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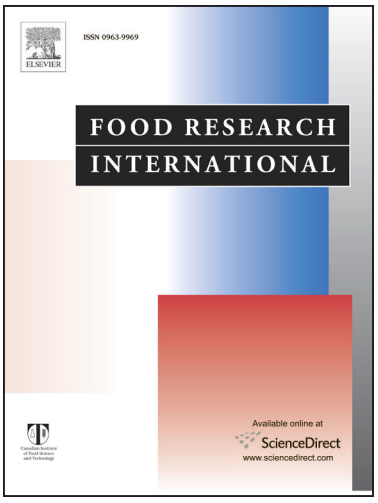

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Peptidomic analysis of the angiotensin-converting-enzyme inhibitory peptides in milk fermented with *Lactobacillus delbrueckii* QS306 after ultrahigh pressure treatment

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ABSTRACT

In this study, we assessed the effect of ultrahigh pressure (UHP) treatment on the concentration of peptides and angiotensin-converting enzyme (ACE) inhibitory activity in milk fermented with *Lactobacillus delbrueckii* QS306. The peptides were identified using peptidomic analysis, and 313 unique peptides were identified. These peptides were derived from 53 precursor proteins. Before and after UHP treatment, 361 (22.2%) peptide sequences exhibited difference, and 53 peptide segments were significantly different. Among them, small peptides (amino acid residues ≤ 6) isoelectric were point at pH 5-6, and the net charge was mainly positive or neutral. With hydrophobicity and ACE inhibitory activity as screening indicators, 214 small peptides with potential ACE inhibitory activity were identified, and 130 new peptides had potential ACE inhibitory activity. A novel ACE inhibitory peptide VAPFP was synthesized, whose in vitro inhibition rate was 10.56 μmol\/L. Therefore, using peptidomics, the changes in peptide sequences and enhancement in ACE inhibitory activity before and after UHP treatment could be effectively identified in milk fermented with *Lactobacillus delbrueckii* QS306. This study provided a convenient method for the discovery and identification of new ACE inhibitory peptides.

Keywords: Peptidomics; Ultrahigh pressure; Angiotensin-converting enzyme inhibitory activity; UPLC-Q-exacitve-HF-X-MS/MS; *Lactobacillus delbrueckii* QS306

1. 1. Introduction

Lactobacilli are essential probiotics in human body, which play an important role in regulation of the composition of human intestinal flora (Lao et al., 2022), prevention of diarrhea (Yang et al., 2021), hyperlipemia (Oh, Koh, Park, Kim, & Kim, 2016), and immune regulation (Yamashita et al., 2014). Dairy products are an important source of nutrition for humans. Casein and whey protein are hydrolyzed into a series of peptides and free amino acids in the fermentation process of dairy products, which provide good nutrition and flavor to the fermented products (Dinika, Verma, Balia, Utama, & Patel, 2020). Moreover, these fermented products exhibit effects such as antihypertensive, hypolipidemic (Rodríguez-Figueroa, González-Córdova, Astiazaran-García, Hernández-Mendoza, & Vallejo-Cordoba, 2013), and antioxidant (S. Li et al., 2014).

Angiotensin-converting enzyme (ACE) inhibitor peptides (ACEIPs), also known as antihypertensive peptides, inhibit ACE to inhibit vasoconstriction and produce antihypertensive effects to alleviate various cardiovascular and cerebrovascular diseases (Geng et al., 2016). At present, ACEIPs have been identified from various food products, including red wine (Alcaide-Hidalgo, Martínez-Rodríguez, Martín-Álvarez, & Pueyo, 2008), vegetables (Yuan et al., 2022), meat (Chen, Yu, Huang, Wang, & He, 2021), seafood (Zhong et al., 2018), and dairy products (S. N. Li, Tang, He, Hu, & Zheng, 2020). These ACEIPs not only play a role in lowering blood pressure but also have high safety, and their versatility is suitable for bee added into that base material of oral medication or functional foods.

