Identification of a novel hypotensive peptide from porcine plasma hydrolysate by *in vitro* digestion and rat model

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ABSTRACT

We separated a novel functional peptide IFPPKPKDTL from porcine plasma hydrolysate by chromatography, HPLC, and identified by Q Exactive LC-MS/MS. Results showed that IFPPKPKDTL had a significant ability of ACE inhibition (76.6%) likely due to the presence of hydrophobic, aromatic, and acidic amino acids that can inactivate ACE by binding Zn^{2+}, providing a hydrogen atom to maintain the link between ACE and the peptide. Furthermore, the ACE inhibition of synthetic IFPPKPKDTL was improved by 15.6% after *in vitro* digestion. Additionally, the systolic blood pressure and diastolic blood pressure of spontaneously hypertensive rats gavaged by the peptide (30 mg/kg). Thereby, ACE inhibitory peptide IFPPKPKDTL from porcine plasma was stable and has potential functional value.

Practical Application

A novel of hypotensive peptide was isolated from porcine plasma hydrolysates and its sequence was identified by Q Exactive LC-MS/MS. The ACE inhibition of the peptide shown it had significant hypotensive properties. Moreover, the ACE inhibitory stability of the peptide was determined by in vitro digestion. Furthermore, spontaneously hypertensive rat (SHR) also was used to confirm the hypotensive properties of the peptide *in vivo*. The results demonstrated a novel hypotensive peptide, which can provide new materials of hypertensive drugs and promote the application of porcine plasma in functional food.

1. Introduction

Animal blood from slaughterhouses is a useful source of protein and bioactives for those who include pork and pork products in their diet. Plasma proteins, including fibrinogen, albumin, and globulins, making up approximately 60% of blood, can be used as functional components in food engineering (Jin et al., 2020). Pigs occupy a large global market that has quadrupled in recent decades and is expected to continue to grow over the next three decades (Luis Lassaletta, et al., 2019), but exploitation of waste streams from pork production have not been considered extensively. Porcine plasma hydrolysates (PPH) demonstrate many bioactivities due to short sequences peptides (2–20 amino acids), which could be used in functional food products, thereby adding value to a waste stream from this food chain(Bernardini et al., 2011; Kim et al., 2018).

Hypertension defined as systolic anddiastolic blood pressure (SBP/DBP) higher than 140/90 mmHg respectively, is a factor of cardiovascular disease together with obesity, diabetes, and high blood lipids, which is a significantpublic health concern and can cause myocardial infarction, stroke and so on (Srikanth and Deedwania, 2016; SharifiRad et al., 2017). About 40% of adults suffer from raised blood pressure and more than 30% of them suffer from complications such as diabetes, which had reported by Norris et al (2020). Renin-angiotensin system (RAS), as one of the blood pressure regulation systems, involves two criticalenzymes called angiotensin I converting enzyme (ACE) and renin (Patricia et al., 2013). Angiotensinogen is firstly converted into angiotensin-I by hydrolysis of renin, and then it is cleaved to produce angiotensin-II by ACE, which can control blood pressure receptors by the combination between angiotensin-II and angiotensin I (Rong et al., 2014). Therefore, inhibiting ACE activity in hypertension treatment is the most effective and commonly used method, such as ACE inhibitors. Herrera Chalé et al. (2014) had reported that ACE inhibitors could significantly reduce the morbidity and mortality of patients with myocardial infarction or heart failure.

However, ACE inhibitors may cause skin rash, angioedema, diarrhea, cough and dizziness, especially antihypertensive drugs, such as captopril tablets (Herrera Chalé et al., 2014). Moreover, because patients with hypertension usually require life-long medical treatment, people have been paying attention to the isolation and identification of ACE inhibition, which can be obtained from various food sources to decrease its side effects. Martina et al. (2017) found functional components in pork protease hydrolysates. Michio et al. (2009) also reported the identification of pro-drug type ACE inhibitory peptide sourced from porcine myosin B. Additionally, the active peptides from different foods usually attract more attention because of their small molecular weight, easy absorption, and almost no toxic side effects. Ji et al. (2020) reported that flaxseed peptides had anexcellentability to lower blood pressure. ACE inhibitory peptide Lys-Arg-Val-Ile-Gln-Try was isolated and identified from porcine skeletal myosin B, which had reported by Muguruma et al (2009). While the variety of amino acid sequence combinations can influence their functions, the peptide sequences that can lower blood pressure need to be discovered and confirmed one by one. Therefore, this manuscript hydrolysate porcine plasma and obtained a new ACE inhibitory peptide from the hydrolysates by Chromatography, HPLC and Q Exactive LC-MS/MS. Furthermore, *in vitro* digestion and spontaneously hypertensive rats (SHR) were used to ensure its functional stability and hypotensive effect *in vivo*.

2. Material and methods

2.1. Materials and chemicals

Fresh pig blood from several adult healthy pigs (6–7 months old) in local slaughterhouses was collected. 24 female spontaneously hypertensive rats (SHRs) were obtained from Beijing Vital River Laboratory Animal Technology Co., Ltd (Beijing, China) and housed in Animal Experiment Center at Zhejiang University of Traditional Chinese Medicine. The rats lived in the animal laboratory until rats increased to 150–170 g. Trypsin and pepsin were purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). The specific activity of trypsin and pepsin were greater than 1,000–2,000 BAEE units/mg solid. Angiotensin Converting Enzyme(ACE) from rabbit lung and N-Hippuryl-His-Leu hydrate (HHL) were purchased from Sigma-Aldrich (Shanghai) Trading Co.Ltd (Shanghai, China). The specific activity of ACE was greater than 2.0 units/mg protein. The purity of HHL is more than 98%.

