



Peptidomic Analysis of ACE Inhibitory Peptides Extracted from Fermented Goat Milk

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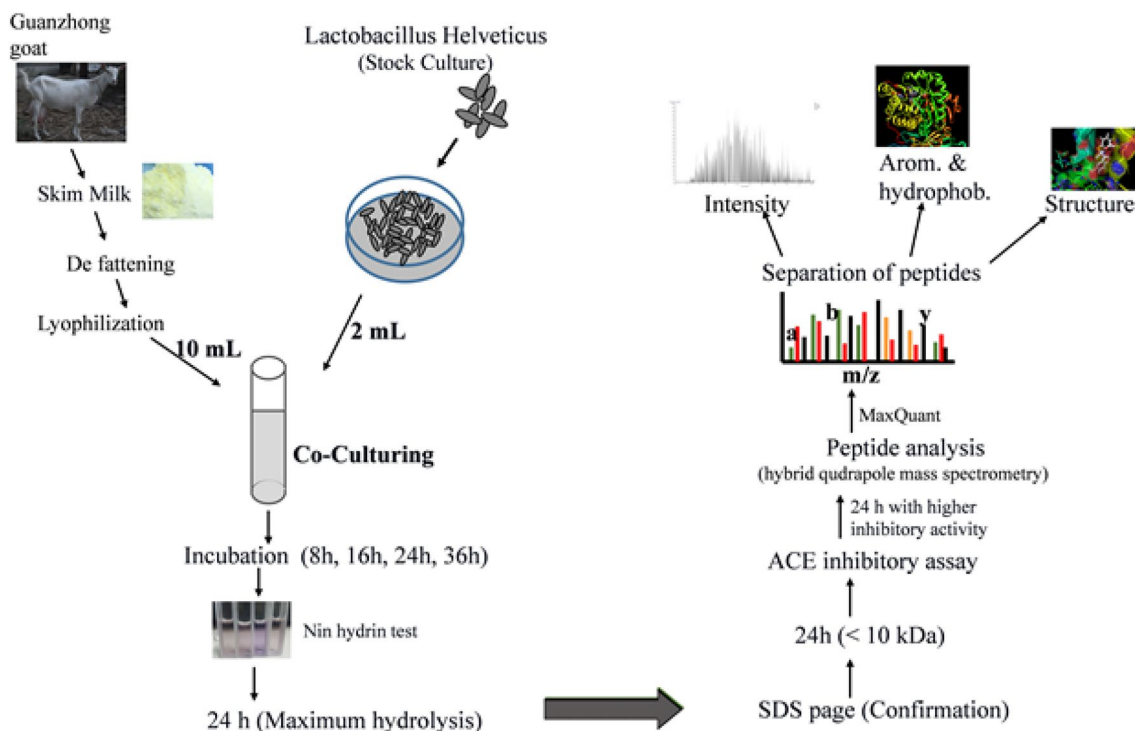
Abstract

Angiotensin converting enzyme (ACE) is considered as main causative agent in growing hypertension and other cardiovascular disorders. Inhibition of ACE by producing and purifying bioactive peptides of fermented goat milk is aimed in this study. Protein extracted from goat milk was hydrolyzed with proteolytic enzymes of LH (*Lactobacillus helveticus*-cicc22171). ACE inhibitory peptides were purified from fermented samples of goat milk protein by optimizing incubation time to 8 h (S-8), 16 h (S-16), 24 h (S-24) and 36 h (S-36), via ultrafiltration. S-8 was used as control to compare the ACE inhibition trend. Molecular weight cut-off; 10000 Da (PM-10) and Ultracel 3K membrane was used to perform ultrafiltration. Sample with 24 h incubation time was considered as best hydrolyzed as compared to others, by applying Nin-Hydrin reaction and SDS-PAGE analysis. ACE inhibitory assay validated the authenticity of S-24 in inhibiting ACE, in vitro. Furthermore, Q executive hybrid quadrupole-orbitrap mass spectrometry was used to determine molecular structure and amino acid sequence of ACE inhibitory peptides. Three peptides, VLPVPQKAVPQ, VLPVPQKVVPQ and TQTPVVVPPFLQPEIMGVPKVKE containing functional amino acid structure, has been identified with highest ACE inhibitory activity on the basis of intensity, size and higher concentration of hydrophobic amino acids as shown in figure as graphical abstract. Fermented goat milk containing these novel bioactive peptides, can be used as nutraceuticals to inhibit ACE and control hypertension in future.

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Graphical Abstract



Keywords ACE-inhibitory peptides · Hypertension · Identification · Purification · Fermentation · Goat milk

Introduction

The renin-angiotensin system (RAS) is a key player in maintaining the cardiovascular health with a continuous series of enzymatic reaction. Angiotensin I is produced in the blood as a result of cleavage in hepatic peptide angiotensinogen by renin. Angiotensin converting enzyme is main player to hydrolyze Ang I into Ang II and this Ang II increases vasoconstriction, inflammation, and oxidative stress in the vascular system via activation of AT₁R (Bader and Ganten 2008). Angiotensin I-converting enzyme (ACE; kininase II; EC 3.4.15.1) is considered as main root cause of hypertension that is carboxy-dipeptidyl-metallopeptidase in nature and main linked enzyme of renin angiotensin system. It was first discovered in horse plasma, and spotted as an enzyme that was responsible to convert Ang I to Ang II (Skeggs et al. 1954; Helmer 1957). It was detected in human plasma a decade later (Yang and Erdös 1967). It is mainly involved in regulation of peripheral blood pressure along with catalyzes the production of Ang II from Ang I (a vasoconstrictor) and inactivation of the vasodilator bradykinin (Gobbetti et al. 2000; Kiom et al. 2004). Recently, ACE2 was discovered in RAS as a homologue of ACE that is responsible to breakdown Ang I into Ang 1–9 and Ang II into Ang 1–7, while

Ang 1–7 is multifunctional bioactive peptide that works as ACE inhibitor. ACE2 is capable of hydrolyzing Ang 1–9 on one hand and generation of Ang 1–7 on the other hand (Mercurio et al. 2008; Oudit and Penninger 2011; Alenina et al. 2008; Bader 2013).

