtration (GF24)	81.47 $\pm$ 0.41	1.59
RP-HPLC (peptide P)	87.99 $\pm$ 2.79	0.20

(Ishak *et al*., 2021)

Table 7. Molecular docking of SWH peptide with ACE protein explaining the intermolecular interactions and binding affinities.

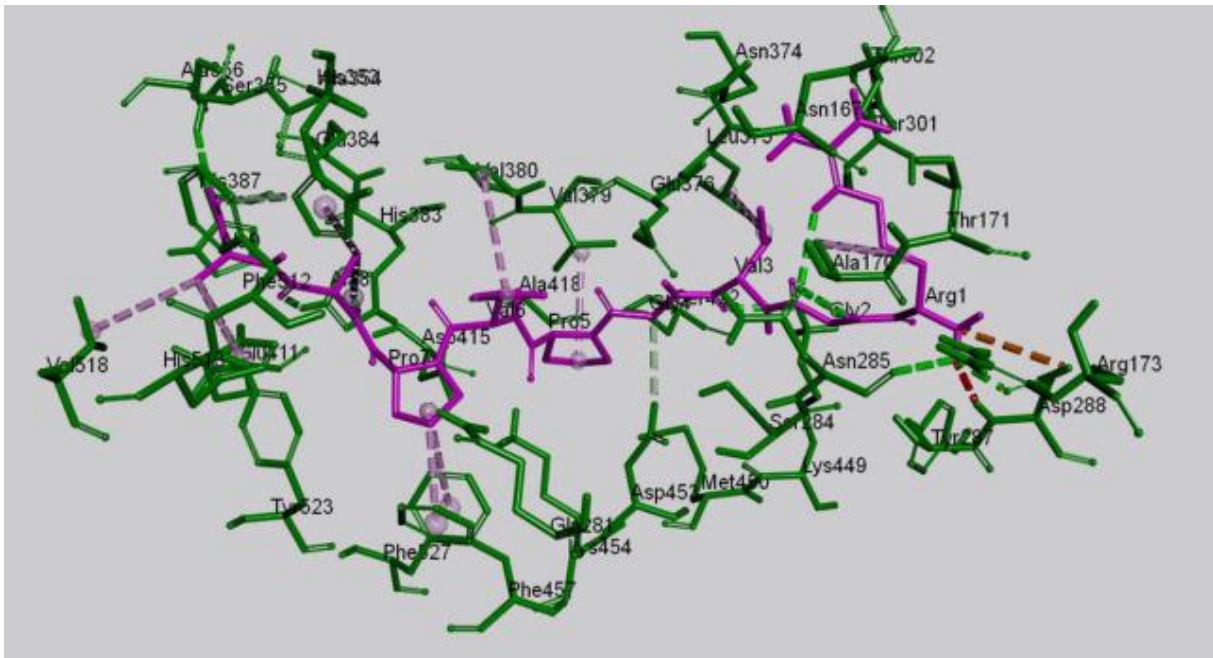| Pi-Interactions			H-Bonds			
ACE-protein residues	ligand residues	Bond length (Å)	ACE - protein residues	ligand residues	Bond length (Å)	Interaction constituents (Donor and acceptor atoms)
His383	Ala8	2.6995	Ala356	Ala9	2.8631	Receptor: Ala356:HN- ligand: Ala9:OCT2
His353	Ala8	5.2033	His383	Ala8	2.1175	Receptor: His383:HE2- ligand: Ala8:O
His383	Ala8	4.9316	Lys449	Val3	2.8	Receptor: Lys449:HZ3- ligand: Val3:O
Phe457	Pro7	4.1956	Asn285	Arg1	2.8441	ligand: Arg1:HT3- Receptor: Asn285:O
His513	Ala9	4.9171	Asp288	Arg1	2.1056	ligand: Arg1:HT3- Receptor: Asp288:OD1
Phe527	Pro7	4.6314	Asn285	Arg1	2.8769	ligand: Arg1:HE- Receptor: Asn285:OD1
			Asn285	Gly2	2.578	ligand: Gly2:HN- Receptor: Asn285:OD1
			His353	Ala9	3.7158	Receptor: His353:CD2- ligand: Ala9:OCT1
			Asp453	Gly4	3.4263	ligand: Gly4:CA- Receptor: Asp453:OD2

(Ishak *et al.*, 2021)

Fig. 10. Inter molecular interactions between amino acids in both ACE and peptide inhibitor.

(Ishak *et al*., 2021)