2.2. Methods

2.2.1. Enzymatic hydrolysis of pig plasma

We were obtained fresh porcine blood from the slaughterhouse and 5% sodium citrate was added to prevent coagulation. The pig blood was centrifuged for 20 min at 4 °C to separate plasma and blood cells. The pig plasma was adjusted to pH 8 after 15 min in a 95 °C water bath, and then 0.2% alkaline protease was added to hydrolyze the mixture at 55 °C for 7 h, with enzymatic hydrolysates collected at 1, 2, 3, 4, 5, 6, and 7 h. The enzyme in the hydrolysate was inactivated by bathing in water at 95 °C for 15 min. After that, the hydrolysate was freeze-dried. The lyophilized hydrolysates were porcine plasma polypeptide powder.

2.2.2. Determination of structure

According to the methods of Li, Zhan, et al. (2020), the structural properties of proteins were analyzed using Fourier Transform Infrared Spectroscopy (FT/IR-4700, Jasco Corp., Tokyo, Japan). The dried plasma polypeptide powder was mixed with KBr powder in a ratio of 1:20. The FT/IR spectra were collected over the range of 500–4,000 cm − 1 with a resolution of 4 cm − 1. The obtained datas were analyzed with Peakfit (Version 4.12, Systat Software Inc., San Jose, California) to evaluate plasma polypeptider secondary structure.

2.2.3. Isolation, purification and identification of thepolypeptide

Isolation, purification and analytical procedures were based on the method of Zhan J. et al (2021). The peptide solution was firstly separated by G-15 gel chromatography (ZX-CXG-800Chromatography Cabinet, Shanghai Zhixin Experimental Instrument Technology Co., Ltd.). The Sephadex G-15 powder was mixed with plenty of water and soaked for 24 h. Pack G-15 Sephadex into a column(diameter: 1.6 cm, length: 60 cm, Shanghai UNTOP Instrument Co.Ltd). According to the chromatographic peaks, the peptide solution was divided into multiple components, and they were collected. The collected components were subjected to functional screening, and the most functional components were selected for separation and purification by HPLC (Agilent High Performance Liquid Chromatography 1260. Chromatographic column: Agilent HC-C18 4.5 mm*250 mm). The obtained components were subjected to functional screening again to selectfunctional components and the most functional component was identifiedby Q Exactive LC-MS/MS. The results of peptide sequences with strong signals and reliable protein sources were selected for synthesis in Sangong Biotech Co., Ltd (Shanghai, China). The purity of the synthetic peptides was greater than 98%.

2.2.4. ACE inhibition

The method from Siriporn et al (2018) with appropriate modification was used. 80 μL of 5 mmol/L HHL and 30 μL of porcine plasma polypeptide gradient solution were added to the centrifuge tube, placing 5 min at 37° C water bath. Then 40 μL of the ACE enzyme was added to incubate at 37° C water bath for 1 h. After incubation, 150 μL of 1 M hydrochloric acid was added to terminate the reaction. And then 700 μL water was added. 30 μL of ultrapure water was as a control group. During the reaction, 150 μL of 1 M hydrochloric acid was added immediately as a blank group after 40 μL of the ACE enzyme. The mixed liquid was filtered through a 0.22 μm aqueous phase filter, and the amount of hippuric acid produced was measured by high-performance liquid chromatography. The conditions of column (CAPCELIPAKCI-SAQS-546150 mm) were 30 °C, mobile phase A (water + 0.2% formic acid), mobile phase B (acetonitrile), flow comparison Example A: B = 85%: 15%, flow rate 1.0 mL/min, detection wavelength 228 nm, injection volume 100 μL, and analysis time 12 min.

The hippuric acid absorption peak appeared at about 6.4 min and the inhibition rate of the ACE enzyme was calculated according to the peak area of the hippuric acid absorption peak. This experiment was repeated three times. The calculation formula is:

$$\text{ACE inhibition rate}(\%) = \frac{(\text{V Control} - \text{V Sample})}{(\text{V Control} - \text{V Blank})} *100$$

Where:

V Control: horse uric acid absorption peak of the control group.
V Blank: horse uric acid absorption peak of the blank group.
V Sample: Horse uric acid absorption peak of the sample group.

2.2.5. In vitro digestion experiments

According to the method of Li et al (2021), with some modifications. 5 mL of the synthetic peptidesolution (1 mg/mL) was adjustedto pH 2.0. Then the 9,600 U/mL pepsin was added for incomplete digestion at a speed of 120 rpm/min for 1 h. After that, the mixture was adjusted to 7.0, and 12,000U/mL trypsin was added for further digestion at a speed of 120 rpm/min for 2 h. After the digestion, the enzyme was inactivated by boiling water bath for 15 min. Then, the functions were detected according to the methods of 2.2.3. This experiment was repeated three times.

