# Prosiding-IOP

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# Detection of native peptides from *Channa striata* extract using de novo sequencing



# Detection of native peptides from *Channa striata* extract using de novo sequencing

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**Abstract.** *Channa striata* or Snakehead fish is well-known as medicinal fish in Asian countries, including Indonesia. It is rich in functional amino acid as well as high protein. Previous study showed the fish extract was active as an ACE inhibitor in vitro, in which 5 -10 kDa fraction of 50% ethanol extract has the potential ACE inhibitory activity (4,76% inhibition of ACE $g^{-1}$ protein). This study aims to identify the native peptide in the *Channa striata* extract using LS-MS/MS. The 5-10 kDa extract fraction was further purified using Sephadex G-50, the significant peak fraction was treated with trypsin and untreated before being analysed using LC-MS/MS. The result showed that the untreated fraction had better result than trypsin treated one, indicating the significant peak fraction contains native peptide. We identified the presence of a 25.8 kDa β-actin fragment using the *Channa striata* database and two peptides GQVITIGNER and VITIGNER that meet the requirement software Proteome Discoverer 2.1. De Novo sequencing resulted 319 peptides with ALC >70%. Bioinformatics study revealed that the native peptide resulting from de novo sequencing was rich in inhibitor ACE peptides. These concluded that *Channa striata* contain a native peptide that is potentially an ACE inhibitor or anti-hypertension.

**Keywords**: ACE inhibitor; anti-hypertension; de novo sequencing; native peptides; snakehead fish

## 1. Introduction

The utilization of fish as healthy food containing high protein and low fat has been known for centuries. *Channa striata,* or snakehead and locally known as ikan gabus for Indonesian and haruan for Malaysian people, has been used for folk medicine, especially for women after giving birth and people after surgery [1, 2]. Research on the ability of *Channa striata* water extract for wound healing instruments has been reported by Rahayu *et al*. [3] and Hendriati *et al*. [4]. Besides, *Channa striata* extract has a protective effect to inhibit cadmium-induced oxidative stress in the liver [5]. The mechanisms of wound healing activity of water extract *Channa striata* were through cell proliferation and pro-angiogenic activity involving four essential proteins/genes, i.e., collagen type XI, actin 1, myosin light chain and myosin heavy chain [6]. Another report by Ghasem *et al*. [7] showed that myofibrillar hydrolysates of *Channa striata* by proteinase K and thermolysin produced two peptides possessing ACE inhibitor, implying that *C. striata* meat was potential for ACE inhibitor and could be used to suppress blood pressure. However, the presence of native peptides in *C. striata* meat has not been reported.

The presence of biologically active peptides in food is essential to be studied since food now is used as a nutrition source and to provide health benefits. Food with high protein, such as fish [8], soybean [9], and milk [10], is a good source of bioactive peptides. Biologically active peptides can be found as natural compounds of food or part of the protein that are inactive in the precursor molecule but are active after hydrolysis and is released with the help of the gut enzymes. Bioactive peptides are peptides that

often have 3 to 20 amino acid residues, having biological activities based on their amino acid composition and sequence [11]. It can be produced by organic hydrolysis, microwave-assisted, chemical hydrolysis, enzymatic hydrolysis. Our previous studies showed that *Channa striata* extract contains native or natural peptides that could inhibit ACE II (angiotensin-converting enzyme) *in vitro* [12, 13]. The peptide of <10 kDa was more effectively extracted with 50% ethanol than the destilled water. Conversely, protein of >10 kDa was better extracted with water compared to 50% ethanol. However, 50% ethanol extract sized 5–10 kDa showed potential ACE inhibitor activity per gram protein (4,76% inhibition of ACE $g^{-1}$ protein). This study aims to identify the native peptide in the *Channa striata* extract using LC-MS/MS. The *de novo* sequenced peptide obtained was further bioinformatically analysed for ACE inhibitor.

## 2. Materials and methods

### *2.1. Channa striata preparation samples*

Wild snakeheads were obtained from Parung, Bogor, West Java, Indonesia. The fishes were sorted to obtain groups with 28–34 cm length and weight about 100–350 g. Fresh fishes were soaked and burrided in ice for 30 minutes then cut into fillet sized. Fish fillets then were kept at -20°C before undergoing extraction and fractionation.

### *2.2. Protein and peptide extraction*

The extraction of protein and peptide of the snakehead was conducted following Budiari et al. [12]. Frozen snakehead fillets were thawed and cut into small pieces with the size of 0,5 × 0,5 × 0,5 cm. The samples were poured with liquid nitrogen and then made into minced fish using a hand blender. As much as 20 g of the meat were macerated using 60 ml of 50% ethanol (fillet:solvent = 1:3) for 10 minutes, kept at <10°C. The suspension was homogenized (Ultra-turrax T25, IKA, USA) for 5 minutes at 13.500 rpm at ice temperature. Homogenates were obtained and put into a centrifuge (Avanti® J-26 XPI, Beckman Coulter, USA) for 10 minutes at 15.344 × g. Finally, the supernatants were kept in storage at -20°C.

### *2.3. Fractionation using ultrafiltration and gel-filtration*

The obtained snakehead extract was fractionated using ultrafiltration membranes with MWCO of 10,000 and 5,000 kDa to separate the fractions of >10 kDa, 5–10 kDa and < 5 kDa. The 5–10 kDa fractions active *in vitro* against ACE were concentrated using a rotary evaporator (Buchi, Swiss) and dried with a freeze dryer (Alpha 1-2 LD, Christ, Jerman). Dried samples were put into storage with a temperature set at -70°C for further study.

A freeze-dried 5–10 kDa of 50% ethanolic extract fraction was added with 2 mL of 50% ethanol. Then, the fraction was further fractionated by using the Gel Filtration system of the AKTA purifier (GE Healthcare Bioscience, Sweden). The matrix used was Sephadex G-50, packed in a chromatography column (1.6×70 cm) (GE Healthcare Bioscience, Sweden). The eluent used was 50% ethanol with the flow rate kept at a constant rate of 0.3 mL $min^{-1}$, and the fractions were gathered in a fraction collector. The absorbance of each eluent was measured at the wavelength of 280 nm in the AKTA purifier system. The fractions were freeze-dried and prepared for LC-MS/MS analysis.

### *2.4. LC-MS/MS analysis*

The major fractions obtained from gel filtration fractionation were freeze-dried and prepared for LC-MS MS in two ways. The first extract was added with trypsin assuming that the sample was in the protein form so that additional preparation (reduction, alkylation and digestion) were conducted to obtain the peptide. Sample (4.9 µg) prepared usingin-solution digestion method following User Guide Thermo In-Solution and Guanidination KIT2. The 2$^{nd}$ sample was prepared without the addition of the trypsin, assuming that the sample or fraction was already in the peptide form. This sample was analyzed without preparation. Peptide resulting from digestion step was cleaned using Pierce C18 spin columns following User Guide Thermo C18 Spin Columns. The cleaned sample was dried in a vacuum concentrator for 2 h.

