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Preparation of ACE inhibitory peptides from *Tenebrio molitor* and antibacterial activity

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Research

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ABSTRACT

This research aimed to obtain active peptides that exert remarkable inhibitory effects on Angiotensin-converting enzyme (ACE). The ACE inhibitory peptides from *T. molitor* were were prepared by hydrolyzing *Tenebrio molitor* powders with papain, separated by using ultrafiltration membranes. Five molecular weight fractions were obtained. Sephadex G-15 was used to separate the fraction with molecular weight < 3 kDa through the different concentrations of the enzyme solutions. Results showed that 100 mg/mL was the optimal concentration, and the elution peaks were divided into three groupsM_1,M_2,M_3. The M_2 component showed the highest activity,which was analyzed and found to be abundant and diverse. The molecular weight of M_2, which was composed of three amino acid residues. The inhibitory activities of the enzymatic hydrolysis solution, the ultrafiltration component, and the chromatography component against indicator bacteria were measured. The enzymatic hydrolysis solution and the component >30 kDa in molecular weight exhibited inhibitory effects on the three indicator bacteria.

Keywords: *Tenebrio molitor*, ACE inhibitory peptide, isolation, purification, antibacterial activity

1. INTRODUCTION

Angiotensin-converting enzyme (ACE) is a two-peptide carboxyl group that regulates human blood pressure. In the renin–angiotensin system and the kallikrein–kinin system, ACE can induce a strong contraction of the vascular smooth muscle and thus an increase in blood pressure (Shi, Jin & Yuan, 2004). Meanwhile, ACE inhibits bradykinin, the kinin with a diastolic blood vessel function in the endogenous blood pressure system of the human body (Cushmanh & Cheung, 1971). Therefore, inhibition of ACE activity plays an important role in blood pressure regulation. Currently, antihypertensive peptides can be prepared through three types of extraction methods, namely, direct extraction, fermentation, and enzymatic hydrolysis. Each extraction method has its inherent advantages and disadvantages.

Antihypertensive peptides derived from food proteins by enzymatic hydrolysis have gained wide attention because of their high safety (Liu & Wu Qiong, 2007). Several studies have reported the preparation of ACE inhibitory peptides from different food protein hydrolysates. Therefore, isolation of ACE inhibitory peptides from foodborne materials is a new developmental trend. At present, ACE inhibitory peptides have been isolated from various foodborne proteins, and these peptides can be divided into two categories (Zhang C&Sun L.C.,2018). One stems from natural plants(Wang.C.&Tu.M.L,2018)., such as peanuts(Shi, A.M& Liu, H.Z,2014), soybean, rapeseed (Ren.H,2011), and corn(Manoharan.S.&Shuib.A.S); the other originates from animals, such as egg white, chrysalis(Deng Z.Z.&Liu Y.J.,2018), and small yellow croaker(Wang X.&Yu H.,2017). All ACE inhibitory peptides that have been reported thus far contain 2–15 peptides, most of which contain hydrophobic and aromatic amino acids with molecular weights ranging from 200 Da to 1500 Da(García-Moreno P.J.&Espejo-Carpio F.J.,2015). Recent studies have focused on the antibacterial activity of peptides because of their potential to be developed as a new type of antibiotics or food preservatives that have the advantages of high safety, nontoxic side effects, simple materials, and green preparation process(Soriano-Santos J, Escalona-Buendía HB,2015).

Tenebrio molitor, also known as large mealworm or bread worm, belongs to the Class Insecta (Coleoptera: quasi Carabidae) (Zhang LS.& Zhang JX.,2009). This insect contains high contents of proteins and amino acids. *T. molitor* has been used to produce health oral liquids containing antioxidant peptides and amino acids. Studies on the ACE inhibitory peptides of *T. molitor* proteins mostly focused on the optimization of enzyme screening and extraction; few reports about the separation and purification of these peptides are available. In the present experiment, *T. molitor* proteins as a source of animal proteins were hydrolyzed with papain to prepare ACE inhibitory peptides. The peptides were isolated and purified via ultrafiltration and Sephadex gel chromatography. The amino acid composition, molecular weight distribution, and ACE inhibitory activity of the ACE inhibitory peptides were then investigated. This study aims to provide a basis for the research and development of ACE inhibitory peptides. The antibacterial activities of the obtained ACE inhibitory peptides were also assessed(Wang.Z.L. & Wang X.M.,2018). This study has a positive significance for the storage, preservation, biological control, and breeding of ACE peptides. It also has broad application prospects in the fields of medicine, health, agriculture, food industry, etc.