2.2.6. SHR model experiments

24 SHR rats were randomly divided into 4 groups, 6 in each group. The SHR rats were synthetic peptide dose group given. We gave SHR rats the different doses of peptide solution (low dose 10 mg/kg, high 30 mg/kg), captopril (10 mg/kg), which were synthetic peptide group and

positive control group respectively, and the model control group (SHR rats) was given the same amount saline. Each rat was weighed once a week, and the amount of gavage was adjusted according to the weight. In the experiment, each rat's blood pressure was measured at 0, 2, 4, 6, and 8 h after gavage, including systolic blood pressure (SBH) and diastolic blood pressure (DBH).

2.3. Statistical analysis

Statistical analysis was performed by one-way analysis of variance (ANOVA), and significant differences were analyzed by Duncan multiple comparisons ($p < 0.05$) using software SAS8.1 (SAS Campus Drive, Cary, NC USA). The results were considered statistically significant at $p < 0.05$.

3. Results and discussion

3.1. Selection of enzymatic hydrolysis time

Plasma proteins contain many proteins, such as hemoglobin, fibrinogen, and so on, which have hypotensive function, especially its hydrolysate (Bernardini et al., 2011). Enzymatic hydrolysis can destroy the peptide bond of protein to produce peptides, including some amino acid residues, which play mean role of function. Additionally, hydrolysis degree (DH) can influence the length and contents of peptides containing some groups with function, such as O–H, C = O and C-O groups (You et al., 2009). To isolate hypotensive peptides with low molecular weight, high activity and easy absorption better, the enzymolysis time should be ensured for high hydrolysis degree and more small peptides. Table 1 shown the hydrolysis degree of hydrolysates raised with the enzymatic hydrolysis time, and reached the maximum after enzymatic hydrolysis 5 h. Moreover, Fig. 1 and Table 1 also shown that hydrolysis could produce small peptides (<8 kDa). The content of peptides increased and the length of peptides decreased respectively, when hydrolysis time increasing until enzymatic hydrolysis time reached 5 h. Additionally, hydrolysis did not eliminate some active groups (Fig. 2), like O–H, C-O and C = O groups represented by the absorption peak at 3289.12 cm^{-1}, 1129.43 cm^{-1} and 1695.78 cm^{-1}, respectively. Therefore, the hydrolysate of 5 h enzymolysis was used to separate hypotensive peptide.

3.2. Isolation and identification of ACE inhibitory peptide

ACE has two active binding sites, one of them which contains Zn^{2+} is the necessary binding site of the influentialgroup of the ACE inhibitor. The activity of ACE will disappear if they bind. Fig. 3c shown the hydrolysate had better ACE inhibition (91.32%), which caused by that the enzymatic hydrolysis released some active amino acid and exposed some residues with some groups, like carboxyl, which could combine with Zn^{2+}, inactivating ACE, which could inhibit the effect of ACE to lower blood pressure. Moreover, some hydrophobic amino acids of peptides produced by hydrolysis could also bind to the catalytic site of ACE and influenced the effect of enzyme, leading to inhibition of ACE (Wijesekara and Kim, 2010; Alemán et al., 2011).

Table 1
Hydrolysis degree and molecular weight of Hydrolysates.

Hydrolysis time (h)	Hydrolysis degree (%)	Mn	Mw
1	10.34 ± 0.36[e]	5749	5898
2	14.23 ± 0.44[d]	5609	6256
3	20.06 ± 0.56[c]	5518	6804
4	25.66 ± 0.66[b]	5507	6947
5	32.93 ± 0.58[a]	5451	6991
6	33.45 ± 0.72[a]	5442	6967
7	33.96 ± 0.61[a]	5414	7060

Note: Different letters represent significant difference ($p < 0.05$); Mn represents average molecular weight, Mw represents peak areas.

After separation, the results (Fig. 3a) shown two fractions P1 and P2. In these two groups, P1 had better ACE inhibitory capability (82.72%) than that of P2 (63.39%) from(Fig. 3c). Therefore, based on the current experimental results, P1 was further separated by HPLC. As a sensitive and rapid method, HPLC can separate smaller molecular weight peptide with molar weights below 1 kDa (López García et al., 2006), which was used to isolate P1. Fig. 3b shown 9 components P1-1 to P1-9 were isolated and the P1-8 had the best ACE inhibition (97.37%) in the 9 components (Fig. 3c). To further explore the relationship of peptides structure and ACE inhibition ability, Q Exactive LC-MS/MS was used to identify the peptide sequence of P1-8.

Q Exactive LC-MS/MS can rapidly resolve peptide components and identify their structure and sequence by ion source, electricfieldandmagneticfield (Shazly, et al., 2017). Q Exactive LC-MS/MS analyzed the component P1-8. After analysis, peptide with 10 determined amino acids identified by Q Exactive LC-MS/MS was synthesized (Fig. 4a and b). Moreover, it showed better ACE inhibition capability (76.59%) in Fig. 4c. Since the active site of ACE cannot accommodate large peptides, the length of amino acid residues could influence ACE inhibition, Blanca et al. (2011) also had reported that peptide with 2–12 amino acids could play mean role of ACE inhibition. While the sequences and species of amino acid in peptides also affected the ACE inhibition (Wang et al, 2017). The presence of hydrophobic amino acids phenylalanine (F), leucine (L), isoleucine (I), proline (P), which could combine with ACE to inhibit its activity (Wijesekara and Kim, 2010). Gu and Wu (2013) also had reported 5 tripeptides IVF, LLF, LNF, LSW, LEF containing hydrophobic amino acids had high ACE inhibitory activity by using Q Exactive LC-MS/MS combined with quantitative structure–activity analysis.