Commonly, synthetic ACE inhibitors like captopril, enalapril, fosinopril, lisinopril, and ramipril are used to minimize the severity of hypertension but these synthetic inhibitors are encrypted with some side effects. So, It is need of hour to explore the natural resources of ACE inhibitors with some health promoting properties (Agostoni and Cicardi 2001; Chen et al. 2013). It has been known long ago about the importance of diet for human health. Recent studies confirmed the role of proteins especially milk proteins in reducing diseases (Groziak and Miller 2000; Das 2001). Milk bioactive peptides are considered as substitute of those synthetic inhibitors and it was reported that hydrolysis of milk protein can produce potent ACE inhibitory peptides (Yamada et al. 2013). Among other dairy species, goat milk is considered as a better choice in the production of ACE inhibitory peptides after fermentation (Parmar et al. 2018).

Recent debate among scientific community regarding exploitation of lactic acid bacteria has gained popularity due to proteolytic activity of LAB in producing bioactive

peptides and functional food (Gobbetti et al. 2002). Proteolytic system, encrypted in Probiotic Lactobacillus, is responsible to break down protein into small peptides. In this way, Lactobacillus are involved in breaking down protein into small peptides during fermentation (Kilpi et al. 2007). When 28 strains of lactobacillus were evaluated to analyze best ACE inhibition in-vitro, only four strains including *L. helveticus* Showed remarkable ACE inhibitory activity (Chen et al. 2012).

Bioactive peptides from bovine milk are not a topic of interest for most of the scientific community due to known fermented profile of peptides and its effect. Now it is time to explore alternative sources like goat or sheep milk to extract bioactive peptides (Bernacka 2011). Due to difference in casein contents, goat milk is considered better than cow milk (Park et al. 2007).

As a second largest source of milk in the world after bovine milk, goat milk is rich in protein, fat, minerals, vitamins, and other nutrients as compared to bovine milk. Its protein profile is considered as close to the human milk due to abundance of smaller protein particles and short chained fatty acids (Belewu and Aiyegbusi 2002; López-Aliaga et al. 2010; Haenlein 2004). This study was designed to prevent the health loss due to cardiovascular diseases especially hypertension, with the introduction of functional food in daily life that can minimize the severity of hypertension. To meet the criteria of this study, fermented goat milk (hydrolyzed by LH in reducing hypertension with preconditions of optimizing incubation time) was used to produce and purify ACE inhibitory peptides on the basis of peptides structure, intensity of peptides in the fermented milk samples and concentration of aromatic and hydrophobic peptides.

Materials and Methods

Materials

Hippuryl-L-histidyl-L-leucine (Hip-His-Leu), purified rabbit lung ACE were purchased from Sigma Chemical Co. Ltd. Real Band 3-color highz Range Protein Marker (9–245 kDa) of reagent grade was purchased from BBI Life Sciences (Sangon Biotech Co. Ltd). Laboratory of Food Sciences (Beijing Forestry University) kindly donated stock culture of LH.

Ethics Statement

All policies mentioned in the code of ethical committee (Beijing Forestry University) for the use and care of animals, were adopted. Animal ethics committee of Beijing Forestry University approved the study design.

Furthermore, Livestock department of Shaanxi province provided resources and manpower for milking.

Milk Collection and Preparation of Samples

Fresh Goat milk from apparently healthy and uninfected guanzhong goats was collected from guanzhong area of Shanxi Province in China. Guanzhong and Xinong Saanen breeds are leading dairy goats in China mainly due to population and milk yield. While, Guanzhong goat is a crossbreed of Xinong Saanen and local white goat of China. Its population is more than any other dairy goat breed reared in China. Concentration of milk protein in guanzhong goat is 3.52% while in Xinong Saanen dairy goat is 3.28%. Guanzhong goat was selected on the basis of its population and greater concentration of milk protein (Xu et al. 2003). Disinfectant solution (Safflon 20%) was used to wash the udder before milk collection. Milking was done in the daytime, 2 h after offering animals total mixed ration. Autoclaveable plastic containers (1000 mL) were used for the collection of samples and sterilized at 121 °C for 20 min (Omer and Eltinay 2008). Whole milk was converted into skim milk by centrifugation at 4000×g for 10 min at 15 °C and then stored at – 20 °C. Freeze-drying (Lyophilization) of skim goat milk was performed by Detianyou FD-1 Freeze Dryer (China Based Company). The freeze-dryer was programmed to operate at – 50 °C with a vacuum pressure of 20 pa (Pascal). After the end of freeze-drying cycle, dried milk was sealed under vacuum and stored at 5 °C until further analysis (Ivanova 2011).

Department of Food Sciences at Beijing Forestry University maintained stock culture of LH. Before experimental use, De Man, Rogosa and Sharpe (MRS) culture medium was prepared and stock culture was propagated for 16 to 18 h at 37 °C. Hemocytometer was used to calculate bacterial colonies and cells were harvested by centrifugation at 6000×g for 10 min at 4 °C and washed three times with distilled water.

Goat milk Protein was co-cultured with the LH (1×10^9 cfu/mL) at the ratio of 10:2 mL respectively. Moreover, Samples were incubated (37 °C) for the interval of 8, 16, 24 and 36 h.

Incubation temperature of LH was optimized at 37 °C because temperature was identified an important factor with higher influence on lactobacillus growth and activity of proteolytic enzyme (Shu et al. 2017). All experiments and co-culturing was done in sterile environment to avoid any contamination by surrounding environment.