Chromatography and liquid chromatography are often used to extract and identify ACEIPs, which is a complex process. The development of peptidomics provides a convenient method for identifying bioactive peptides. Peptidomics can help to assess whether pasteurized human milk is as beneficial as breastfed milk in providing biologically active peptides and to verify which biologically active peptides are biologically significant for infants (Wada & Lönnerdal, 2015). In 10 commercial dairy products, 2117 unique peptides were identified using peptidomics; this the most comprehensive milk-peptide spectrum reported to date (Bhattacharya, Salcedo, Robinson, Henrick, & Barile, 2019).

Peptidomics was used to analyze the undigested and gastrointestinally digested antioxidant peptides and ACE inhibitory peptides in soy milk fermented by Lactobacillus delbrueckii WS4 (Chourasia, Chiring Phukon, Minhajul Abedin, Sahoo, & Kumar Rai, 2022). The application of peptidomics to screen antihypertensive peptides is the future line of research.

Ultrahigh pressure (UHP) technology refers to nonthermal sterilization treatment technology using 100-1000 MPa pressure to destroy microbial cells in food and to inactivate enzymes and proteins at room temperature or lower (J. Zhou et al., 2019). The UHP technology has made remarkable achievements in experimental equipment, production equipment, processing, sterilization, preservation, and other aspects (Georget, Miller, Callanan, Heinz, & Mathys, 2014). UHP treatment of oil palm fruit significantly increased total phenolic and flavonoid contents of three phenolic fractions, antioxidant activities, and intracellular inhibition of reactive oxygen species and provided cytoprotective effects (J. Zhou et al., 2019). The application of UHP technology to improve the antihypertensive activity of fermented milk has not been studied to date.

Previous studies have reported that UHP has a good effect on the ACE inhibition activity, apparent viscosity, antioxidant activity, and volatile aromatic content of milk fermented with *Lactobacillus delbrueckii* QS306. In addition, UHP-treated fermented milk maintained a high ACEIP activity and good quality during storage (Wu, Zhao, Wang, & Shuang, 2022). However, it is difficult to identify a large amount of ACEIPs by conventional extraction identification methods. Till now, studies on the effect of UHP treatment on the promotion of various biological activities in fermented milk are scarce. Thus, the purpose of this study was to investigate the changes in the concentration and sequence of ACEIPs before and after UHP treatment in milk fermented with *Lactobacillus delbrueckii* QS306 using peptidomics and to identify more ACEIPs with potentially high activity.

2 Materials and methods

2.1 Materials

Lactobacillus delbrueckii QS306 was initially isolated from Xilin Gol League traditionally fermented

milk (yogurt fermented by herdsmen) and was provided by the National Food Research and Development Team of the College of Food Science and Engineering, Inner Mongolia Agricultural University. All other chemical reagents were obtained commercially and were of analytical or chromatographic grade.

2.2 Cultivation of Strains and Preparation of Fermented Milk

We cultured *Lactobacillus delbrueckii* QS306 till three subcultures in de Man, Rogosa, and Sharpe (MRS; Guangdong Huankai Microbial Sci. & Tech. Co., Ltd., Guangzhou, China) broth at 37°C for 24 h. Further the cells were centrifuged (3000 *g* for 15 min at 4°C) and resuspended in sterilized saline water to obtain cell density of approximately 10^8 cfu/mL. Overall, 3% (v/v) cell suspension was added to sterile reconstituted skim milk (11%; w/v), and this was incubated at 37°C for 48 h.

2.3. High Pressure Processing

Fermented milk was filled in 100 mL PET (polyethylene terephthalate) bottles, sealed in the super clean table (SW-GJ-2D, Suzhou Zhijing Purification Equipment Co., Ltd., Suzhou, China), and treated with UHP at room temperature. Pressure (HPP 600 Mpa/30 L, Baotou Kefa High Pressure Tech. Co., Ltd., Baotou, China) was 400 MPa, and pressure holding time was 10 min. Fermented milk before and after UHP treatment was stored at 4℃, and the activity of ACEIPs was measured in time.