Furthermore, the stereo conformation of peptide also could influence the combination of peptide and ACE and the ACE inhibitory peptides mainly combined with the amino acid residues in the active center of the ACE molecule, such as Glu384, Tyr520, His387, etc. by hydrogen bonds (Wang et al, 2017). The different sequences of peptides could influence the effect of the combination of peptide and ACE.Rawendra et al (2014) conducted simulated molecular docking of turtle protein-derived ACE inhibitory peptide IVR and found that the role of hydrogen bonds between the C-terminus of IVR and the amino acid residues Lys511, Tyr520 of tACE molecule were related to its degree of inhibition. Li et al (2014) found that the amino acid residues Arg522 and Asp358 of ACE could form stable hydrogen bonds with the ACE inhibitor peptide ACKEP. Zhou et al (2012) found that LKP and IKP, both as tripeptides, had different ACE inhibition rates of L and I amino acid residues at the N-terminus and cACE, which affected the interaction between peptides and ACE. Therefore, the hydrophobic amino acids L appeared at C-terminal (Fig. 4b), which might better combine with the amino acid residues of ACE, playing the role of inhibiting, and Paiva et al.(2016) also found the same conclusion.

Additionally, the amino acid F, P with imidazole ring and K, L with –NH$_2$ and –CH$_3$ groups could provide hydrogen atom to form hydrogen binding, enhancing the stable of combination between ACE and IFPPKPKDTL. Moreover, D had carboxyl which could inhibit ACE by binding to Zn^{2+}. In addition, aromatic amino acids K, L and acidic amino acid D also could chelate with other peptides and metal ions (Jae-Young et al, 2007), which could result in that peptide chelate combining with other binding sites of ACE played better steric hindrance to the binding of angiotensin I and ACE to prevent ACE from working. Wang et al (2017) also reported the 21-peptide derived from tuna had much higher ACE inhibitory activity than tripeptides derived from rapeseed oil, which might cause by that macromolecules could play a steric hindrance to ACE.

3.3. ACE inhibitory properties of IFPPKPKDTL after digestion

The ACE inhibitor peptide can exert the hypotensive effect in the body if it enters the bloodstream in an active form. Thus, peptide needs
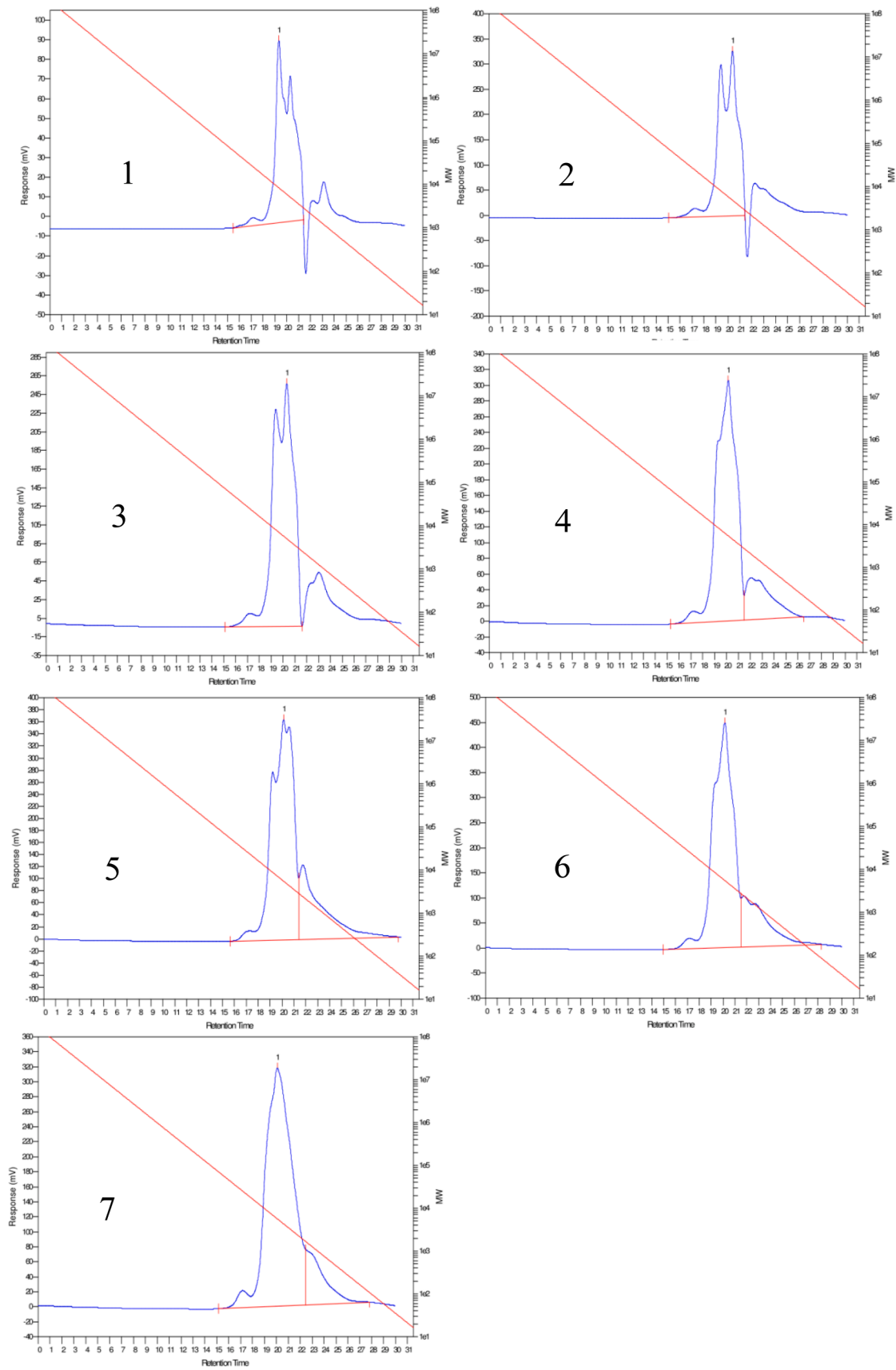
Fig. 1. The GPC of porcine plasma hydrolysates. Note: 1, 2, 3, 4, 5, 6, 7 represent the enzymatic hydrolysis time (h).
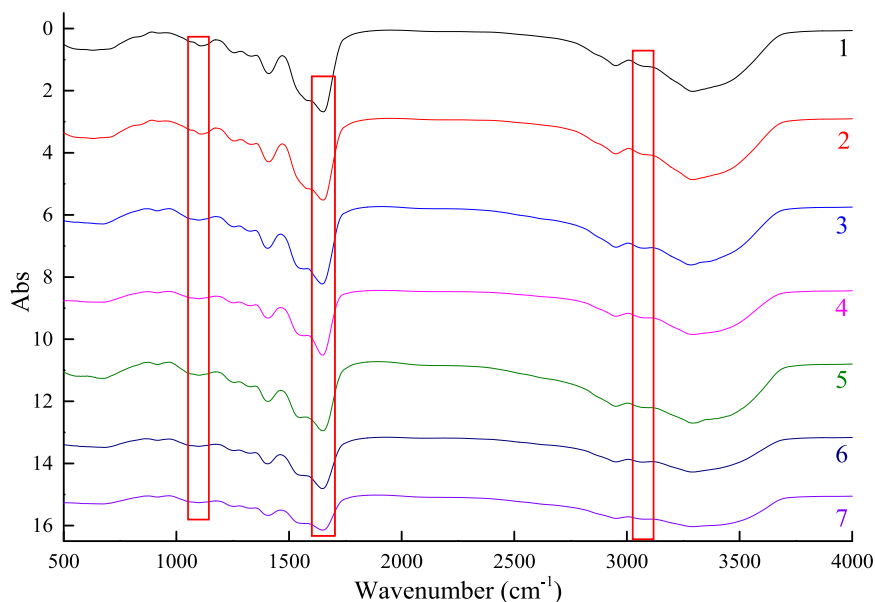
Fig. 2. FT/IR spectrum of different enzymatic hydrolysis time. Note: 1, 2, 3, 4, 5, 6, 7 represent enzymatic hydrolysis 1, 2, 3, 4, 5, 6, 7 h respectively.