2. MATERIALS AND METHODS

2.1 Materials

Materials	Company
Defatted powder of Tenebrio molitor protein (protein content 63.17%)	Chongqing Fujiang Biological Technology Co. Ltd.
Papain PTN6.0S （1.89×104 U/g）	Sigma company in American
ACE	Sigma company in American
N-[3-(2-furylacryloyl)]-L- phenyalanyl-glycyl-glycine	Sigma company in American
Sephadex gel chromatography	Sigma company in American

2.2 Equipment's

Equipment's	Company
Vivaflow tangential flow ultrafiltration	Germany Sartorius Group Co. ltd.;
HHS4 thermostatic bath	Shanghai Pudong Yuexin science instrument factory
LB941 enzyme standard instrument	Germany Berthold company
Bench top refrigerated centrifuge ;FD-1 freeze dryer	Thermo Fisher Scientific company in America
PH PHS-25 instrument	Shanghai Precision Scientific Instrument Co., Ltd.
FA10004A electronic balance	Shanghai precision instrument factory
glass column (1.6 * 60cm)	Shanghai Jinhua chromatography equipment factory
BSZ–100 Automatic partial collector DHL-A Computer constant current pump	Qingpu Shanghai West Instrument Factory
UV spectrophotometer	Beijing Purkinje General Instrument Co. company
L-8900 amino acid analyzer	Hitachi, japan
4800 Plus MALDI TOF/ TOF™ Mass spectrometer	AB SCIEX company in America
SPX-80BSH- II biochemical incubator	Shanghai CIMO Instrument Manufacturing Co. ltd.
HPS-250 Biochemical culture box	Dongming Harbin medical instrument factory
TomySX-500 high pressure sterilization pot	Shanghai Mai Medical Technology Co., ltd
HFsafe-1500 manual type bio safety cabinet	Hongkong force Kang Development Co., ltd

2.3 Methods

2.3.1 Preparation of ACE inhibitory peptides from T. molitor

A certain amount of *T. molitor* protein was configured to a 7 g/100 mL substrate concentration of protein solution, and the pH was adjusted to 6.5. Then, 1% papain was added and then placed under a constant temperature bath of 55 °C to induce enzymatic hydrolysis for 7 h. The enzyme solution was placed in a boiling water bath for 10 min to inactivate enzymes and then centrifuged at 10 000 r/min for 10 min at 4 ° C. The supernatant was lyophilized to prepare highly active *T. molitor* ACE inhibitory peptides for the determination of ACE inhibition rate.

2.3.2 Determination of ACE inhibition rate

Shalaby's method was employed with modifications (Shalaby, Zakora & Otte, 2006). FAPGG was used as the substrate, and a microplate reader was used to determine ACE inhibition rate.

ACE solution was prepared as follows. Distilled water (1 mL) was slowly injected into 0.1 U of ACE glass bottle, mixed, and then set aside. In specific, 10 μL of aqueous ACE and 10 μL of ACE inhibitory peptide sample solution (1 mg/mL) were added to the enzyme label plate, followed by 150 μL of substrate solution (1.0 mmol/L FAPGG dissolved in 50 mmol/ L Tris HCl containing 0.3 mol/L NaCl, pH 7.5). The solution was then preheated at 37 °C for 5 min to initiate the reaction. The microhole plate was placed in the enzyme standard instrument, and the absorbance of A1 at 340 nm was recorded. The absorbance at 340 nm was determined again after 30 min. The blank group comprised 10 μL of the substrate instead of the ACE inhibitory peptides of the sample solution. The initial and final absorbance values of the blank group were recorded as A_{01} and A_{02}, respectively.