2.4 Preparation of Original Peptide Samples

The pH of the fermented milk (FM) or UHP-FM was adjusted to 4.6 using 1 M HCl. Further, the samples were centrifuged at 3000 *g* and 4°C for 15 min. The pH of the supernatants was adjusted to 8.3 using 1 M NaOH. They were centrifuged at 3000 *g* and 4°C for 5 min. The supernatants were used to determine ACE inhibition activity.

2.5 Ultrafiltration

The original peptide samples were separated using 10 kDa UF centrifuge tubes (Amicon Ultra-50 mL, Millipore, Billerica, USA), and two fractions were obtained as per the molecular weight (MW > 10 kDa and < 10 kDa). The fraction of < 10 kDa was further separated using 3 kDa UF centrifuge

tubes (Amicon Ultra-50 mL, Millipore, Billerica, USA), and fractions with MW < 3 kDa were collected.

2.6. ACE inhibitory activity assay

The pH of the samples was adjusted to 8.3, and ACE inhibitory activity was determined using the method by Wu Nan (Wu et al., 2022) with slight modifications (Table 1).

From 10 μL of reaction solution, the peak area of hippuric acid (HA) generated from the reaction was measured. The liquid phase conditions were set as follows. Mobile phase A was 0.05% TFA aqueous solution, and mobile phase B was acetonitrile. The column was balanced with 98% of solution A. The flow rate was 0.8 mL/min. The detection duration was 10 min. The detection wavelength was 228 nm. The liquid phase gradient was set from 0 to 10 min with 75% for mobile phase A and 25% for mobile phase B. The inhibition rate of the ACEIPs was calculated according to the following formula:

ACE inhibition rate (ACEI %) = [(Aa − Ab)/Aa) × 100

where Aa is peak area of HA generated without sample addition; Ab is peak area of HA generated by reaction group.

2.7 Peptide sequence analysis using UPLC-Q-exactive-HF-X-MS/MS

Overall, 0.1% TFA solution was added to the UHP-FM and FM samples with molecular weights < 3 kDa for desalination on C18 Cartridge (100 μm × 20 mm, 5μm, Dr. Maisch GmbH), and peptides were quantified. The appropriate amount of peptide solution from each sample was run on a chromatographic system (Easy nLC 1200, Thermo Fisher Scientific Inc., Waltham, MA) with nanoliter flow rate. Buffer A was 0.1% formic acid aqueous solution, and buffer B was 0.1% formic acid in acetonitrile aqueous solution (acetonitrile was 95%). Peptides were loaded onto a trap column (100 μm × 20 mm, 5μm, C18, Dr. Maisch GmbH) before separating on a reverse phase column (75 μm × 150 mm, 3μm, C18, Dr. Maisch GmbH); flow rate was 300 nL/min. Liquid phase gradients were as follows: 0-2 min, B: 4%-7%; 2-92 min, B: 7%-20%; 92-110 min, B: 20-35%; 110-112 min, B: 35%-90%; and 112-120 min, B: 90%. Eluted peptides were analyzed using a Q Exactive HFX

Orbitrap mass spectrometer (Thermo Scientific). Full-scan MS spectra were acquired with a scan range of 350–1800 m/z, resolution of 70,000 at m/z 200 to distinguish precursor ions of molecular species with similar m/z value, and AGC target of 1×10^6 ions or maximum injection time of 50 ms. Mass spectra were collected in data-dependent mode with one precursor scan followed by 15 MS/MS scans. MS2-scan MS/MS spectra were acquired with a scan range of 200-2000 m/z, resolution of 17,500, and AGC target of 1×10^5 ions or maximum injection time of 100 ms. MS/MS fragmentation was performed using higher energy collision–induced dissociation with normalized collision energy of 27, and isolation window was 1.6 Th.

2.8 Peptide quantification

The mass spectrometry database retrieval software used in this study was MaxQuant 1.6.1.0 (Cox et al., 2011). Uniprot (https://www.uniprot.org/) protein database: Uniprot-Bos Taurus (Bovine) [9913]-46728-20201102. The species was Bovine, with a total of 46,728 protein sequences. Two max missed cleavage sites and main search peptide tolerance was set to 4.5 ppm, first search peptide tolerance and MS/MS Tolerance of 20 and 20 ppm, respectively. The percolator node was used to validate identified peptide-spectral matching (PSM) and to filter the data with parameters of a strict target false discovery rate (FDR) of 0.01 and a relaxed target FDR of 0.01. Protein quantification was considered valid when the MS spectra matched to razor. Unique peptides were used for protein quantification.