to resist the hydrolysis of gastrointestinal enzymes and maintain its biological activity through the intestinal wall after oral administration (Escudero et al, 2010). Thereby the biological activity of peptides can be activated or inactivated due to the degradation of enzymes. After *in vitro* digestion, IFPPKPKDTL shown better ACE inhibitory capability (88.53%) than undigested peptide (76.59%) in Fig. 4c, meaning that the ability of ACE inhibition of IFPPKPKDTL was stable in the digestive environment of the gastrointestinal tract. The peptide bond composed of aromatic amino acids or acidic amino acids were the main part of pepsin action, while the trypsin specifically acts on the peptide bond composed of essentialamino acids arginine and lysine (K) carboxyl groups (Wen et al, 2015). Thereby, pepsin could destroy the peptide binding of F, P and D, while trypsin could destroy that of K, and the smaller peptides IFP, PK, DTL and free amino acids P, K, D might produce to continue to play the role of ACE inhibition due to the dissociation of IFPPKPKDTL. Moreover, tripeptides or dipeptides could better bind to the active site of ACE (Blanca et al., 2011) and free amino acids P, K, D had higher mobility, which could better provide hydrogen atom and chelate with other amino acids and Zn^{2+} (Jae-Young et al, 2007) to inhibit ACE activity. Stuknytė et al. (2015) had reported the type and number of ACE-I peptides in cheeses changed from VPP, IPP, RYLGY, RYLG, AYFYPEL, AYFYPE, LHLPLP and HLPLP to only VPP, IPP, HLPLP and LHLPLP after *in vitro* static gastrointestinal digestion, which meant the digestion could enhance or eliminate the ACE inhibitory capability of the peptide by dissociation. Escudero et al (2014) also had reported the ACE inhibitory peptide IAGRP of Spanish dried ham retained almost the same ACE inhibitory activity before and after *in vitro* digestion, which caused by the peptide was partially degraded into smaller peptides, maintaining antihypertensive biological activity. Hwang (2010) also had reported that small ACE inhibitory peptides obtained from tuna cooking juice still could maintain the activity after digestion.