The change (△A Inhibitors= $A_1 - A_2$, △Blank= $A_{01} - A_{02}$) in the absorbance value was used to calculate the inhibition rate of ACE.

2.3.3 Ultrafiltration separation of ACE inhibitory peptides

Membranes with different molecular weights were loaded in the ultrafiltration system.

Before adding the sample, the membranes were washed with pure water at room temperature for ultrafiltration. Enzymatic hydrolysates were separated though 5, 10, 3, and 30 kDa ultrafiltration membranes by using the Vivaflow ultrafiltration system. After ultrafiltration, enzymolysis fluid was divided into five parts: group I (M > 30 kDa), group II (10 kDa < m < 30 kDa), group III (5 kDa < m < 10 kDa), group IV (3 kDa < m < 5 kDa), and group V (m < 3 kDa). After vacuum freeze drying, the ACE inhibition rate of each component was determined.

2.3.4 Gel chromatography separation of ACE inhibitory peptides

Sephadex G-15 was treated and then placed into a 1.6 cm × 60 cm glass column. The *T. molitor* ACE inhibitory peptides were dissolved in distilled water, and solutions with different concentrations of 100, 200, and 300 mg/mL were prepared. A 3 mL sample was added and then flowed with distilled water to 0.3 mL/min. One tube was collected every 10 min, and a total of 50 tubes were collected. Absorbance was measured at 280 nm by using a UV spectrophotometer. The optimal elution peaks were collected and frozen dry. The inhibition rate of ACE was determined.

2.3.5 Molecular weight distribution of T. molitor ACE inhibitory peptides

To determine the molecular weights of the ACE inhibitory peptides, the highly active M_2 component purified by Sephadex G-15 was identified through matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS). First, the trypsin enzyme of horse heart myoglobin was used as the standard peptide for the external standard calibration of the instruments (error ≤ 0.1u, relative standard deviation ≤ 10 ppm). MS parameter settings were as follows: a mass of data acquisition using the MS-positive-2KV reflection mode, MALDI source as the laser source, scanning range m/z of 120–760, laser energy of 5000, and positive ion mode. The instrument control software used was 4800 Explorer TM, and the mass-to-charge ratio (m/z) was a single-isotope mass number. The spotting method was as follows. A sample was dissolved in ultrapure water to a concentration of 5 μg/μL, 0.5 μL points were taken on the stainless steel MALDI target plate after natural drying, and 0.5 μg/μL α-cyano-4-hydroxy cinnamic acid served as a matrix. After natural drying, test samples were submitted for MS detection(Ibrahim HR&Ahmed AS,2017).

2.3.6 Amino acid analysis of T. molitor ACE inhibitory peptides

The amino acid contents of the samples were analyzed through the following four steps: tryptophan hydrolysis, peroxyformic acid removal, drying, and determination. To oxidize the samples completely, 0.100 g of *T. molitor* ACE inhibitory peptides were placed in a hydrolysis tube, added with 4.2 mol/L of sodium hydroxide hydrolysis tryptophan samples and 1.0 mL of formic acid solution, and then incubated at 25 °C for 3 h. The oxidation reaction was terminated by adding 1 mL of ethanol. The dried flask into which the remaining liquid sample from the hydrolysis tube was poured was connected and placed in the vacuum unit to dry the samples. After drying under reduced pressure termination, the flask was rinsed with ultrapure water and then dried. A second drying step was conducted, and the above process was repeated thrice to volatilize the residual peroxyformic acid sufficiently. HCl (10 mL, 6 mol/L) was added to the rest of the sample solution, three drops of phenol were added with a glue head dropper, and the vials were frozen in liquid nitrogen for 3–5 min. Then, the vials were connected with pump. After drying the vials, they were pumped with nitrogen, and the operation was repeated thrice. In this state, the vials were sealed and placed in the oven at 110 °C for 22 h. Samples that were sufficiently hydrolyzed were removed from the loft drier and cooled to room temperature. The sample tubes were opened, the hydrolyzing liquid was filtered into a 50 mL volumetric flask, and flushing fluid was obtained by washing the vials repeatedly with ultrapure water. A 1.0 mL aliquot of the sample was transferred from the 50 mL volumetric flask into a 5 mL volumetric flask and then placed in a vacuum dryer.