2.9 Peptides hydrophobicity, activity score, and previously reported sequences

The hydrophobicity was predicted as per the method by Adler-Nissen (Adler-Nissen, 1986) and using the following equation:

$$Q = \sum (\frac{M_i}{M} \times \Delta f_i)$$

where Q was the hydrophobicity value of peptides (kJ/mol); M_i was the molar mass of amino acids (g/mol); M was the molar mass of peptides (g/mol); and Δf_i was the side-chain hydrophobicity value of amino acids (kJ/mol). Peptides with hydrophobicity > 6 kJ/mol were further analyzed.

The UHP-FM and FM peptides were further evaluated using Peptide Ranker (http://distilldeep.ucd.ie/PeptideRanker/) to predict the activity score. Peptides with activity scores > 0.5 were further analyzed.

AHTPDB (http://crdd.osdd.net/raghava/ahtpdb/) database was used to query the previously reported sequences.

2.10 Prediction of peptide stability and Fmoc solid phase conjugation of peptide

The specificity of protease digestion sites (for example, the known hydrolysis sites of trypsin are lysine and arginine) was considered to predict the types of peptide segments that may be produced by proteins with known amino acid sequences. ExPASy PeptideCutter server (https://web. express.org /peptideCutter/) predicts the results of a single and multiple proteases by simultaneously hydrolyzing a certain protein to produce peptides. Pepsin and trypsin digestion of peptides in both FM and UHP-FM with activity > 0.5 and hydrophobicity > 0.6 was simulated in PeptideCutter. Solid-phase synthesis and purification of Fmoc were completed by Gil Biochemical (Shanghai, China) Co., Ltd.

2.11 Data statistics and analysis

All measurements in this study were conducted in triplicates, and data were expressed as mean ± standard deviation (SD). Duncan's new multiple range test was performed to determine the significant differences among samples. $P < 0.05$ was considered significant. The online tool PI (https//web.expasy.org/compute.pi/) was used to calculate the isoelectric point (pI). Peptides venny map was generated using Venny 2.1.0 (https://bioinfogp.cnb.csic.es/tools/venny/index.html). Microsoft Excel 2019 (Microsoft Co., USA) software and GraphPad Prism 6.0 programs (GraphPad Software Inc., La Jolla, CA, USA) were used to generate graphs.

3. RESULTS AND DISCUSSION

3.1 ACE inhibitory activity of crude isolated components of milk fermented with *Lactobacillus delbrueckii* QS306

After UHP treatment, the ACE inhibitory activity of whey in milk fermented with *Lactobacillus delbrueckii* QS306 was significantly higher than untreated whey (Table 2). The ultrafiltration separation with MW < 3 kDa of whey was significantly higher than the component with MW < 10 kDa whey (Table 2). The ACE inhibitory activity of each component under the same separation conditions or the same concentration after UHP treatment was significantly higher than that of the untreated group (Table 2).

3.2 Peptides identify of UHP-FM and FM using peptidomics

The concentration determination and sequence analysis of the samples with MW < 3 kDa in UHP-FM and FM were performed using peptidomics. The peptide concentration in UHP-FM was significantly higher than that in FM (0.921 ± 0.04 and 0.84 ± 0.05 μg/μL). The total number of peptides in the components with MW < 3 kDa in UHP-FM and FM was 1463 and 1431, respectively, containing 270 and 264 unique peptides, respectively. Overall, 77.8% of the peptide sequences of the components with MW < 3 kDa in UHP-FM and FM were identical and 22.2% were different (Fig. 1 A). UHP not only increased the concentration of polypeptides in the fermented milk but also changed the sequence of the peptides. UHP causes deprotonation of charged groups and disrupts hydrophobic interactions, thereby causing conformational and structural changes in proteins (Knudsen, Otte, Olsen, & Skibsted, 2002). Proteins are hydrolyzed and denatured during fermentation; further UHP treatment breaks the covalent and noncovalent bonds, leading to the generation of new bioactive peptides.