3.4. Hypotensive effects of peptide IFPPKPKDTL under SHR model

SHR is recognized internationally as the most similar animal model of hypertension to humans, especially in cardiovascular complications, the pathogenesis of hypertension (Yang et al, 2019). Therefore, SHR can be used to select and develop antihypertensive drugs. Fig. 5a showedthatthe positive control captopril (10 mg/kg) could decrease systolic blood pressure (SBP) and diastole blood pressure (DBP) after SHR took it

for 2 h. Moreover, the antihypertensive effect reached maximum after 4 h that the SBP and DBP decrease by 10.52% and 14.91% respectively, while the effect began to weaken after 6 h.However, the peptide IFPPKPKDTL (30 mg/kg) could lower SHR blood pressure and reached the maximum effect after 6 h, and the effect of the peptide was better than that of Captopril. Moreover, the best hypotensive effect of the peptide (30 mg/kg) with 6 h improved by about 33.33% in SBP and DBP, comparing with Captopril (10 mg/kg) with 4 h, which indicated that the peptide IFPPKPKDTL had the hypotensive effect comparable to the positive control Captopril. Moreover, IFPPKPKDTL could be digested to smaller peptides and free amino acids by the gastrointestinal tract and quicklyenter the blood, enhancing its function (Fig. 4c). Additionally, blood pressure was mainly regulated by the renin-angiotensin aldosterone system (RAAS), which contained two antagonistic pathways activated by ACE and ACE2: ACE-AngII-AT-1R and ACE2-Ang- (1–7) -MAS-R, which played the opposite role in maintaining cardiovascular homeostasis. ACE can convert AngI into the vasoconstrictor AngII, mediating its biological function by binding to two G protein-coupled receptors: AT1 and AT2. The receptor AT1 and AT2 are related to vasoconstriction and vasodilation, respectively. While ACE2 can produce the vasodilator Ang-(1–7), inhibiting AngII-induced vasoconstriction by binding the Mas receptor, lowering