The residue was dissolved with ultrapure water and then dried; this process was repeated twice. Finally, the residue after evaporation was dissolved with 1 mL of disodium hydrogen phosphate–citrate buffer (pH 2.2) before being assayed by a machine. A 0.2 mL aliquot of the mixed standard amino acid liquid was extracted and placed in the 5 mL volumetric flask containing phosphate dibasic–citrate buffer (pH 2.2) to a constant volume. The diluent concentration of 1.0 nmol/10μL was used as the standard amino acid. Subsequently, ACE inhibitory peptides were injected into the amino acid analyzer to measure the amino acid content of the samples.

2.3.7 Antimicrobial testing of T. molitor ACE inhibitory peptides

A 300 mg sample of the ACE inhibitory peptides dissolved in 1 mL of sterile ultrapure water was taken in a clean bench. After 30 min of irradiation with ultraviolet rays, the sample was filtered twice through a 0.22 μm microfiltration membrane. Water agar culture medium (10 mL, 50 °C) was added in the sterilization culture dish. After cooling, four Oxford cups were placed equidistantly in a Petri dish. The LB solid medium (20 mL) was removed from the oven, cooled to approximately 50 °C, and then added with 1 mL of the test bacteria (1×10^6 CFU/mL). The mixture was slowly poured into the center of the dish, and the Oxford cups were pulled out after the mixture cooled and solidified. One hole was served as the negative control, which contained 200 μL of sterile ultrapure water. Each of the three other holes was added with 200 μL of the *T. molitor* ACE inhibitory peptides that were obtained through different separation–purification methods. The culture dish was developed through incubation at 37 °C incubator for 24 h.

The inhibition zone diameter was measured with a Vernier caliper behind the culture dish under irradiation reflected light, and the average was calculated after three times of vertical measurement.

3. RESULTS AND DISCUSSION

3.1 Ultrafiltration of *T. molitor* ACE inhibitory peptides

As shown in Fig. 1, the inhibition rates of the ACE inhibitory peptides with different molecular weights differed after ultrafiltration. The ACE inhibitory effect of the component > 3 kDa in molecular weight was weaker than that of the enzymatic liquid. Only the component < 3 kDa in molecular weight (component V) showed a higher inhibition rate than the enzymatic liquid. Such results indicate that the most effective ingredients of *T. molitor* ACE inhibitory peptides originated from the component V. The results also confirmed that the ACE inhibitory peptides mainly constitute small peptides. Hence, the component V was adopted for all subsequent purification experiments.

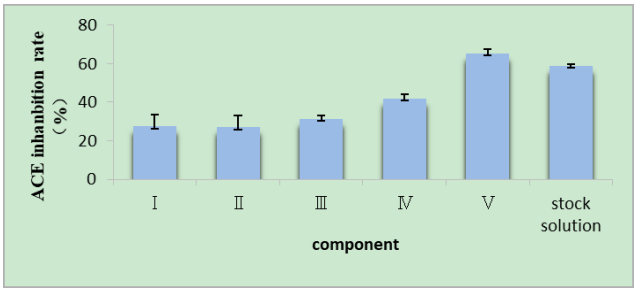

Fig. 1 The ACE inhibitory activity of isolated fractions from Tenebrio molitor protein power after ultrafiltration