Furthermore, samples were centrifuged at 15,000×g for 30 min and supernatant was filtered by using 0.45 µL (microliter) filter tip and stored at – 20 °C for further analysis.

Degree of Hydrolysis

Nin-Hydrin reaction was applied to estimate the degree of hydrolysis of protein into peptides. To prepare 20 mL solution, reagents were prepared according to standard protocol with 2 g (gram) of $\text{Na}_2\text{HPO}_4 \cdot 10\text{H}_2\text{O}$, 1.2 g $\text{NaH}_2\text{PO}_4 \times 2\text{H}_2\text{O}$, 0.1 g Nin-Hydrin and 0.06 g Fructose.

Each sample (S-8, S-16, S-24 and S-36) with the quantity of 0.05 mL was added into 15 mL of deionized water to dilute it further; 0.5 mL aliquot was taken and mixed with 1 mL reagent solution. Mixture was allowed to heat on 100 °C for 15 min. After heating, the color of samples turned into purple and its wavelength was examined at 570 nm by using Spectrophotometer (PGENERAL T-6 New century, China).

SDS-PAGE (Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis)

Gel electrophoresis was performed in a 10% separating gel along with 12% stacking gel. Separating and stacking gel was prepared according to standard protocol devised by Laemmli (1970). Electrophoretic separations was carried out by using a Bio-Rad Mini-Protein II Electrophoresis Cell System with a constant power supply of 18 mA/gel for 130 min after adding 15 μL samples into each well.

Polyacrylamide gel was stained with 0.25% (w/v) Coomassie R-250 Blue in 50% (v/v) methanol and 7.5% (v/v) acetic acid for 15–30 min (Laemmli 1970). Gel was destained in 20% (v/v) methanol and 7.5% acetic acid.

Ultrafiltration

Fermented goat milk peptides were divided into large and small (< 10,000 Da) molecular weight fractions through ultrafiltration. Extraction of bioactive peptides was performed according to the Jang and Lee method with some modifications (Jang and Lee 2005). Briefly, fermented milk samples were centrifuged at 10,000 $\times g$ for 20 min at 4 °C. The supernatant was ultra-filtrated by PM-10 membrane, (molecular weight cut off 10,000; Millipore Corp., Billerica, MA) by optimizing temperature at 4 °C. Samples were centrifuged again, at 4000 $\times g$ for 45 min at 4 °C and filtered using an Ultracel 3K membrane (MWCO, 3000; Millipore Corp., Billerica, MA).

Assay for ACE Inhibitory Activity

ACE inhibitory activity of the goat milk peptides was measured according to Cushman and Cheung (1971) with

some modifications. Briefly, 50 μL of each sample (5 mM Hip-His-Leu in 0.1 M borate buffer containing 0.3 M NaCl, pH 8.3) was incubated with 50 μL of ACE at room temperature for 10 min. To this mixture, 200 μL of 5 mM Hip-His-Leu was added and incubated at 37 °C for half an hour. The reaction was then stopped by the addition of 1 N HCL weighing 200 μL . By the addition of 500 μL ethyl acetate, released hippuric acid was extracted and remaining ethyl acetate was removed by evaporation. Moreover, hippuric acid was redissolved in 1 mL distilled water and measured spectrophotometrically at 228 nm. Each sample was tested in triplicate. Without protein hydrolysate, the mixture was used as control.

$$\text{ACE inhibition\%} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100$$

Identification of Peptides

Q executive hybrid quadrupole-orbitrap mass spectrometry was used to identify peptides in the sample. This is a bench top LC-MS/MS system combines quadrupole precursor ion selection with high-resolution, accurate-mass Orbitrap detection; mass range from 50 to 6000 m/z and scan range up to 12 Hz. Chromatographic separations were performed with Acclaim™ PepMap™ 100 C18 LC Columns (300 μm i.d. \times 5 mm, packed with Acclaim PepMap RSLC C18, 5 μm , 100 Å, Nano Viper, Acclaim PepMap 75 μm \times 150 mm, C18, 3 μm , 100Å). Thermo Scientific™ Acclaim™ PepMap™ 100 C18 LC Column can produce high-resolution analysis of all type of peptides. Along with LC-MS/MS, these columns are often used for protein identification, decoding amino acid sequence in protein and systems biology. Equipped With the property of high loading capacity, these columns are right choice for the analysis of low abundant peptides in complex proteomics samples.

The mobile phase consisted of 0.1% formic acid in pure water (Solvent A) and 0.1% formic acid, 80% ACN (Solvent B), formed with gradient elution with following parameters: 0–5 min, 0–5% B; 5–25 min, 5–5% B; 25–30 min, 5–50% B; 25–30%, 50–90% B; 30–35 min, 90% B; 35–45 min, 5% B. The total run time was 40 min with a constant flow rate of 300 nL/min.

Some parameters in Orbitrap were as follows: spray voltage, 2.0 kV; capillary temperature, 250 °C; m/z (mass to charge ratio) range (ms), 350 to 1800. AGC (Automatic gain control) ion injection targets for first level FTMS (Fourier Transform Mass Spectrometry) scan were 70,000 (40 ms max injection time) with 3e6 AGS target and for second level mass spectrometry were 17,500 (60 ms max