Dried samples were identified for peptides using LC-MS/MS. Dried samples solubilized in a 50 µL dissolving solution (2% acetonitrile, 1% formic acid in water LC-MS grade). An 2 µL peptide (about 160 ng) was fractionated in the Thermo Pepmap RSLC C18 (2 µm, 100 Å, 75 µm x 25 cm) column (Thermo RSLC nano 3000 instruments), eluted with 100% water LC-MS grade, 0.1% formic acid (A) and 20% ACN LC-MS grade, 0.1% formic acid (B) 0.3 µL/min. Gradient elution used was: 0-5% B for 5 min., 5-99% B for 31 min., 99% B for 10 min and 5% B for 10 min. The fractionation result was analyzed using Thermo Q-Exactive Mass Spectrometry. Ion polarity was in positive mode (resolution used was 70,000 for MS scan and 17,500 for MS/MS scan). The range scan used was 200-2000 m/z with an isolation window of 4 m/z. Fragmentation energy used in MS/MS scan was 30 eV. Results from LC-MS/MS instrument were analyzed using two software: Proteome Discoverer 2.1 (Thermo Scientific) for protein identification based on database and Peaks Studio 8.5 (Bioinformatics Solutions Inc,) for *de novo* sequencing. Software Thermo Proteome Discoverer 2.1 used search engine Sequest HT with database *Channa striata* (Taxonomy ID: 64152), *Channa* (Taxonomy ID: 33789) and *Channa argus* (Taxonomy ID: 215402). An enzyme used was trypsin with maximum missed cleavage 2. Modification used was oxidation (for amino acid Met), acetyl (for N-terminal) and carbamidomethyl (for amino acid Cys). In Software Peaks Studio 8.5, enzim trypsin with maximum missed cleavage three was used. For unprepared samples (sample without additional enzyme trypsin) used unspecified enzyme (in Proteome Discoverer 2.1) and none (in Peaks Studio 8.5).

*2.5. Bioinformatics study*

All peptides detected in *de novo* sequencing from samples without trypsin and confirmed ACE inhibitors' bioactivity based on a search in the BIOPEP database (www.uwm.edu.pl/biochemia/index.php/pl/biopep) [14]. The physicochemical properties of each detected amino acid peptide sequence, including sequence length, molecular weight, isoelectric point, net charge and hydrophobicity, were calculated using the PepDraw (http://www.tulane.edu/~biochem/WW/PepDraw/) [15].

## 3. Result and discussion

*3.1 Ultrafiltration and gel filtration fraction*

Previous studies reported that the low molecular weight fraction (5 – 10 kDa) of crude extract of snakehead fish produced by fractionation with ultrafiltration membranes had the most potential ACE inhibitory activity [12]. These results are like the statement of Erdmann *et al*. [16], which states that most ACE inhibitors have low molecular weight proteins or peptides (< 3 kDa) with 3-13 amino acids. The potential fraction was then further fractionated using gel-filtration chromatography (Sephadex G-50). Figure 1 shows the fractionation chromatogram of 5–10 kDa extract. The chromatogram shows one dominant peak and a very small peak before and after the dominant peak. These results indicate the protein represented as amino acids that absorb 280 nm was in that major fraction. The fraction was further freeze-dried and analysed to LC-MS/MS.

3.2 *Peptide identification using LC-MS/MS*

We used Software Proteome Discoverer 2.1 to identify the sequence of peptides based on sequence peptide compatibility from an experiment with that of the database used. The limitation of using this software was can only identify peptides available in the database. As alternatives, we used *de novo* sequencing to determine amino acid sequence based on calculation fragments ion obtained from LC-MS/MS, with the help of software Peaks Studio 8.5. Using the software Proteome Discoverer 2.1, *Channa striata* database, we could not identify protein atau its fragment presented in the samples. However, the protein β-actin (41.7 kDa) when it used Database Channa (Taxonomy ID: 33789) followed by Database *Channa argus* (Taxonomy ID: 215402). We also detected two peptides, i.e. AGFAGDDAPR and GILTLK. However, only the first peptide meet the requirement since the value of XCorr > 1.5 (1.89). XCorr value is the value of cross correlation between peptides resulting from an experiment with peptides in the database in which the minimum standard of the XCorr for publication is > 1.5. The identified amino acid analyzed the position of the peptide in the β-actin protein in the peptide compared to the whole amino acid composing the β -actin protein. Figure 2 shows the position

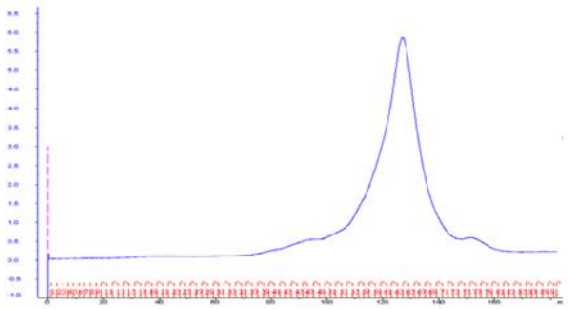

**Figure 1.** The chromatogram profile of 5 – 10 kDa snakehead fish extract.

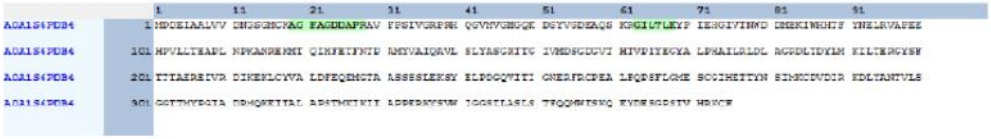

**Figure 2.** Location of the peptide sequence identified in the β-actin protein (green) and sequence of the whole amino acid in β-actin protein of *Channa argus* (black color).

of the peptide identified, which were in amino acids 19-28 and 63-68, which was 4% of the amino acid composing β-actin.

Protein and peptides identified were not from *Channa striata* but *Channa argus* (Northern Snakehead). This condition might be that the data available in Uniprot (http://www.uniprot.org) is not complete for *C. striata*. When we did alignment (Figure 3) we detected two fragments of protein β-actin of *Channa striata* (B2YKW3dan V9TMK0) in the β-actin of *Channa argus* position 131 – 362, while the identified was in position 19-28 and 63-68. That is the reason when using the *C. striata* database, and the peptide was not identified. Myofibrillar protein or structural protein in snakehead fish has been reported and identified as actin (alpha1, skeletal muscle), actinin (alpha2), actin (alpha 2, smooth muscle, aorta), fast skeletal myosin light chain 3, myosin heavy chain (fragment) and skeletal muscle actin (fragment) [17].