3.2 Sephadex G15-isolated ultrafiltration fraction of *T. molitor* ACE inhibitory peptides

As shown in Figures 2, 3, and 4, the V constituent was obtained after ultrafiltration of *T. molitor* ACE inhibitory peptides. Different concentrations of the sample were selected, and three chromates appeared through Sephadex G15. The effect of the peak at the 200 mg/mL concentration was not satisfactory, whereas that of the peak at 300 mg/mL was not enough to separate the gel filtration profiles. The sample concentration of 100 mg/mL was selected on the basis of the effect of the elution peak(M_1, M_2, and M_3). The liquid of the elution peak was repeatedly isolated and collected, concentrated, and then freeze-dried. Then, ACE inhibition rate was determined. Figure 5 shows the separation by Sephadex G15. The M_2 constituent showed the highest ACE inhibition rate (i.e., approximately 72.11%), with an IC_{50} of 0.6052 mg/mL. The isolated liquid was subjected to chromatography, concentrated, and then freeze-dried. Finally, the remaining liquid was used for further analyses.

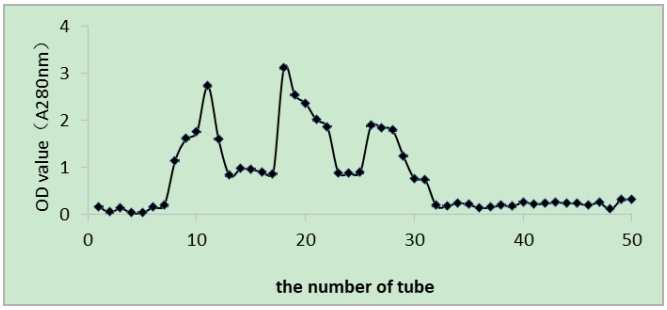

Fig. 2 Glucan gel chromatography spectrum diagram at the concentration of 100 mg/mL

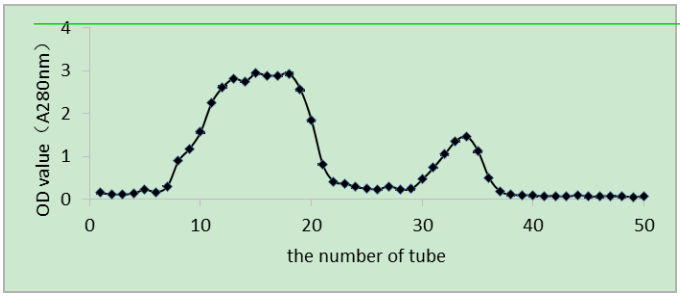

Fig. 3 Glucan gel chromatography spectrum diagram at the concentration of 200 mg/mL

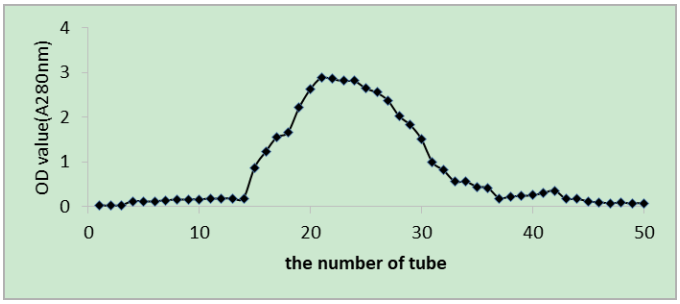

Fig.4 Glucan gel chromatography spectrum diagram at the concentration of 300 mg/mL

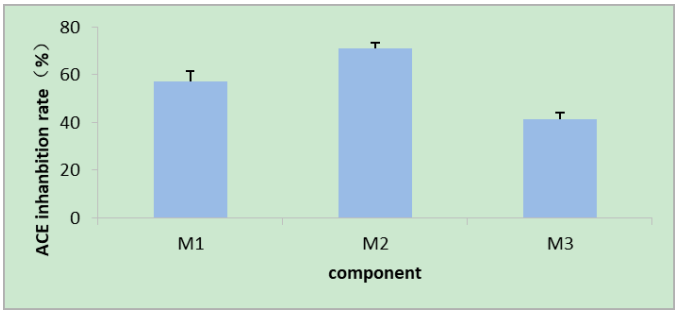

Fig.5 The ACE inhibitory activity of isolated fractions from Tenebrio molitor protein power after Sephadex G–15 gel purify at the concentration of 100 mg/mL