blood pressure (Wu et al., 2016). The peptide IFPPKPKDTL with the hypotensive effect might be caused by that it might bind to AngII, inhibiting the vasoconstriction induced by the combination of AT1 and AngII. Peptides RPYL from lactoferrin could also lower blood pressure as an AT1 receptor antagonist, reported by Fernandez-Musoles et al (2013). In addition, IFPPKPKDTL also could decrease ACE (Fig. 4c), lowing blood pressure by inhibiting the role of the pathway ACE-AngII-AT-1R. SAGGYIW and APATPSFW from wheat gluten could regulate ionic and hydrophobic interactions at the catalytic site of ACE, decreasing the ACEactivity to lower blood pressure, which had been reported by Zhang et al (2020). He et al (2017) also found dendrobium flowers aqueous extract could inactivate ACE to decrease AngII, lowering blood pressure. Tao (2019) also had reported synthetic ACE polypeptides from Chinese Wolfberry could regulate blood pressure by reducing ACE to weaken the effect of the pathway of ACE-AngII-AT-1R.
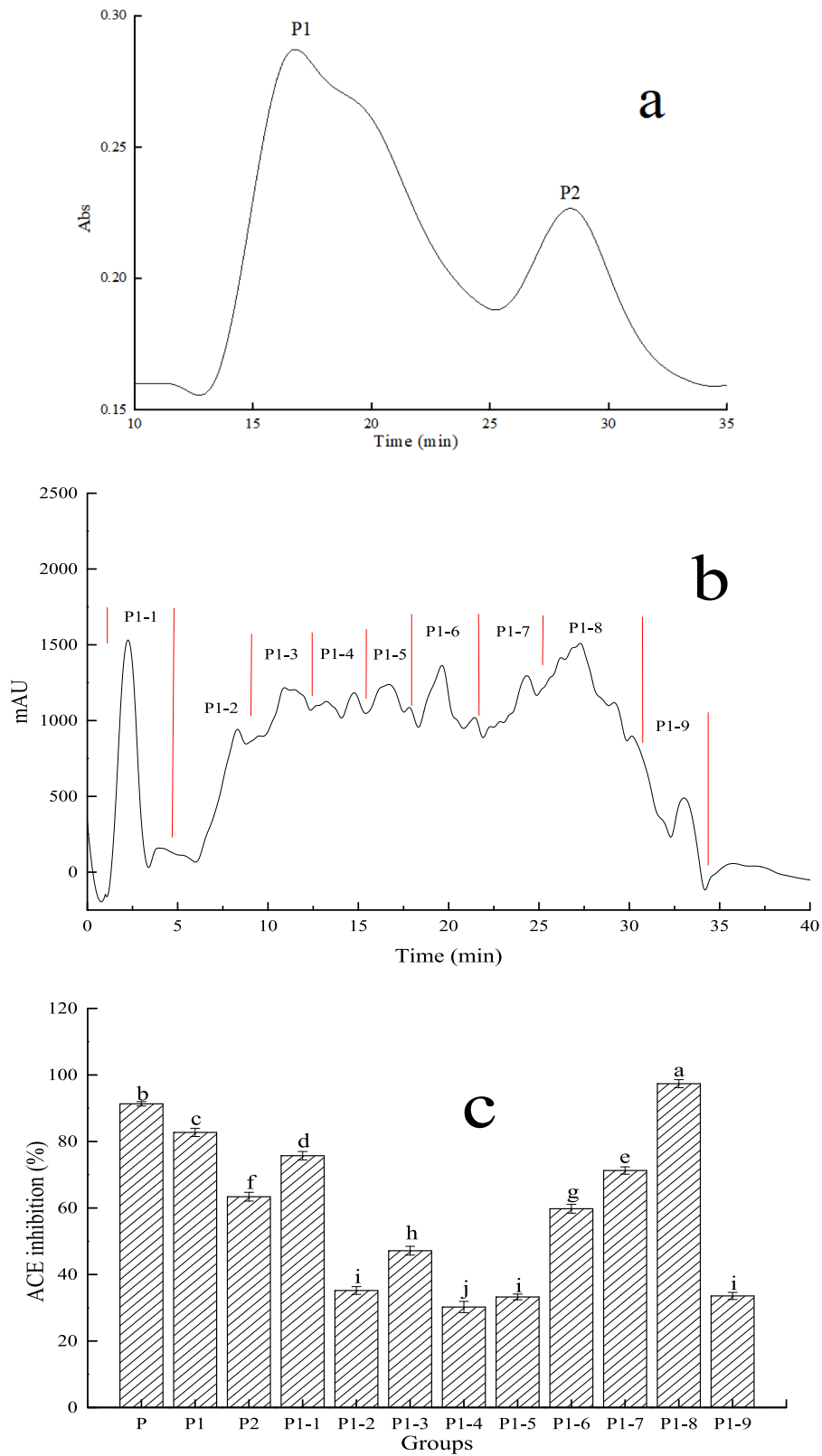
Fig. 3. ACE inhibition of peptide after chromatography and HPLC. Note: Different letters represent significant difference ($p < 0.05$); In the Figure a, b and c, P1, P2 represent the components corresponding to the different absorption peaks of chromatography; In the Figure c, P represents hydrolysate after 5 h enzymatic hydrolysis. P1-1 to P1-9 represent the components corresponding to the different absorption peaks of HPLC of P1.
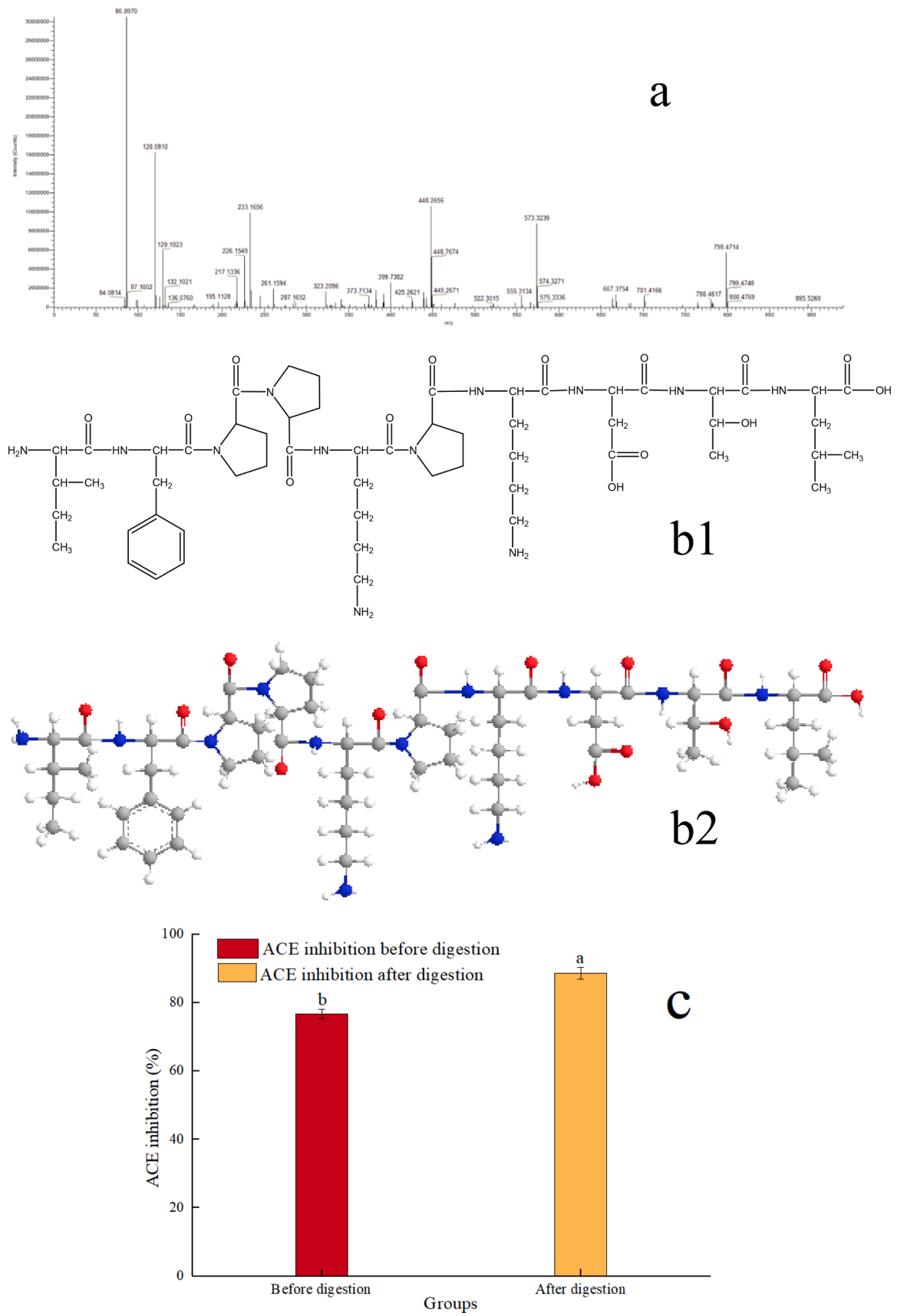
Fig. 4. ACE inhibition of synthetic peptide IFPPKPKDTL before and after digestion. Note: Different letters represent significant difference ($p < 0.05$).
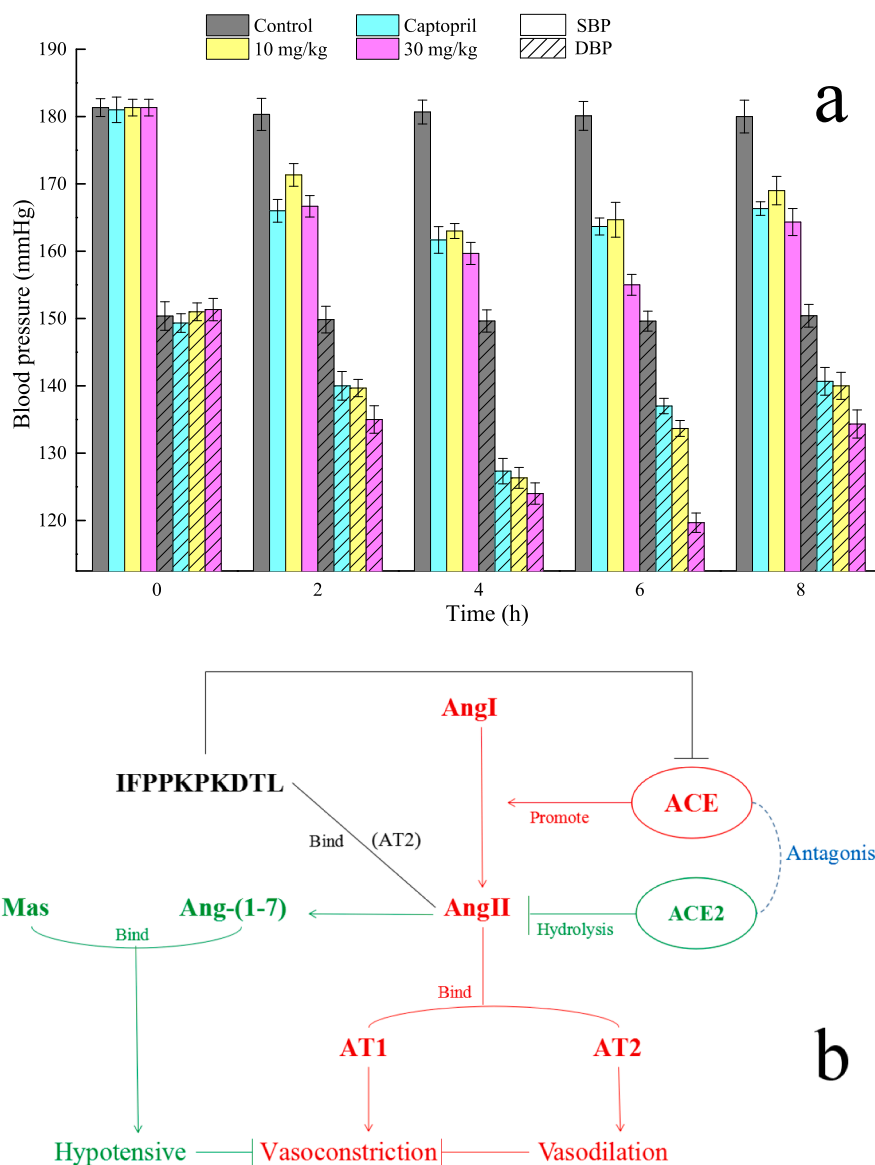
Fig. 5. Effect of samples on blood pressure of SHR. Note: Figure a is the Changes in blood pressure within 8 h of intragastric administration in rats. Figure b explains the mechanism by which peptides lower blood pressure.