When we used the sample without preparation (without the addition of trypsin enzyme) assuming that our sample was already in the form of peptides, using the database of *Channa striata*, we were successfully identified β-actin (fragment, 25.8 kDa) with three unique peptides within Score Sequest HT 38.12. This result was better than the samples with trypsin preparation, confirming that our samples contained native peptides. Figure 4 shows three identified peptides, i.e., GQVITIGNER, VITIGNER and DIRKDL. The previously two peptides meet the requirement of XCorr values of 2.46 and 1.6, while the third was not. The peptides sequence could be seen in the amino acids 115-124, 117-124, and 158-163, which was 6.9 % from the whole amino acid composing protein β-actin fragments (figure 4).

*3.3 De novo sequencing and bioinformatics study*

In this step, the peptide was sequenced based on ALC (average local confidence) value, presenting the confidence limit of the peptide from De Novo sequencing. The higher the ALC value, the higher the confidence level. We identified 224 peptides with ALC > 70%, about 4x of the peptide number identified from samples with the addition of trypsin treatment. Based on a bioinformatics study using the BIOPEP

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CLUSTAL O(1.2.4) multiple sequence alignment

TR|B2YKW3|B2YKW3_CHASR          ------------------------------------------------------------
TR|V9TMK0|V9TMK0_CHASR          ------------------------------------------------------------
TR|A0A1S6PD84|A0A1S6PD84_9TELE  MDDETAALVVDNGSGMCKAGFAGDDAPRAVFPSIVGRPRHQGVMVGMGQKDSYVGDEAQS 60

TR|B2YKW3|B2YKW3_CHASR          ------------------------------------------------------------
TR|V9TMK0|V9TMK0_CHASR          ------------------------------------------------------------
TR|A0A1S6PD84|A0A1S6PD84_9TELE  KRGILTLKYPIEHGIVTNWDDMEKIWHHTFYNELRVAPEEHPVLLTEAPLNPKANREKMT 120

TR|B2YKW3|B2YKW3_CHASR          ------------------------------------------------------------
TR|V9TMK0|V9TMK0_CHASR          ----------AMYVAIQAVLSLYASGRTTGIVMDSGDGVTHTVPIYEGYALPHAILRLDL 50
TR|A0A1S6PD84|A0A1S6PD84_9TELE  QIMFETFNTPAMYVAIQAVLSLYASGRTTGIVMDSGDGVTHTVPIYEGYALPHAILRLDL 180

TR|B2YKW3|B2YKW3_CHASR          ---------------------------------IKEKLCYVALDFEQEMGTAASSSSLEKSY 29
TR|V9TMK0|V9TMK0_CHASR          AGRDLTDYLMKILTERGYSFTTTAEREIVRDIKEKLCYVALDFEQEMGTAASSSSLEKSY 110
TR|A0A1S6PD84|A0A1S6PD84_9TELE  AGRDLTDYLMKILTERGYSFTTTAEREIVRDIKEKLCYVALDFEQEMGTAASSSSLEKSY 240
                                                 ****************************

TR|B2YKW3|B2YKW3_CHASR          ELPDGQVITIGNERFRCPEALFQPSFLGMESCGIHETTYNSIMKCDVDIRKDLYANTVLS 89
TR|V9TMK0|V9TMK0_CHASR          ELPDGQVITIGNERFRCPEALFQPSFLGMESCGIHETTYNSIMKCDVDIRKDLYANTVLS 170
TR|A0A1S6PD84|A0A1S6PD84_9TELE  ELPDGQVITIGNERFRCPEALFQPSFLGMESCGIHETTYNSIMKCDVDIRKDLYANTVLS 300
                                ************************************************************

TR|B2YKW3|B2YKW3_CHASR          GGTTMYPGIADRMQKEITALAPSTMKIKIIAPPERKYSVWIGGSILASLSTFQQMWISK- 148
TR|V9TMK0|V9TMK0_CHASR          GGTTMYPGIADRMQKEITALAPSTMKIKIIAPPERKYSVWIGGSILASLSTFQQMWISKQ 230
TR|A0A1S6PD84|A0A1S6PD84_9TELE  GGTTMYPGIADRMQKEITALAPSTMKIKIIAPPERKYSVWIGGSILASLSTFQQMWISKQ 360
                                ************************************************************

TR|B2YKW3|B2YKW3_CHASR          ---------------
TR|V9TMK0|V9TMK0_CHASR          EY------------- 232
TR|A0A1S6PD84|A0A1S6PD84_9TELE  EYDESGPSIVHRKCF 375
```

**Figure 3.** Alignment amino acid of β-actin protein from *C. striata* and *C. argus* database.

| Accession | Description | Coverage | # Peptides | # PSMs | # Unique Peptides | # AAs | MW [kDa] | calc. pI | Score Sequest HT |
|---|---|---|---|---|---|---|---|---|---|
| V9TMK0 | Beta-actin (Fragment) OS=Channa striata PE=2 SV=1 | 7% | 3 | 21 | 3 | 232 | 25.8 | 5.30 | 38.12 |
| T1QKQ6 | LOC562320 (Fragment) OS=Channa striata GN=K0AA1239 PE=4 SV=1 | 3% | 1 | 2 | 1 | 307 | 35.8 | 7.96 | 0.00 |
| E5LA24 | NADH-ubiquinone oxidoreductase chain 5 (Fragment) OS=Channa striata GN=ND5 PE=3 SV= | 25% | 2 | 2 | 2 | 339 | 37.7 | 7.20 | 0.00 |
| V8ND48 | Cathepsin B OS=Channa striata GN=CathB PE=2 SV=1 | 4% | 1 | 1 | 1 | 330 | 36.1 | 6.58 | 0.00 |
| T1QSZ3 | Uncharacterized protein (Fragment) OS=Channa striata GN=GCS1 PE=4 SV=1 | 2% | 1 | 2 | 1 | 309 | 35.5 | 6.33 | 0.00 |
| A0A0I1VDK6 | ATP synthase subunit a OS=Channa striata PE=4 SV=1 | 4% | 1 | 1 | 1 | 227 | 25.1 | 9.41 | 0.00 |
| K9LH64 | Calponin OS=Channa striata PE=2 SV=1 | 5% | 1 | 1 | 1 | 317 | 35.1 | 6.57 | 0.00 |
| A0A077H055 | NADH dehydrogenase iron-sulfur protein 2 OS=Channa striata PE=2 SV=1 | 2% | 1 | 1 | 1 | 466 | 53.0 | 6.38 | 0.00 |

| Sequence | # PSMs | Master Protein Accessions | Theo. MH+ [Da] | XCorr Sequest | Charge Sequest HT | m/z [Da] Sequest HT | RT [min] Sequest HT |
|---|---|---|---|---|---|---|---|
| GQVITIGNER | 19 | V9TMK0 | 1086.59020 | 2.46 | 2 | 543.79852 | 17.1215 |
| VTIGNFR | 1 | V9TMK0 | 901.51016 | 1.60 | 2 | 451.25836 | 13.9445 |
| DIRKDL | 1 | V9TMK0 | 759.43593 | 1.05 | 2 | 380.22180 | 18.2125 |