The fraction of the peptides with MW < 1500 Da in the MW < 3 kDa fraction of UHP-FM and FM was 74.91% (1096 peptides) and 74.00% (1059 peptides), respectively (Fig. 1B). Overall, 29.46% (431 peptides) and 28.23% (404 peptides) peptides in UHP-FM and FM were with MW < 650 kDa, respectively. pI is the pH value at which a molecule has no charge on its surface. The fermentation by lactic acid bacteria reduced the pH of milk. pI of casein is 4.6 (Yen, Lin, & Tu, 2015). The spatial structure of protein changed, and protein molecules gathered together to produce curd. Peptides with pI < 7 in UHP-FM and FM accounted for 56.94% (833 peptides) and 57.65% (825 peptides) of total

peptides, respectively (Fig. 1C). The pI of peptides in UHP-FM and FM were mostly in the pH range of 5–7 (Fig. 1E). UHP-FM and FM contained 53 and 49 precursor proteins, respectively. Fig. 3D shows the top 20 precursor proteins with a large number of polypeptides. P02666 (β-casein), P02662 (α-S1-casein), P02663 (α-S2-casein), and A0A3Q1M5U9 (κ-casein) were the main precursor protein sources of identified peptides. The relative abundance of the terminal amino acid classes of the polypeptides in UHP-FM and FM is shown in Fig. 1F. At the C-terminal of peptides in UHP-FM and FM, amino acids Phe, Lys, Leu, Pro, Arg, Val, Glu, Asn, Gln, and Thr were relatively abundant. At the N-terminal of peptides in UHP-FM and FM, amino acids Ala, Phe, Gly, Lys, Leu, Pro, Arg, Val, Tyr, Glu, Gln, and Ser were relatively abundant. The aliphatic, aromatic, and hydrophobic amino acids were mainly distributed at N-terminal with relatively high abundance.

Overall, 52 peptides were significantly different between UHP-FM and FM, and 22 and 30 peptides were significantly up- and downregulated, respectively (Fig. 2A). The 22 significantly upregulated peptides (a fold change > 1.5, $P < 0.05$) corresponded to 8 proteins (P02666, P02662, P02663, P02453, P02465, A0A3Q1M5U9, A0A3Q1MMV3, and F1MF78), and EVKITVDDKHYQ and GPAGERGEQGPA were the unique significantly upregulated peptides. The 30 significantly downregulated peptides ($P < 0.05$) corresponded to 11 proteins (A0A452DHW7, A0A3Q1M5U9, P02663, P02666, P02662, F1MR22, E1BNY9, A0A3Q1LZU0, P80195, P02668, and A0A3Q1LWV4), and EVKITVDDKH, GVSKEAMAPKKHKEMPFPYPVEPFTERQ, LSKELTPKAKDKN and SQNPKLPLSIL were the unique significantly downregulated peptides. Among the significantly different peptide fragments, HQPHQPLPPTVM, QSWMHQPHQPLPPT, SDKIAKYIPI, SWMHQPHQPLPPT, VAPFPEVFGKEK, WMHQPHQPLPPT, FSD, VYPFPGPIHNSLPQN (Contreras, Carrón, Montero, Ramos, & Recio, 2009), HKEMPFPKYPVEP (Robert, Razaname, Mutter, & Juillerat, 2004), QLL (Quirós, Hernández-Ledesma, Ramos, Amigo, & Recio, 2005), YQEPVLGPVRGP (Pihlanto, 2013), and YVRYL (Lau, Abdullah, & Shuib, 2013) shared the same fragments with the validated ACEIPs, and these 12 peptides exhibited ACE inhibitory activity. In addition, these 12 peptides corresponded to casein, including the five forms of

casein (A0A3Q1M5U9, A0A452DHW7, P02666, P02662, and P02663) .