3.3 Amino acid composition of *T. molitor* ACE inhibitory peptides

Table 1 shows the rich amino acid contents of the *T. molitor* ACE inhibitory peptides; in particular, the peptides contained 18 amino acids and seven essential amino acids. The amino acid with the highest content in the larvae of *T. molitor* was Pro (29.04%), followed by Glu (18.06%) and Val (6.78%). The three amino acids accounted for approximately 55% of the total amino acids. The discovery of protein food derived from antihypertensive peptides has proven that peptides whose N-terminal contains Ala, Val, Leu, and Gly residues and whose C-terminal contains Arg, Tyr, Phe, and Pro residues can lower blood pressure. The *T. molitor* ACE inhibitor peptides containing a high content of the above amino acids can be inferred to possess a relatively satisfactory ACE inhibition effect.

Table 1: Amino acid composition of M_2 part (%) (g/100g protein)

Amino	Content	Amino	Content
Asp	1.10	Leu	5.26
Thr	1.74	Tyr	6.21
Ser	1.59	Phe	3.86
Glu	18.06	Lys	0.45
Gly	5.49	His	3.18
Ala	6.40	Arg	2.88
Val	6.78	Pro	29.04
Met	1.63	Trp	0.08
Ile	3.75	Cys	2.50

3.4 Molecular weight distribution of *T. molitor* ACE inhibitory peptides

Strong signal peaks were observed in the mass spectrogram. Among the three signal peaks, the 379.1 signal peak was the molecular ion peak $(M + H)^+$, which indicated that the molecular weight of the peptide segment was 378 Da. The ACE inhibitory peptides may be synthesized by the dehydration condensation of three amino acids (i.e., tripeptide). The molecular weight of the tripetide comprising Gly, Arg, and Phe was 378 Da, and the individual molecular weights of these three amino acids were 75, 174, and 165 Da, respectively. The tripeptide had six compound modes through the dehydration condensation of amino acids: Gly–Arg–Phe, Gly–Phe–Arg, Arg–Gly–Phe, Arg–Phe–Gly, Phe–Arg–Gly, and Phe–Gly

–Arg. Basing from the sequence of the peptide segments, we concluded that Phe was a C-terminal amino acid through the characteristic ion 166.0033 in the mass spectrogram; that is, Phe provided a H^+ in a $-NH_2$ during the formation of the tripeptide. Meanwhile, Arg was the intermediate amino acid of the peptide through the characteristic ion 156.0923 in the mass spectrogram; that is, Arg provided a H^+ in a $-NH_2$ and a OH^- in a –COOH during the formation of the tripeptide. Therefore, Gly may provide OH^- and then produce the characteristic ion 58. However, data collection was started after 120. Thus, the characteristic ion 58 was not observed in the mass spectrum. Basing from the characteristic ion 232.0958, we inferred that the ions formed after -Gly-Arg-Phe lost -Phe residue. Therefore, the amino acid sequence of the tripeptide may be Gly-Arg-Phe.