```
                1         11        21        31        41        51        61        71        81        91
V9TMK0        1 AMYVAIQAVL SLYASGRTTG IVMDSGDGVT HTVPIYEGYA LPHAILRLDL AGRDLTDYLM KILTERGYSF TTTAEREIVR DIKEKLCYVA LDFEQEMGTA

V9TMK0      101 ASSSSLEKSY ELPDGQVITI GNERFRCPEA LFQPSFLGME SCGIHETTYN SIMKCDVDIR KDLYANTVLS GGTTMYPGIA DRMQKEITAL APSTMKIKII

V9TMK0      201 APPERKYSVW IGGSILASLS TFQQMWISKQ EY
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**Figure 4.** Location of peptide sequence identified from protein β-actin fragment (green color) and the whole sequence of protein β-actin fragment of C. *striata* (black color).

database (http://www.uwm.edu.pl/biochemia/index.php/en/biopep, 215 of the 224 peptides identified have bioactivity as ACE inhibitors (Table 1). Aluko *et al.* [18] stated that bioactive peptides with specific amino acid sequences could act as ACE inhibitors through hydrophobic interactions on the enzyme's active site. The mechanism occurs in peptides containing hydrophobic amino acids (Try, Phe, and Trp) and positively charged (Lys and Arg) at the C-terminal [19]. Several potent ACE inhibitors have also been reported to have a Pro residue at the C-terminal position [20] Ghassem *et al.* [7] also stated the presence of aromatic (W, Y, F) and aliphatic (I, A, L, and M) residues in dipeptides and tripeptides or the polypeptide (4-10 amino acids) in the last three amino acids of the C-terminal sequence is very

**Table 1.** List of peptides resulted from de novo sequencing from samples without addition of trypsin with their ACE inhibitors activities, predicted using BIOPEP.

| Peptide sequences | Number of amino acids | Molecular weight (Da) | Isoelectric point (PI) | Hydro-phobicity | Net charge | Bioactivities |
|---|---|---|---|---|---|---|
| TYKN | 4 | 524.3 | 9.5 | 11.09 | 1 | YK |
| ALYLR | 5 | 634.4 | 9.97 | 7 | 1 | LY, LR |
| EFAVL | 5 | 577.3 | 3.2 | 8.61 | -1 | AV, AVL, EF |
| EPKLR | 5 | 641.4 | 10.01 | 15.03 | 1 | KL, LR |
| FAGDD | 5 | 523.2 | 2.78 | 15.12 | -2 | AG, GD |
| KELRE | 5 | 673.4 | 6.69 | 18.52 | 0 | KE, LR |
| KEREL | 5 | 673.4 | 6.69 | 18.52 | 0 | KE, ER |
| KEWVL | 5 | 673.4 | 6.53 | 10.53 | 0 | EW, KE |
| KLESL | 5 | 588.3 | 6.53 | 12.29 | 0 | KL |
| KRLEP | 5 | 641.4 | 9.8 | 15.03 | 1 | RL, KR |
| KRLPE | 5 | 641.4 | 9.8 | 15.03 | 1 | RL, KR |
| LRKEP | 5 | 641.4 | 10.14 | 15.03 | 1 | KE, LR |
| LRKPE | 5 | 641.4 | 10.14 | 15.03 | 1 | KP, LR |
| LSYLR | 5 | 650.4 | 9.91 | 6.96 | 1 | SY, LR |
| NPPKF | 5 | 601.3 | 9.63 | 10.12 | 1 | NPP, PPK, KF, PP |
| ACFPAH | 6 | 644.3 | 7.17 | 9.64 | 0 | FP, CF, AH |
| AFSHHT | 6 | 698.3 | 8.11 | 12.06 | 0 | AF, HHT |
| APFDVK | 6 | 675.4 | 6.76 | 12.81 | 0 | VK, AP |
| APFQLT | 6 | 675.4 | 5.37 | 6.6 | 0 | AP, FQ |
| EAYSFD | 6 | 730.3 | 2.82 | 13.71 | -2 | AY, EA, SF |
| ELQANK | 6 | 701.4 | 6.71 | 15.2 | 0 | NK, LQ |
| EPLSVK | 6 | 671.4 | 6.71 | 13.22 | 0 | PL, VK |
| EVKDRL | 6 | 758.4 | 6.83 | 18.07 | 0 | RL, VK, EV |
| FVCPPH | 6 | 698.3 | 7.15 | 8.32 | 0 | PP, PH |
| GFLVVT | 6 | 634.4 | 5.36 | 5.42 | 0 | GF |
| KEDVEH | 6 | 755.3 | 4.31 | 23.47 | -2 | VE, KE |
| KNQLAE | 6 | 701.4 | 6.53 | 15.2 | 0 | LA |
| KPLLCT | 6 | 673.4 | 8.67 | 8.57 | 1 | PL, KP |
| KPLSTE | 6 | 673.4 | 6.53 | 13.93 | 0 | PL, KP, TE, ST |
| KPLYGP | 6 | 673.4 | 9.48 | 10.17 | 1 | LY, YG, GP, PL, KP |
| KPPTVT | 6 | 641.4 | 9.8 | 11.02 | 0 | KP, PT, PP |
| KPYLGP | 6 | 673.4 | 9.48 | 10.17 | 1 | LGP, GP, LG, KP |
| KVTEAL | 6 | 659.4 | 6.53 | 13.37 | 0 | EA, TE |
| LDDKLR | 6 | 758.4 | 6.89 | 17.29 | 0 | KL, LR |
| LDDKRL | 6 | 758.4 | 6.89 | 17.29 | 0 | KL, LR |