3.3 Analysis of small peptides (2-6 amino acid residues)

3.3.1 pI, charge, and hydrophilicity

At the pI of UHP-FM, 258 small peptides had pH < 7, accounting for 56.70% of the small peptides in UHP-FM (Figs. 3 A and B). At the pI of FM, 249 small peptides had pH < 7, accounting for 58.58% of the small peptides in FM. The pI of small peptides was in the range of 5-6, with 159 and 152 peptides accounting for 34.95% and 35.76% of the total peptides, respectively. The pI of peptides in UHP-FM and FM was mostly in the range of 5-6 (Figs. 4 D and F). Overall, 384 and 357 small peptides existed in UHP-FM and FM, respectively. They were mainly positively charged or uncharged, accounting for 84.40% and 84.00% of the small peptides in UHP-FM and FM, respectively (Figs. 3 C and D). And 295 and 272 small peptides in UHP-FM and FM had the hydrophilicity index of peptide segment < 0, accounting for 64.84% and 64.00% of the small peptides in UHP-FM and FM, respectively (Figs. 3E and F).

3.3.2 Analysis of amino acids at N- and C-terminals in small peptides

Overall, 455 peptides were present in UHP-FM, among which 24.9% (141) were new peptides (Fig. 4 A). The amount of tripeptides, tetrapeptides, and pentapeptides increased after UHP treatment. The relative abundance of the amino acids at the N- or C-terminal in small peptides is given in Fig. 4G. Statistical analysis of the relative abundance revealed that Phe, Lys, Leu, Pro, Arg, Val, Trp, Tyr, and Gln were relatively highly abundant at N-terminal and Phe, Lys, Leu, pro, Arg, Glu, and Gln were relatively highly abundant at C-terminal. The peptides with Ala, Glu, Lys, Leu, Arg, Val, and Tyr at N-terminal or with Ala, Phe, Pro, Leu, Tyr, Val, and Trp at C-terminal exhibited high ACE inhibitory activity. The somatic isoform of ACE contains C- and N-active sites, and sequences of the two active sites have 60% similarity. The amino acid residues at N- and C-terminals exhibit a great impact on the activity of ACEIPs (Bernstein et al., 2011). However, the hydrolysis of angiotensin I by C-domain is three times higher than that by N-domain. Studies have reported that many inhibitors have C-domain selectivity (Jimsheena & Gowda, 2011). The amino acid residues at N- and C-terminals (Fig.

4E) have stronger affinity with ACE active sites (Song et al., 2021). In UHP-FM and FM, N-Leu, N-Val, N-Tyr, C-Phe, C-Pro, and C-Leu were the terminal amino acids with the highest abundance and potentially high ACE inhibitory activity after UHP treatment. The number of N- and C-terminal peptides in UHP-FM and FM with potentially high ACE inhibitory activity was 115 and 116, respectively (Figs. 4B and C).

3.4 Differential Analysis of Small Peptides in UHP-FM and FM

Based on the prediction of function of peptides in the precursor protein, the potential biological function of differential peptides in small peptides was evaluated. Among them, the number of small peptide precursor proteins reached more than 10% of the total difference (Fig. 5A), including microtubule-based movement, microtubule-based process, cell projection morphogenesis, dynein complex, microtubule associated complex, microtubule motor activity, motor activity, nucleoside-triphosphatase activity, and pyrophosphatase activity. In Gene Ontology (GO) analysis, the differential peptides in small peptides were divided into the first five biological processes related to the movement of cell or subcellular component, organelle organization, cellular developmental process, cellular component assembly, and cell projection organization (Fig. 5B). In terms of cellular components, the first five highly enriched subcategories were catalytic complex, microtubule associated complex, cell projection part, ciliary plasm, collagen trimer, and motile cilium (Fig. 5C). In terms of molecular function, the first five highly enriched subcategories were nucleoside phosphate binding, nucleotide binding, anion binding, anion binding and hydrolase activity, and acting on acid anhydrides (Fig. 5D).