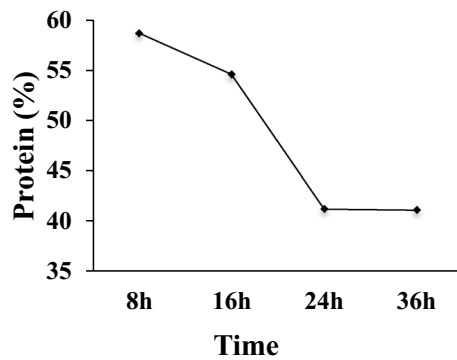


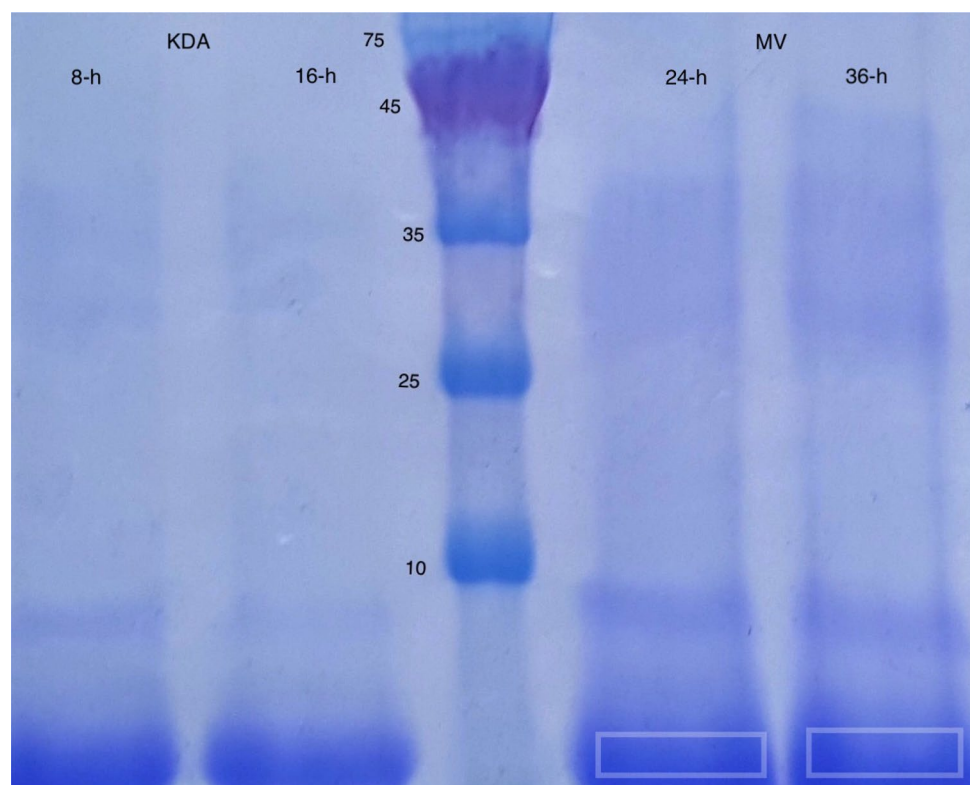
Fig. 1 Time period was indicated in hours on x-axis while y-axis represents percentage of protein hydrolysis. Protein percentage declined from 60 to 40% with the passage of time that indicates the hydrolysis of protein into peptides by the increasing colonies of LH. Four biological replicates were used for each sample and then average percentage of each sample was plotted on this graph according to increase in fermentation time

injection time) with $1e5$ AGS target; 27NCE (normalized collisional energy) and 20 TopN.

Database Search

The original mass spectrum file was processed by MM File Conversion software, and then used MaxQuant to search the database.

Fig. 2 Molecular weight (MW) of peptides was indicated in kDa while samples with 8 h and 16 h incubation showed the presence of small peptides with less concentration while samples with 24 h and 36 h incubation period showed greater concentration of small peptides with molecular weight less than 10 kDa. White small rectangle shapes were used to highlight the specific area where concentration of small peptides was high after 24 and 36 h of fermentation



Results and Discussion

Protein Hydrolysis and Peptides Purification

Main Aim of this study was to purify and identify Small peptides after hydrolysis of protein. To verify that proteins are hydrolyzing into peptides, Nin-Hydrin reaction was applied because it is widely used to analyze amino acids, peptides, and proteins in food and microbiological studies (Friedman et al. 1984; Pearce et al. 1988). Enzymes of LH were used to break down the protein into peptides. Degree of protein degradation into peptides by hydrolysis is called degree of hydrolysis (DH). It is one of most authentic mechanism to measure the hydrolysis process (Adler-Nissen 1979). It is also used as indicator of comparing different protein hydrolysates (Mahmoud 1994). Protein concentration decreased with its degradation into peptides as the incubation time rises and maximum hydrolysis was observed after 24 h. Degree of hydrolysis determined at various incubation times with LH is shown in Fig. 1.

Results of DH show the gradual decrease in protein contents from 8 to 24 h and it remains almost constant from 24 to 36 h, with negligible difference in protein degradation. It can be assumed on the basis of DH that 24 h were enough to hydrolyze milk protein with the help of LH enzymes.

SDS-PAGE was applied as next step to quantify the molecular mass of hydrolyzed protein. SDS-PAGE was

mainly applied to determine the molecular mass of the hydrolyzing peptides, just like it was used in previous studies (Garcia-Mora et al. 2015). A representative SDS-PAGE of the samples with different incubation time is shown in Fig. 2. This result endorses the outcome of Fig. 1.

Major changes while performing SDS-PAGE included decrease in several bands indicated greater enzymatic breakdown by 24-h incubation. Samples with 24 h and 36 h incubation time, represented almost same picture of hydrolysis that indicated importance of 24-h incubation sample.

Moreover, filtration of samples with PM-10 membrane (molecular weight cut-off; 10 kDa) was done to further purify ACE inhibitory peptides. Samples were lyophilized after purification and concentrated in distilled water. Different steps in separation and purification techniques were recommended in previous study, were followed (Jinyu 2006). For ultra-filtration, Ultracel 3K-membrane was used (under 3000 Da) and small peptides were purified. Membrane technology is used to purify peptides on larger scale.

ACE Inhibition Pattern

All four samples were tested to evaluate their potency to inhibit ACE production by applying ACE assay, in vitro.