| Peptide sequences | Number of amino acids | Molecular weight (Da) | Isoelectric point (PI) | Hydro-phobicity | Net charge | Bioactivities |
|---|---|---|---|---|---|---|
| LDELER | 6 | 773.4 | 3.72 | 18.11 | -2 | ER |
| LDELER | 6 | 773.4 | 3.72 | 18.11 | -2 | ER |
| LDWNAR | 6 | 773.4 | 6.74 | 11.36 | 0 | AR |
| LHVKVT | 6 | 695.4 | 10.14 | 11.11 | 1 | VK |
| LLLYGT | 6 | 678.4 | 5.35 | 4.84 | 0 | LY, YG, GT |
| LLPDEP | 6 | 682.4 | 2.8 | 12.95 | -2 | LLP |
| LSDEVH | 6 | 698.3 | 3.99 | 16.25 | -2 | EV |
| PPLESA | 6 | 612.3 | 3.21 | 11.52 | -1 | PL, PP, PPL |
| QEAAKK | 6 | 673.4 | 9.66 | 18.9 | 1 | AKK, AA, EA |
| QFLLGP | 6 | 673.4 | 5.17 | 5.75 | 0 | LGP, GP, LG |
| QLLAPK | 6 | 668.4 | 9.84 | 9.61 | 1 | LAP, AP, LA |
| QNKLAE | 6 | 701.4 | 6.56 | 15.2 | 0 | LA, NKL, KL, NK |
| QQTLPH | 6 | 722.4 | 7.59 | 10.9 | 0 | PH |
| SLDVEH | 6 | 698.3 | 3.99 | 16.25 | -2 | VE |
| SLFFPS | 6 | 696.3 | 5.38 | 4.29 | 0 | FP, LF, FF |
| STVVAL | 6 | 588.3 | 5.5 | 6.94 | 0 | ST |
| TELEMT | 6 | 722.3 | 2.92 | 13.74 | -2 | TE |
| TLEEMT | 6 | 722.3 | 2.92 | 13.74 | -2 | LEE |
| TPYMVL | 6 | 722.4 | 5.46 | 5.2 | 0 | TP |
| TVFVMT | 6 | 696.4 | 5.28 | 5.1 | 0 | VF, VM |
| AEWLALA | 7 | 772.4 | 3.21 | 8.44 | -1 | LA, EW, WL |
| AGLFLGP | 7 | 673.4 | 5.24 | 6.63 | 0 | LF, LGP, GP, GL, AG, LG |
| APKLDAP | 7 | 710.4 | 6.76 | 14.37 | 0 | AP, DA, KL |
| APKLDPA | 7 | 710.4 | 6.76 | 14.37 | 0 | AP, KL |
| APKLPEG | 7 | 710.4 | 6.86 | 15.01 | 0 | AP, EG, KL, KLP |
| ATPFDVK | 7 | 776.4 | 6.76 | 13.06 | 0 | VK, TP |
| ELTKLAH | 7 | 810.5 | 7.74 | 14.91 | 0 | LA, KL, AH |
| KEALTPS | 7 | 744.4 | 6.53 | 14.43 | 0 | EA, KE, TP |
| KEDLLGA | 7 | 744.4 | 4.01 | 17.12 | -1 | GA, LG, KE |
| KENGVAK | 7 | 744.4 | 9.63 | 19.17 | 1 | GV, NG, KE |
| KGPVYVT | 7 | 762.4 | 9.48 | 10.61 | 1 | VY, GP, GPV, KG, YV, KGP |
| KPALDAP | 7 | 710.4 | 6.44 | 14.37 | 0 | AP, DA, KP |
| LDENGVK | 7 | 773.4 | 4 | 18.26 | -1 | VK, GV, NG |
| LKPADAP | 7 | 710.4 | 6.71 | 14.37 | 0 | LKP, AP, DA, KP |
| LKPAPEG | 7 | 710.4 | 6.81 | 15.01 | 0 | LKP, AG, EG, PAP, KP |

| Peptide sequences | Number of amino acids | Molecular weight (Da) | Isoelectric point (PI) | Hydro-phobicity | Net charge | Bioactivities |
|---|---|---|---|---|---|---|
| QATLNAK | 7 | 744.4 | 9.84 | 12.32 | 1 | LN |
| QDYMAAP | 7 | 794.3 | 2.93 | 12.07 | -1 | AAP, AP, AA, DY |
| SGLDVEH | 7 | 755.3 | 3.99 | 17.4 | -2 | GL, SG, VE |
| SGLDVHE | 7 | 755.3 | 3.99 | 17.4 | -2 | GL, SG |
| TACTLFT | 7 | 755.4 | 5.04 | 6.17 | 0 | LF |
| TAPFDVK | 7 | 776.4 | 6.46 | 13 | 0 | TAP, VK, AP |
| TELKLAH | 7 | 810.5 | 7.57 | 14.91 | 0 | LKL, LA, KL, TE, AH |
| TTCAFLT | 7 | 755.4 | 5.04 | 6.17 | 0 | AF, AFL |
| TTRLLAH | 7 | 810.5 | 10.79 | 10.54 | 1 | RL, LA, AH |
| TVSDKPL | 7 | 758.4 | 6.46 | 13.48 | 0 | PL, KP |
| AFLWVDAH | 8 | 957.5 | 4.98 | 9.36 | -1 | LW, AF, DA, AH, AFL |
| AGPALVSP | 8 | 710.4 | 5.24 | 9.08 | 0 | GPA, VSP, GP, AG, AGP |
| ALFTTVGP | 8 | 804.4 | 5.24 | 6.77 | 0 | LF, GP, VG, VGP |
| ALYFHLLL | 8 | 988.6 | 7.85 | 3.31 | 0 | LY, HLL, HL |
| AVLGVWGP | 8 | 797.4 | 5.24 | 6.58 | 0 | VW, GVW, GP, GV, WG, LG, AV, AVL, LGV |
| DPDAQFQY | 8 | 982.4 | 2.88 | 14.94 | -2 | DA FQ |
| EPSLLNSP | 8 | 855.4 | 3.01 | 11.08 | -1 | LN |
| FALWVDAH | 8 | 957.5 | 4.98 | 9.36 | -1 | LW, DA, FAL, AH |
| KEAKAAGA | 8 | 744.4 | 9.63 | 20.28 | 1 | AA, GA, AG, EA, KA, KE |
| KSSGGLGP | 8 | 701.4 | 9.8 | 13.96 | 1 | LGP, GP, GL, GG, SG, LG |
| LEPAPAVK | 8 | 823.5 | 6.81 | 13.9 | 0 | VK, AP, PAP, AV |
| LNLLMSDT | 8 | 905.5 | 3 | 8.68 | -1 | LN |
| SPELLNSP | 8 | 855.4 | 3.01 | 11.08 | -1 | LN |
| SPMPLLPH | 8 | 890.5 | 7.63 | 7.94 | 0 | LLP, PL, PH |
| TEFYDDPP | 8 | 982.4 | 2.69 | 16.92 | -3 | FY, TE, PP, EF |
| TMLYPLYR | 8 | 1055.5 | 9.39 | 5.51 | 1 | LY, YP, PL, LYP |
| TPLSLLGP | 8 | 796.5 | 5.17 | 6.29 | 0 | LGP, GP, PL, LG, TP |
| TTALLPGP | 8 | 768.4 | 5.17 | 7.83 | 0 | LLP, LPG, GP, PG |
| TTFMPTKH | 8 | 961.5 | 9.82 | 11.54 | 1 | PT, TF |