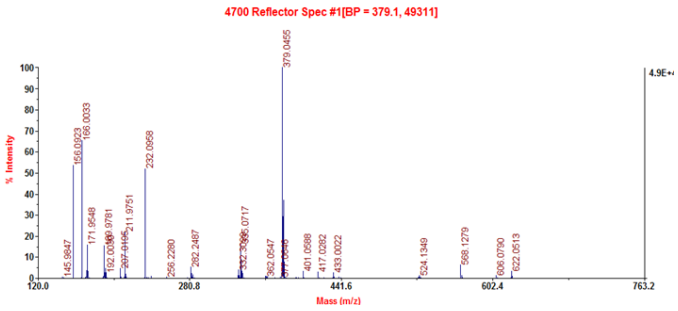

Fig.6 Mass spectra (c) of M_2

3.5 Inhibitory activity of *T. molitor* ACE inhibitory peptides toward *Alternaria* fungus

As shown in Table 2 and Figure 7, the ACE inhibitory peptides that underwent enzymolysis and purification showed fungal inhibitory activities. The ACE inhibitory peptides > 10 kDa and < 30 kDa in molecular weight displayed the strongest inhibitory effect, with an inhibition zone of approximately 5.15 mm. The M1 component exhibited a stronger antibacterial effect compared with the hydrolysates < 3 kDa in molecular weight. This result may be attributed to the fact that peptides of low molecular weight have less polypeptide fragments with inhibitory effects on *Alternaria* fungus. A. enzymatic hydrolysis solution B. molecular weight greater than 30 kDal C. molecular weight between10 kDal and 30 kDal D molecular weight between 5 kDal and 10 kDal E. molecular weight between 3 kDal and 5 kDal F. molecular weight less than 3 kDal G. chromatography M_1 H. chromatography M_2 I. chromatography M_3.

Table 2: The antibacterial against Alternaria alternata of Tenebrio Molitor ACE inhibitory peptide

Types	**Diameter of antibacterial circle（mm）**
enzymatic hydrolysis solution	4.55±0.50
M>30 kDal	2.76±0.50
10 kDal<M<30 kDal	5.15±0.14
5 kDal<M<10 kDal	4.29±0.28
3 kDal<M<5 kDal	1.87±0.42
<3 kDal	2.77±0.09
M_1	4.49±0.28
M_2	none
M_3	none

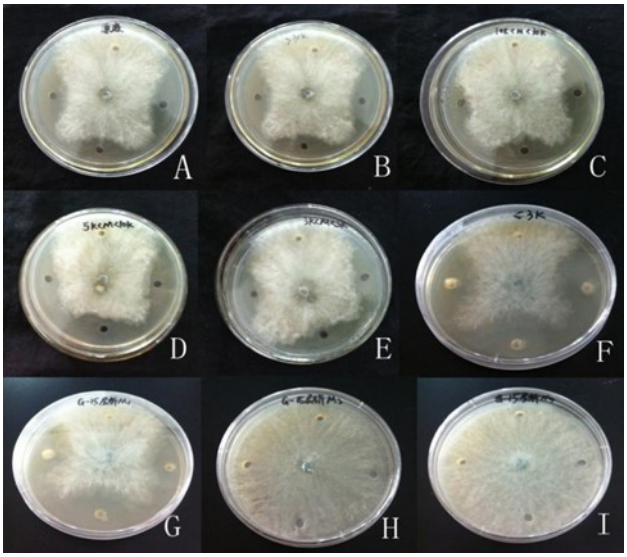

Fig. 7: The antibacterial effect against Alternaria alternata of ACE inhibitory peptide from tenebrio molitor

A. enzymatic hydrolysis solution B. molecular weight greater than 30 kDal C. molecular weight between10 kDal and 30 kDal D molecular weight between 5 kDal and 10 kDal E. molecular weight between 3 kDal and 5 kDal F. molecular weight less than 3 kDal G. chromatography M_1 H. chromatography M_2 I. chromatography M_3

3.6 Inhibitory activity of *T. molitor* ACE inhibitory peptides on *Escherichia coli*

As shown in Table 3 and Figure 8, the ACE inhibitory peptides that underwent enzymolysis and purification demonstrated a certain inhibitory effect on *E. coli*. The ACE inhibitory peptides > 30 kDa in molecular weight exhibited the strongest inhibitory effect, with an inhibition zone of approximately 7.37 mm. The M1 component showed a stronger antibacterial effect compared with the hydrolysates < 3 kDa in molecular weight. The inhibitory effect was satisfactory, with an inhibition zone of 7.22 mm. This result may be attributed to the fact that the peptides have polypeptide fragments with inhibitory effects on *E. Coli.*

Table 3: The antibacterial against Escherichia coli of Tenebrio Molitor ACE inhibitory peptide