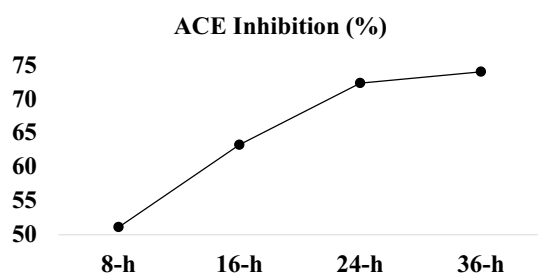


Fig. 3 Time period in hours, was indicated on x-axis while Y-axis represented the ACE inhibitory percentage with increasing incubation time. Samples were mixed with their replicates and four samples (D-8, D-16, D-24 and D-36) were prepared. Each sample was tested in triplicate to check their effect in the inhibition of ACE in-vitro

Fig. 4 The MS/MS spectrum of charged ions with m/z 1267.72 (TQTPVVVPPFLQPEIMGVPKVKE), 459.76 (TLTDVEKL), 588.36 (VLPVPQKAVPQ), 651.32 (REQEELNVVGE) and 602.38 (VLPVPQKVVVPQ) have been identified in a–e. The MS/MS spectrum was acquired with Q executive hybrid quadrupole-orbitrap mass spectrometry. The peptide sequence is indicated on the bottom with collision-induced fragmentation pattern. The b and y ions are indicated in blue and red, respectively. X-axis shows the m/z and y-axis shows the relative abundance of each amino acid. (Color figure online)

Although, It was confirmed that S-24 was best hydrolyzed by previous tests and expected to show better inhibitory activity due to greater concentration peptides, but all samples were tested against ACE to further authenticate results. A clear increasing trend of ACE inhibitory activity has been observed with increasing incubation time of fermented samples, and after 24 h, increasing trend was as slow as negligible. It was reported previously that proteolytic activity of lactobacillus (*Lactobacillus Plantarum*) is at its peak at 24 h of fermentation (Gonzalez-Gonzalez et al. 2011). It was clear after running assay that S-24 was the sample to further evaluate for its peptides profile. Figure 3, indicated the increasing trend of ACE inhibition percentage with increasing time.

After 8 h of incubation 51.11% ACE inhibitory percentage was observed that increased gradually with time and after 16-h, it rose to 62.23%. Further increase in time to incubate samples, showed increase in inhibition of ACE by 72.32% after 24 h and 73.98% after 36 h.

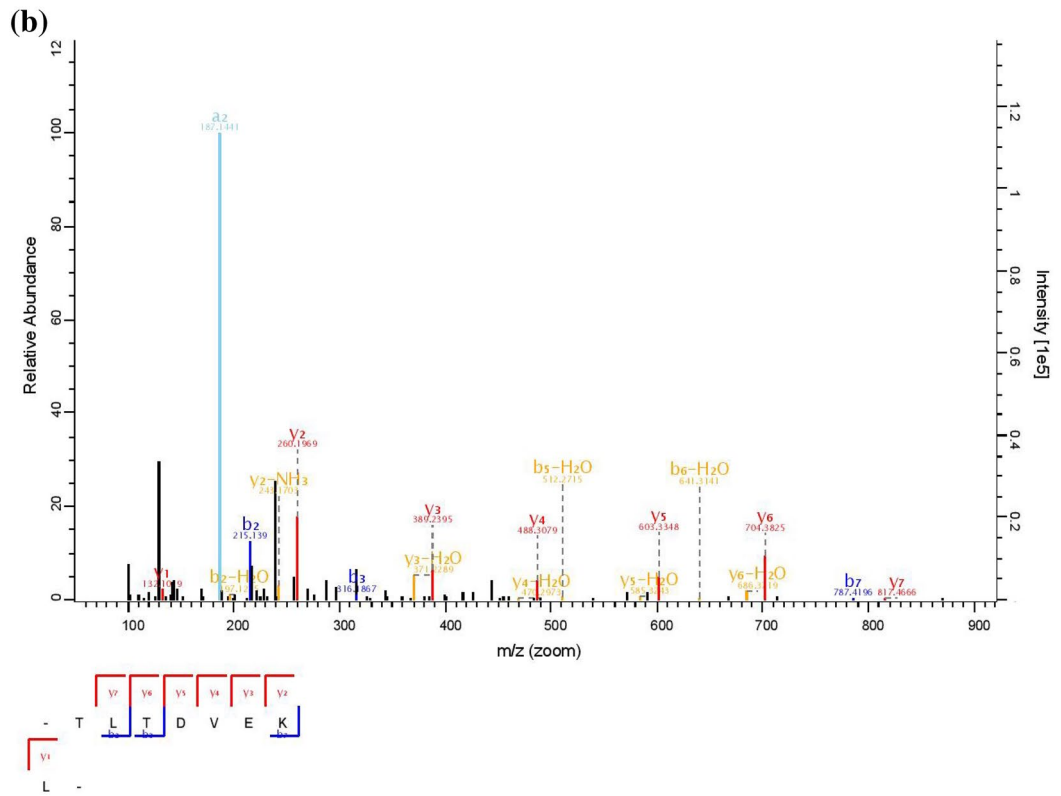
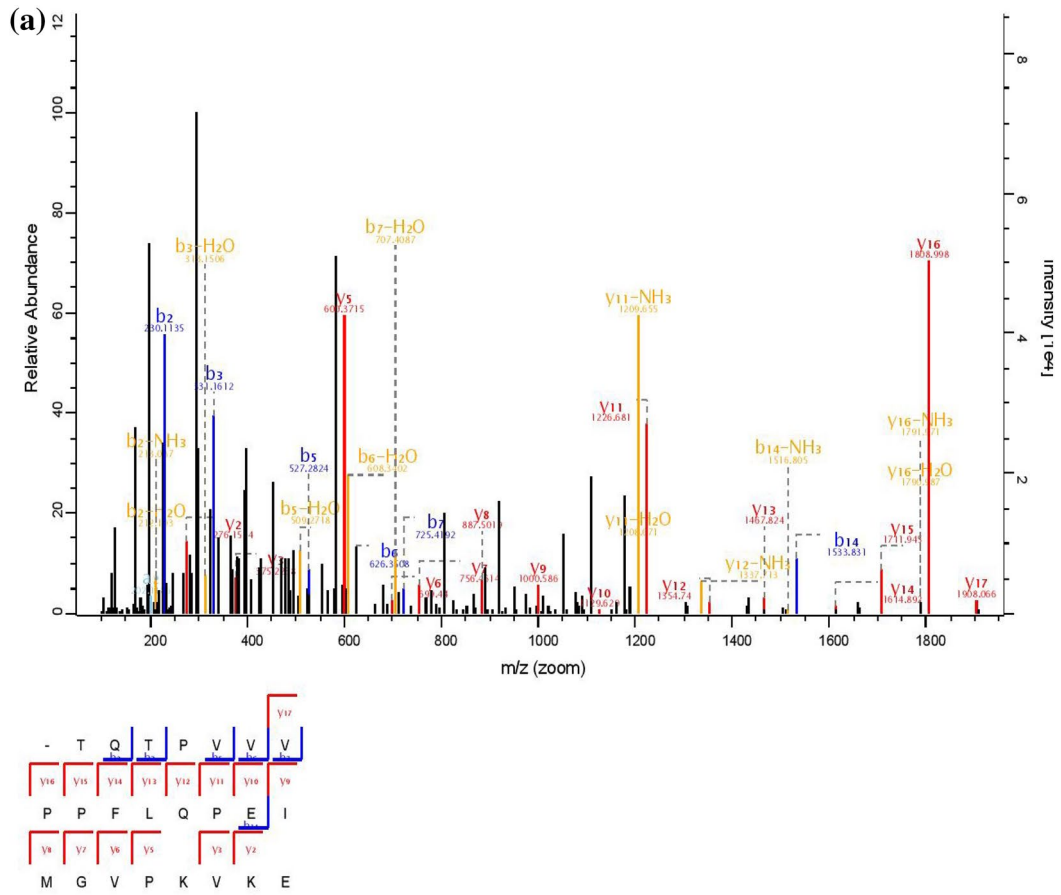
Identification of Peptides