| Peptide sequences | Number of amino acids | Molecular weight (Da) | Isoelectric point (PI) | Hydro-phobicity | Net charge | Bioactivities |
|---|---|---|---|---|---|---|
| TTMDRVWH | 8 | 1044.5 | 7.57 | 12.96 | 0 | VW |
| TTVVDDPR | 8 | 901.4 | 3.91 | 16.71 | -1 | PR |
| TVLCATPE | 8 | 832.4 | 3.09 | 10.94 | 1 | TP |
| TVMFNFLS | 8 | 957.5 | 5.36 | 3.66 | 0 | MF, NF, FNF, VM |
| TVREEPAV | 8 | 899.5 | 4.09 | 16.94 | -1 | VR, AV |
| TYDFEDPP | 8 | 982.4 | 2.69 | 16.92 | -3 | PP, DF |
| YMDAEMCQ | 8 | 989.3 | 2.91 | 14.37 | -2 | DA |
| AEVVDTPAV | 9 | 899.5 | 3.01 | 15.18 | -2 | EV, AV, TP |
| EANDPEWAP | 9 | 1027.4 | 2.72 | 18.84 | -3 | AP, EA, EW, WA |
| EPPWAAHAP | 9 | 974.5 | 5.06 | 13.69 | -1 | AP, AA, AH, PP, WA |
| ESEEMEAYQ | 9 | 1114.4 | 2.79 | 22.77 | -4 | AY, EA, ME |
| FETTFEDPP | 9 | 1081.5 | 2.72 | 16.16 | -3 | PP, TF |
| GFAGDDAPR | 9 | 904.4 | 3.91 | 18.72 | -1 | PR, AP, GF, AG, DA, GD, AGDDAPR |
| LFYCALLLT | 9 | 1055.6 | 5.06 | 1.21 | 0 | LF, FY |
| LSMGLNMAT | 9 | 936.4 | 5.36 | 7.27 | 0 | GL, MG, LN |
| QFAGDDAPR | 9 | 975.4 | 3.91 | 18.34 | -1 | PR, AP, AG, DA, GD, AGDDAPR |
| QFAGDDPLN | 9 | 975.4 | 2.85 | 15.63 | -2 | PL. AG, GD, LN |
| QGEDYLEVP | 9 | 1048.5 | 2.72 | 18.44 | -3 | VP, GE, QG, EV, DY |
| TCAATDAPR | 9 | 904.4 | 6.06 | 15.47 | 0 | PR, AP, AA, DA |
| TGRMLTGPA | 9 | 902.5 | 10.79 | 11.23 | 1 | GPA, GP, GR, TG, TGP, LTGP |
| TPEECEYDF | 9 | 1131.4 | 2.77 | 20.38 | -4 | EY, TP, DF |
| TPEEHFEGH | 9 | 1081.4 | 4.35 | 23.28 | -3 | GH, EG, TP |
| TVMTLADAH | 9 | 957.5 | 4.98 | 12.99 | -1 | LA, DA, AH, VM |
| TVVDEAPAV | 9 | 899.5 | 3.01 | 15.18 | -2 | AP, EA, EV, EAP |
| TVVPPPPAP | 9 | 873.5 | 5.17 | 8.43 | 0 | VPP, AP, VP, PAP, PP, VVPP, PPP |
| TYEMYTEPP | 9 | 1129.5 | 2.85 | 13.85 | -2 | MY, TE, PP, YE |
| AGFAGDDPAR | 10 | 975.4 | 3.91 | 19.22 | -1 | GF, AG, GD, AR |
| AGFAGDDPLN | 10 | 975.4 | 2.85 | 16.51 | -2 | PL, GF, AG, GD, LN |
| DFDPDLVYVQ | 10 | 1209.6 | 2.77 | 15.14 | -3 | VY, LVY, DF, YV |

| Peptide sequences | Number of amino acids | Molecular weight (Da) | Isoelectric point (PI) | Hydro-phobicity | Net charge | Bioactivities |
|---|---|---|---|---|---|---|
| DPTPEDFYVK | 10 | 1209.6 | 3.68 | 19.26 | -2 | FY, VK, PT, TP, DF, YV, DFY |
| DVVPDEMYVK | 10 | 1193.6 | 3.68 | 18.99 | -2 | MY, VK, VP, YV |
| EPPDEEAYPQ | 10 | 1173.5 | 2.76 | 23.41 | -4 | AY, YP, EA, PP, PQ |
| EPVCTLKPAP | 10 | 1053.6 | 6.16 | 13.77 | 0 | LKP, AP, PAP. KP |
| ESMCNGEAQY | 10 | 1130.4 | 2.97 | 17.49 | -2 | GE, EA, NG |
| EVEAAPVVSP | 10 | 996.5 | 2.85 | 15.52 | -2 | AAP, VSP, AP, AA, EA, EV, VE |
| FDDPETPYVK | 10 | 1209.6 | 3.68 | 19.26 | -2 | VK, TP, YV |
| FDDPMKYTPP | 10 | 1209.5 | 3.91 | 15.56 | -1 | MKY, KY, PP, TP |
| KANPDEMYVK | 10 | 1193.6 | 6.63 | 20.42 | 0 | MY, VK, KA, YV |
| KGVLFCHSVP | 10 | 1085.6 | 8.67 | 10.88 | 1 | LF, VP, KG, GV |
| KPGLLPECVT | 10 | 1055.6 | 6.14 | 13.03 | 0 | LLP, PGL, GL, PG, KP |
| KRAKKFVEDT | 10 | 1220.7 | 10.28 | 23.96 | 2 | AKK, KR, RA, KF, VE |
| SPEPPYMYVK | 10 | 1209.6 | 6.55 | 12.66 | 0 | MY, VK, PP, YV |
| SPEVTNLVAP | 10 | 1025.5 | 3.01 | 11.7 | -1 | VAP, AP, EV |
| TAETHSLCFA | 10 | 1078.5 | 5.06 | 12.84 | -1 | CF |
| TDNPVVLLSP | 10 | 1053.6 | 2.93 | 9.96 | -1 | LSP, VVL, |
| TLEAHPPEVL | 10 | 1104.6 | 4.08 | 15.56 | -2 | EA, EV, AH, PP, HP |
| TLVPDEMYVQ | 10 | 1193.6 | 2.91 | 12.78 | -2 | MY, VP, YV |
| TPDCEEENAP | 10 | 1103.4 | 2.66 | 24.29 | -4 | AP, TP |
| TPDPDEFYVK | 10 | 1209.6 | 3.68 | 19.26 | -2 | FY, VK, TP, YV, EF |
| TPDPPYMYVQ | 10 | 1209.5 | 3.04 | 10.43 | -1 | MY, PP, TP, YV |
| TPDVTNLVAP | 10 | 1025.5 | 2.93 | 11.5 | -1 | VAP, AP, TP |
| TVCDAYSAGP | 10 | 982.4 | 2.93 | 13.35 | -1 | AY, GP, AG, DA, AGP |
| TVTVHAMCFA | 10 | 1078.5 | 7.14 | 8.41 | 0 | CF |
| VVDPDEFYVQ | 10 | 1209.6 | 2.79 | 15.92 | -3 | FY, YV, EF |
| VVDPDEMYVK | 10 | 1193.6 | 3.68 | 18.99 | -2 | MY, VK, YV |
| VYDPMYKHGT | 10 | 1209.5 | 7.73 | 15.66 | 0 | MY, VY, GT, HG, YK |
| WAPAPAPAHA | 10 | 987.5 | 7.72 | 11.06 | 0 | AP, PAP, AH, WA |