Numbers	Types	Diameter of antibacterial circle （mm）
1	enzymatic hydrolysis solution	6.22±0.18
2	M>30 kDal	7.37±0.49
3	10 kDal<M<30 kDal	none
4	5 kDal<M<10 kDal	none
5	3 kDal<M<5 kDal	none
6	<3 kDal	3.23±0.39
7	M_1	7.22±0.25
8	M_2	none
9	M_3	none

Fig. 8 The antibacterial effect against Escherichia coli of ACE inhibitory peptide from tenebrio molitor

1. enzymatic hydrolysis solution 2. molecular weight greater than 30 kDal 3. molecular weight between10 kDal and 30 kDal 4. molecular weight between 5 kDal and 10 kDal 5. molecular weight between 3 kDal and 5 kDal 6. molecular weight less than 3 kDal 7. chromatography M_1 8. chromatography M_2 9. chromatography M_3

3.7 Inhibitory activity of *T. molitor* ACE inhibitory peptides on *Staphylococcus aureus*

As shown in Table 4 and Figure 9, only few of the ACE inhibitory peptides that underwent enzymolysis and purification exerted inhibitory effects on *S. aureus*. Only the enzymatic hydrolysis solution and the ACE inhibitory peptides > 30 kDa in molecular weight displayed antimicrobial effects. This result may be attributed to the fact that only the macromolecular polypeptides contain polypeptide fragments with inhibitory effects on *S. Aureus*.

Table 4: The antibacterial against Staphylococcus aureus of Tenebrio Molitor ACE inhibitory peptide

Numbers	Types	Diameter of antibacterial circle（mm）
1	enzymatic hydrolysis solution	6.65±0.25
2	M>30 kDal	7.33±0.28
3	10 kDal<M<30 kDal	none
4	5 kDal<M<10 kDal	none
5	3 kDal<M<5 kDal	none
6	<3 kDal	none
7	M_1	none
8	M_2	none
9	M_3	none

Fig. 9: The antibacterial effect against Staphylococcus aureus of ACE inhibitory peptide from tenebrio molitor

1. enzymatic hydrolysis solution 2. molecular weight greater than 30 kDal 3. molecular weight between10

kDal and 30 kDal 4. molecular weight between 5 kDal and 10 kDal 5. molecular weight between 3 kDal and 5 kDal 6. molecular weight less than 3 kDal 7. chromatography M_1 8. chromatography M_2 9. chromatography M_3

4. CONCLUSION

Hydrolysis of *T. molitor* proteins by papain increased the ACE inhibition rate added up to 72.11% and yielded an IC_{50} of 0.6052 mg/mL on the basis of ultrafiltration and Sephadex G15 separation. The inhibition rate of the proteins after separation and purification significantly increased ($p < 0.05$). The polypeptides that displayed ACE inhibitory effect possessed a relatively small molecular weight (378 Da), contained a high amino acid content, and comprised three amino acid residues whose sequence may be Gly-Arg-Phe. *Alternaria* was selected as the indicator bacteria, and *E. coli* and *S. aureus* epiphyte were selected as the indicator epiphyte. The enzymatic hydrolysis solution and the liquid component were subjected to the inhibitory test after isolation and purification. The ACE inhibitory peptides, particularly the polypeptide fragments with a large molecular weight, elicited antibacterial effects. The enzymatic hydrolysis solution and the component > 30 kDa in molecular weight showed certain inhibitory effects on the three indicator epiphytes. The strongest bacteriostasis to *Alternaria alternata* was depicted by the 10–30 kDa enzyme solutions, with an inhibition zone of up to 5.15 mm. The strongest bacteriostasis to *E. coli* and *S. aureus* was exhibited by the 30 kDa enzyme solutions, with inhibition zones of 7.37 and 7.33 mm, respectively. The *T. molitor* ACE inhibitory peptides may be further separated and purified by a liquid equipment to provide a powerful evidence for the study of *Tenebrio* ACE inhibitory peptide QSAR and new synthetic methods of peptide segments. *T. molitor* as a raw material for the preparation of bioactive peptides can be used to process natural antibacterial agents.

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