A total of 1257 small and large peptides were identified in this experiment. Among those, five peptides were identified with greater concentration and smaller peptides structure while the rest of peptides were detected in very lower concentration. Milk sample (D-24) used for the detection of peptide profile was collected from four different sources of same breed and then mixed after fermentation and hydrolysis

Table 1 Five most abundant peptide with low molecular weight

Peptide ID	Peptide sequences	Mass (Da)	Intensity (10^8)	C-term cleavage window	N-term cleavage window
1073	TQTPVVVPPFLQPEI-MGVPKVKE	2548.397	3.46	MGVPKVKETMVPKHKE	LPQNILPLTQTPVVVP
1045	TLTDVEKL	917.506	3.13	TLTDVEKLHLPLVQ	PFTESQSLTLTDVEKL
1136	VLPVPQKAVPQ	1174.707	3.01	VPQKAVPQRDMPIQAF	VLSLSQPKVLPVPQKA
826	REQEELNVVGE	1300.625	2.98	EELNVVGETVESLSSS	CLVALAIAREQEELNV
1138	VLPVPQKVVVPQ	1202.738	2.72	VPQKVVVPQRDMPIQAF	VLSLSQPKVLPVPQKV

Peptide ID was assigned on the basis of MaxQuant search results, and peptide sequence was obtained in the result of running MS/MS. Molecular mass of peptides is expressed in Da (Dalton)