| Peptide sequences | Number of amino acids | Molecular weight (Da) | Isoelectric point (PI) | Hydro-phobicity | Net charge | Bioactivities |
|---|---|---|---|---|---|---|
| APTPTGPLEAP | 11 | 1049.5 | 3.01 | 13.49 | -1 | GPL, GP, PL, AP, TG, EA, PT, EAP, TP, TGP |
| EAHQVVFEDPP | 11 | 1266.6 | 3.72 | 20.05 | -3 | VF, EA, AH, PP, VVF |
| ENCCDGEAPPP | 11 | 1130.4 | 2.72 | 21.68 | -3 | AP, GE, EA, DG, PP, EAP, PPP |
| EYCDNGSGLVK | 11 | 1183.5 | 4 | 19.14 | -1 | VK, GL, GS, SG, NG, EY |
| FSEVTVAMVHT | 11 | 1219.6 | 5.06 | 11.56 | -1 | EV |
| KANGAADLVAP | 11 | 1025.6 | 6.44 | 16.77 | 0 | VAP, AP, AA, GA, NG, KA |
| PATPTGDVVPP | 11 | 1049.5 | 2.93 | 13.33 | -1 | VPP, VP, GD, TG, PT, PP, VVPP, TP |
| QGVLTTNASVP | 11 | 1085.6 | 5.17 | 10.10 | 0 | VP, GV, QG, TTN |
| TAKRMCNAPSP | 11 | 1174.6 | 9.86 | 14.66 | 2 | AP, KR |
| TLAAAVVPESP | 11 | 1053.6 | 3.01 | 11.85 | -1 | LAA, LA, VP, AA, AV, AVV |
| TPAPTGALEPP | 11 | 1049.5 | 3.01 | 13.49 | -1 | AP, GA, TG, PAP, PT, PP, ALEP, LEPP, TP |
| TPAPTGELPAP | 11 | 1049.5 | 3.01 | 13.49 | -1 | AP, GE, TG, PAP, PT, TP |
| TPAPTGLAEPP | 11 | 1049.5 | 3.01 | 13.49 | -1 | AP, LA, GL, TG, PAP, PT, PP, TP |
| TPDPDEVLDPP | 11 | 1193.5 | 2.61 | 21.55 | -4 | EV, PP, TP |
| TPDPDPVFEPP | 11 | 1209.6 | 2.69 | 17.59 | -3 | VF, PP, FEP, TP |
| TPDPPVDFPEP | 11 | 1209.6 | 2.69 | 17.59 | -3 | FP, PP, TP, DF |
| TPDPPVFEDPP | 11 | 1209.6 | 2.69 | 17.59 | -3 | VF, PP, TP |
| TPVDEPAATGF | 11 | 1103.5 | 2.92 | 15.93 | -2 | AA, GF, TG, TP |
| TTLLTPSGPAP | 11 | 1053.6 | 5.17 | 8.68 | 0 | GPA, GP, AP, SG, PAP, TP, SGP |
| VVDPDPVFEGH | 11 | 1209.6 | 3.66 | 19.4 | -3 | VF, GH, EG |
| EAWAPEPAPAHA | 12 | 1245.6 | 4.08 | 18.32 | -2 | AW, AP, EA, PAP, AH, WA |
| EPAPAPEPAPEP | 12 | 1200.6 | 2.75 | 21.13 | -3 | AP, PAP |
| KNVFAPEQHSVP | 12 | 1351.7 | 7.55 | 16.89 | 0 | VF, FAP, AP, VP |
| KRGDRDVYCCVT | 12 | 1413.6 | 8.19 | 21.33 | 1 | VY, KR, GD, RG |
| PEVGNDPPEQMK | 12 | 1339.6 | 3.73 | 23.66 | -2 | VG, EV, PP |