4. Conclusion

The research has shown that the porcine plasma hydrolysate had a good ACE inhibitionrate (91.32%) due to active amino acids and carboxylbinding to Zn^{2+} to inactivate ACE. The fraction P1 from PPH also had better ACE inhibition (82.72%) in the two fractions isolated by chromatography. Moreover, the P1-8 with the best ACE inhibitory capability (97.37%) in all components separated by HPLC was purified. Additionally, the IFPPKPKDTLwas identified and synthesized from the component P1-8, whichhad a good ACE inhibition rate (76.59%) due to hydrophobic amino acids and aromatic amino acids providing hydrogen atom to enhance the combination of peptide and ACE. Moreover, it still showed ACE inhibitory properties (88.53%) after *in vitro* digestion because IFPPKPKDTL could be hydrolyzed to smaller active peptides and free amino acids. It (30 mg/kg) could also decrease the SBP and DBP of SHR to play the role of hypotensive effect by might binding to AngII to produce vasodilation and decrease the level of ACE. Thenovel type of ACE inhibitory peptide could be potentially used in formulating functional foods.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author Contributions

In the course of this research, Junqi Zhan was responsible for the separation, purification and functional evaluation of samples. Gaoshang Li is responsible for rat experiments and data processing. Professor

Daodong Pan and Professor Yali Dang guided the writing and analysis of this manuscript.

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