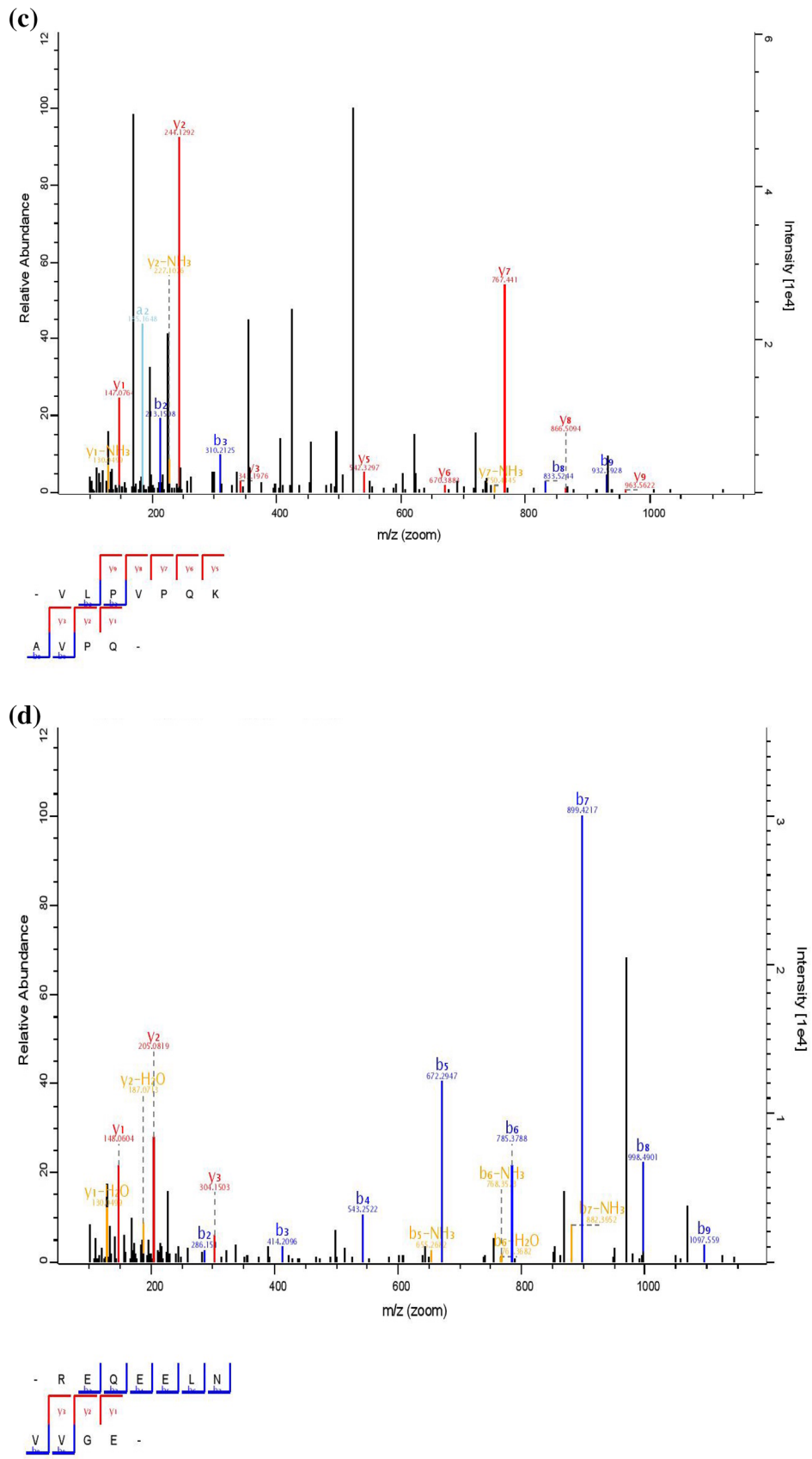


Fig. 4 (continued)

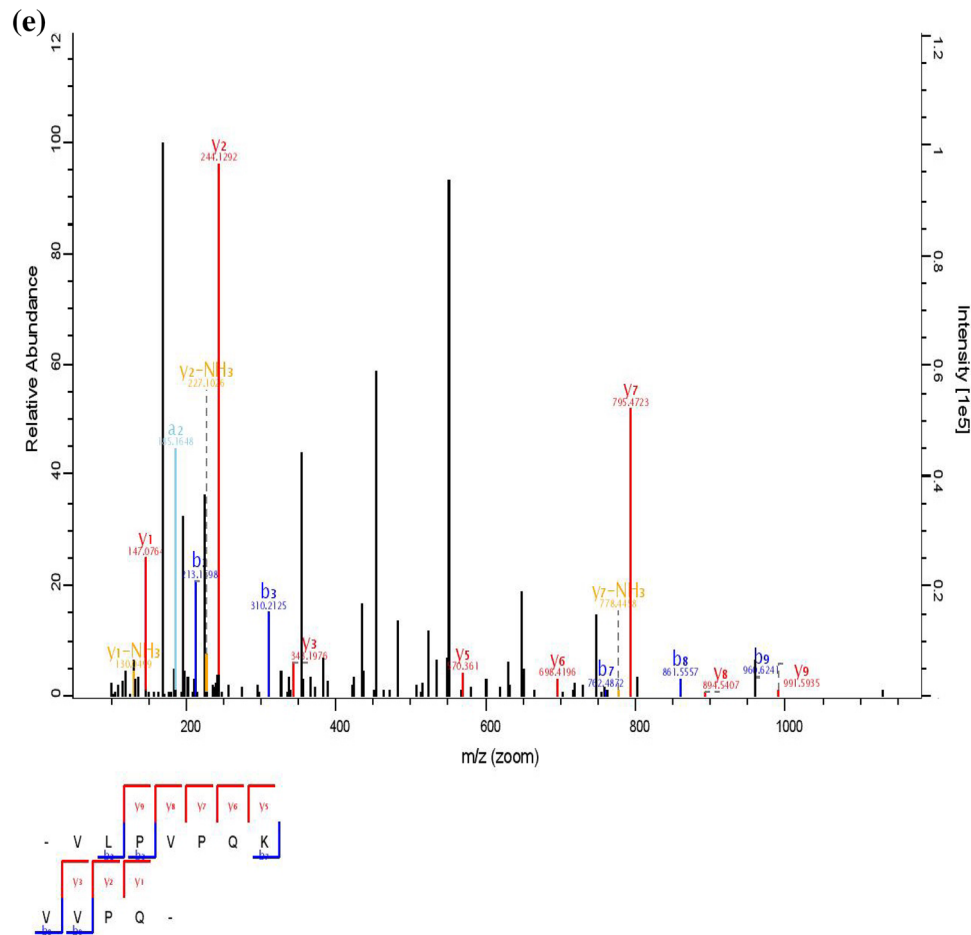


Fig. 4 (continued)

step because replicates of each sample was showing almost same hydrolysis percentage and peptides concentration.

Five most abundant Peptides with low molecular weight were identified as shown in Table 1.