| Peptide sequences | Number of amino acids | Molecular weight (Da) | Isoelectric point (PI) | Hydro-phobicity | Net charge | Bioactivities |
|---|---|---|---|---|---|---|
| QNVFAPEKHSVP | 12 | 1351.7 | 7.58 | 16.89 | 0 | VF, FAP, AP, VP, EK |
| RTTVTSPPELPP | 12 | 1293.7 | 6.51 | 13.4 | 0 | LPP, PP |
| TLASNVTAVESE | 12 | 1219.6 | 2.92 | 16.2 | -2 | LA, VE, AV |
| TLDDVTFDPVAP | 12 | 1288.6 | 2.66 | 16.22 | -3 | VAP. AP, TF |
| TPDSKSYPDGGK | 12 | 1250.6 | 6.59 | 23.82 | 0 | YP, GK, GG, DG, SY, TP |
| TPVDPEPAAHAP | 12 | 1200.6 | 3.99 | 19.35 | -2 | AP, AA, AH, TP |
| TVDAPEPAAHAP | 12 | 1174.6 | 3.99 | 19.71 | -2 | AP, AA, DA,AH |
| EGETEAGDEDDDP | 13 | 1377.5 | 2.46 | 40.17 | -8 | AG, GE, GD, EG, EA, TE |
| EPAAPAPEPATEQ | 13 | 1306.6 | 2.86 | 22.37 | -3 | AAP, AP, AA, PAP, TE |
| EPAPAPAEPATEQ | 13 | 1306.6 | 2.86 | 22.37 | -3 | AP, PAP, TE |
| EPAPEPAEWTPAE | 13 | 1422.6 | 2.75 | 22.64 | -4 | AP, PAP, EW, TP |
| TPVGEPAPAEHAP | 13 | 1271.6 | 4.07 | 20.49 | -2 | GEP, AP, VG, GE, PAP, TP |
| EAPAWEPAPEAHAP | 14 | 1471.7 | 3.78 | 22.09 | -3 | AW, AP, EA, PAP, AH, EAP |
| EPAAWEPEPAAHAP | 14 | 1471.7 | 3.78 | 22.09 | -3 | AW, AP, AA, AH |
| EPAPAFPAHPAPCP | 14 | 1400.7 | 5.06 | 14.97 | -1 | FP, AFP, AF, AP, PAP, AH, HP |
| EPAPEPAEPEASAE | 14 | 1422.6 | 2.7 | 29.07 | -5 | AP, EA, PAP |
| TVPDAPAPAAAHPA | 14 | 1284.6 | 4.98 | 17.22 | -1 | AP, VP, AA, DA, PAP, AH, HP |
| WAHAHAPEAHGAAH | 14 | 1461.7 | 6.52 | 23.05 | -1 | AP, AA, GA, HG, EA, AH, WA |
| EPAPAFAPAPHPACP | 15 | 1471.7 | 5.06 | 15.47 | -1 | FAP, AF, AP, PAP, HP, PH |
| EPAPEPATTTYLPAE | 15 | 1585.8 | 2.82 | 19.64 | -3 | AP, PAP |
| PEAGHAEPAHAAHAP | 15 | 1461.7 | 5.77 | 26.72 | -2 | AP, AA, AG, GH, EA, AH |
| TVPPADAPEAHPAAP | 15 | 1439.7 | 3.99 | 20.49 | -2 | AAP, VP, AP, VP, AA, DA, EA, AH, PP, HP |
| TVPPASMPAPHPACP | 15 | 1471.7 | 7.14 | 12.13 | 0 | VPP, AP, VP, PAP, PP, HP, PH |
| EPAPAEPECAEPAPAE | 16 | 1606.7 | 2.7 | 29.23 | -5 | AP, PAP |
| EPAPAHEAPEGCSPSL | 16 | 1590.7 | 3.79 | 23.98 | -3 | AP, EG, EA, PAP, AH, EAP |

| Peptide sequences | Number of amino acids | Molecular weight (Da) | Isoelectric point (PI) | Hydro-phobicity | Net charge | Bioactivities |
|---|---|---|---|---|---|---|
| EPAPAPFAPAHPAPCP | 16 | 1568.7 | 5.06 | 15.61 | -1 | FAP, AP, PAP, AH, HP |
| EPAPAPFCSAHPAPAE | 16 | 1590.7 | 4.07 | 19.42 | -2 | AP, PAP, AH, HP |
| EPPAAPFAPAHPAPCP | 16 | 1568.7 | 5.06 | 15.61 | -1 | AAP, FAP, AP, AA, PAP, AH, PP, HP |
| GHCPAPEPAFTPAPAE | 16 | 1590.7 | 4.07 | 19.86 | -2 | AF, AP, GH, PAP, TP |
| HGCPAPFAPAHPAPAE | 16 | 1568.7 | 6.05 | 18.81 | -1 | FAP, AP, HG, PAP, AH, HP |
| PAEPAPEPAFTFGASC | 16 | 1590.7 | 2.84 | 16.14 | -2 | AF, AP, GA, FG, PAP, TF |
| EPAPAPAFAPAPHAPCP | 17 | 1639.8 | 5.06 | 16.11 | -1 | FAP, AF, AP, PAP, PH |
| PAEPAPEPAEPSCPASL | 17 | 1661.8 | 2.93 | 21.28 | -3 | AP, PAP, ASL |

important and determines the binding process of the ACE active site. Val and Ile residues at the N-terminal position are also strongly suspected of ACE inhibitor activity [21].

The bioactivity of a peptide is not only determined by the presence of a particular amino acid in its sequence. Physical and chemical properties such as sequence length, molecular weight, isoelectric point, net charge, and hydrophobicity also determine the bioactivity of a peptide [15]. De novo analysis showed that the detected peptides composed 4-17 amino acids with a 490-1661 Da molecular weight. Analysis using Pepdraw (http://pepdraw.com/) showed that the detected peptides had hydrophobicity values ranging from 1.21 to 40.17 Kcal $mol^{-1}$, isoelectric point ranging from 2.46 to 10.79 and net charge ranging from -8 to 2. Gonzalez-Gracia *et al*. [22] stated that peptides with strong ACE inhibitory activity have short amino acid chains (<30 amino acids) with a molecular weight of less than five kDa. Darewicz *et al*. [23] also stated that small peptides had better ACE inhibitory activity. This condition is related to the binding process with the active site of ACE. Long-chain peptides are more difficult to bind to the active site, resulting in decreased inhibitor activity [24]. The in silico study by Tamam *et al*. [15] stated that the ACE inhibitor peptide has several characteristics such as sequence length of about 7-8 amino acids, molecular weight in the range of 800-900 Da, isoelectric point in the range of 6-7, net charge in the range of 0, and hydrophobicity in the range of 10-11 kcal $mol^{-1}$.

## 4. Conclusion

Analysis using software Proteome Discoverer 2.1 of LC-MS/MS output data from samples without preparation (without the addition of trypsin) shows better results than the prepared samples (with the addition of trypsin), confirming) confrming that samples were already in the peptide or protein fraction using *C. striata* database. Fragment Beta-actin (25.8 kDa) and three peptides have been identified in the samples using the *C. striata* database. Protein Beta-actin (41.7 kDa) has been identified from the *Channa argus* database. Samples contain peptides identified using *Channa argus* and *Channa striata* database as AGFAGDDAPR, GQVITIGNER, VITIGNER, TEAPLNPKAN, KDSYVGDEAQ, GFAGDDAPR, TEAPLNPKA, DSYVGDEAQSK, FAGDDAPR, KSYELPDG, SYELPDGQ. De novo sequencing resulted in 319 peptides with ALC > 70% presented in the fraction samples. Bioinformatics study shows that the peptide identified was rich in peptide ACE inhibitor based on the BIOPEP database.

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