They were categorized on the basis of intensity and abundance of peptide in the sample, presence of hydrophobic and aromatic amino acids. Peptide sequence, with molecular weight of 2532.4026 Da and PID-1073 (peptide identification number), was categorized as the most abundant peptide on the basis of mass to charge ratio (m/z) which was recorded 1267.72 in Fig. 4a. It was identified with retention time of 41.785, 41.824, 44.933 and 44.944 min. Low molecular weight peptide (917.506 Da) with the retention time of 25.411, 27.137 and 27.93 min was identified thrice in S-24. Mass spectrum of PID-1045 was 459.76 m/z as shown in Fig. 4b. Third and fifth peptides with PVP, VVP sequence contested for potential ACE inhibitory peptides. PID-1136 with two times spectroscopic identification and retention time of 21.494 and 21.519 min while PID-1138 with one time identification with retention time of 25.318 min, was

explained in Fig. 4c, e. Molecular weight of PID-1136 and 1138 was 1174.707 and 1202.738 Da respectively; PID-1136 with leading razor protein number Q712N8 was identified after 35 scans containing 6 isotopic peaks while PID 1138 with leading razor protein number Q95L76, was identified after 52 scans containing 5 isotopic peaks. Furthermore, PID 826 was identified once with retention time of 21.648 min. Its molecular weight was 1300.625 Da explained spectroscopically in Fig. 4d. PID 826 with leading razor protein number Q95L76 was identified once after 26 scans containing 6 isotopic peaks.

It was reported earlier that many ACE inhibitory peptides consist of 2–12 amino acids (Byun and Kim 2002), and peptides less than 3 kDa could be responsible for most of the ACE inhibitory activity (Moreno-Montoro et al. 2017; Espejo-Carpio et al. 2013). In this study, results indicated that out of five most abundant peptides, four (TLTDVEKL, VLPVPQKAVPQ, REQEELNVVGE, VLPVPQKVVPQ) consisted of less than 12 peptides and all five peptides are smaller than 3 kDa. Short chain peptides are less prone to

proteolytic enzymes of small intestine after oral administration and easily absorbable to the absorptive cells because intestinal cells are restricted to absorb ultra-small peptides. Peptides with higher concentration of hydrophobic peptides and Proline residues are expected to pass the digestive enzymes intact (Meisel 2004; Ibrahim et al. 2017) due to their resistance against digestive enzymes (Vermeirssen et al. 2004; Norris et al. 2014) while higher concentration of Proline in PID 1073 (TQTPVVVPPFLQPEIMGVPKVKE), PID 1136 (VLPVPQKAVPQ), and PID 1138 (VLPVPQKVVPQ) showed that those peptides can have the ability to bypass digestive enzymes if tested in-vivo. On the other hand, peptides detected in S-24 showed ACE inhibitory activity, in vitro and it might assume that these peptides will remain intact in the gut and intestine of human, rather than degrading by proteolytic enzymes and might be absorbed easily through intestinal cells because of their smaller structure with low molecular weight.

Some ACE inhibitory peptides contain tyrosine, phenylalanine and tryptophan on C-terminal (Shahidi and Mine 2005) and presence of phenylalanine on C-terminal of PID 1136 and 1138 indicates its ACE inhibitory role. C-terminal hydrophobic amino acid residues specially methionine and valine, was observed on the in PID 1073, 1136, 1138 respectively, while it was stated in a study that C-terminal hydrophobic amino acid residues are responsible for ACE inhibition that comes from goat milk (Ibrahim et al. 2017). It is stated in some studies that ACE-inhibitory peptides encoded in natural foods and products contain basic amino acid residues (Suetsuna and Nakano 2000; Pripp et al. 2004) that further supports our finding by exhibiting the presence of Lysine in all peptides with higher presence of Proline in PID 1073 followed by PID 1136 and 1138. Greater quantity of amino acid with aromatic side chains in a sample may increase the ACE-inhibitory activity (Cian et al. 2013; Chalé et al. 2014; Udenigwe and Aluko 2011), while our findings are in agreement by the identification of amino acid with higher concentration of aromatic amino acid residues on C-terminal of PID 1136 and 1138. Methionine, Tryptophane, and Valine in P-1073, 1045, 1136 and 1138 on C-terminal indicated the greater hydrophobicity of this peptide and greater hydrophobicity of C-cleavage shows the greater ACE inhibitory activity (He et al. 2012). N-terminal with Aliphatic amino acid might increase ACE inhibitory activity (Cheung et al. 1980), While in this study, aliphatic amino acid Leucine and Valine was identified in PID-1073, 1136 and 1138 respectively. Leucine and Valine, with small N-terminal amino acids and strong hydrophobicity are more appropriate for ACE inhibitory activity (Wu et al. 2006) and same amino acids were found on N-terminal of P-1073, 1136 and 1138.

Conclusion

Incubation of goat milk protein with LH for 8 h was considered as control, related to other samples. With increase in fermentation time, increase in protein hydrolysis into peptides was observed. In this way, LH showed its prominent role to hydrolyze protein into bioactive peptides. Further, spectroscopic analysis of those peptides showed that molecular mass of peptides (peptide length) was not the only important factor to evaluate its functional activities; other factors including presence and intensity of aromatic and hydrophobic amino acid and amino acid sequence played an important role. In this study, fermented goat milk peptides, by optimizing incubation time to 24 h, were proved to exhibit remarkable ACE- inhibitory activity. Three ACE-inhibitory peptides, PID-1073 (TQTPVVVPPFLQPEIMGVPKVKE), PID-1136 (VLPVPQKAVPQ) and 1138 (VLPVPQKVVPQ), with functional group attached were identified by Q executive hybrid quadrupole-orbitrap mass spectrometry after 24 h of fermentation. Furthermore, structural analysis, intensity and presence of hydrophobic/aromatic amino acid concentration provided clear picture of their mode of action. In the light of previous research and our results, it can be documented that peptides with smaller molecular size with greater concentration are not only responsible in ACE inhibition in-vitro but amino acid sequence also play significant role also. So, It is concluded that Goat milk peptides fermented with LH after optimizing incubation time to 24 h can be used as functional food or nutraceuticals in treating hypertension and consumption of this fermented goat milk may lead to prevent the cardiovascular disorders. This bioactive food without side effects, can replace synthetic drugs.

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Compliance with Ethical Standards

Conflict of interest I declare no conflict of interest.

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