In addition, pathway analysis revealed that the first four networks of these peptide precursor proteins were extracellular matrix (ECM) receptor interaction, focal adhesion, platelet activation, and protein digestion and absorption (Fig. 6A). These involved signaling molecules and interactions, cellular community-eukaryotes, immune system, and digestive system, which were consistent with the significantly different peptide pathways. The Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway corrected by P value ($P < 0.01$) was ECM receptor interaction and advanced glycosylation

end products-receptor of AGEs (AGE-RAGE) signal pathway (Fig. 6B). ECM is composed of a complex mixture of structural and functional macromolecules, which play an important role in the morphogenesis of tissues and organs and in maintaining the structure and function of cells and tissues. These interactions lead to direct or indirect control of cell activities, such as adhesion, migration, differentiation, proliferation, and apoptosis, and the expression level of ECM is related to the formation of malignant tumors (Bao et al., 2019). Excessive deposition of advanced glycation end-products (AGEs) will destroy the structure of ECM, change its biochemical characteristics and metabolism, and lead to covalent cross-linking of proteins. AGE-RAGE signaling pathway and disease AGE-RAGE-signal-mediated diabetes complications include diabetes neuropathy, diabetes nephropathy, diabetes vascular complications, and diabetes foot syndrome (Kay, Simpson, & Stewart, 2016). In addition, AGEs play an important role in most age-related diseases such as Alzheimer's disease, cancer, cardiovascular disease, kidney disease, hypertension, stroke, visual impairment, and skin disease.

3.5 Small peptides with potential ACE inhibitory activity

Overall, 566 small peptides in UHP-FM and FM and small peptides with activity scores > 0.5 and hydrophobicity > 6 kJ/mol are given in Table 3; these small peptides were from 214 peptides derived from 33 types of proteins. The peptides in β-casein, α-S1-casein, and midasin proteins were the most abundant (29, 30, and 30, respectively). Casein is the main protein source in milk. β-casein and α-S1-casein are different structural forms of casein. Midasin protein is present in various organisms and is related to protein hydrolysis, protein folding, protein unfolding, membrane transport, and assembly of macromolecular complexes in the nucleus (Garbarino & Gibbons, 2002). Overall, 84 peptides with the same or similar sequences were obtained from reviewed literature and confirmed to have the ACE inhibitory activity (Table 3); moreover, 130 new peptides had potential ACE inhibitory activity.

As shown in Fig. 7, 11 new peptides with good stability were screened from the small peptides of FM and UHP-FM using PeptideCutter. The ACE inhibitory activity of VAPFP (97.91% ± 0.14%) was significantly ($P < 0.05$) higher than that of HLPLP (96.58% ± 0.39%%), FPPQ (64.83% ± 0.27%),

FPPQs (74.54% ± 0.74%), LNPW (62.18% ± 2.31%%), LPPL (65.41% ± 2.31%%), NPW (74.57% ± 3.10%%), PLPN (63.96% ± 0.67%%), PPF (73.67% ± 1.58%%), WR (90.09% ± 0.43%%), and HGF (79.30% ± 2.72%). The half maximal inhibitory concentration (IC_{50}) of VAPFP was 10.56 μmol/L.

4. Conclusion

In conclusion, UHP treatment enhanced ACE inhibitory activity, increased peptide concentration, and changed peptide sequence in milk fermented with *Lactobacillus delbrueckii* QS306. Moreover, the changes in peptide sequence before and after UHP treatment were characterized at the molecular level using peptidomics, and 130 new peptides were reported with potential ACE inhibitory activity. Overall, 11 small peptides exhibited good stability. The ACE inhibitory activity of VAPFP was 10.56 μmol/L. Studies are ongoing investigating the active sites and in vivo mechanisms of action of ACE inhibitory peptides.

CRediT authorship contribution statement

Nan Wu: Conceptualization, Data curation, Formal analysis, Methodology, Project administration, Resources, Writing - original draft. **Fengmei Zhang:** Conceptualization, Resources, Project administration. **Quan Shuang:** Conceptualization, Resources, Project administration.

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.

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analysis using UPLC-Q-exactive-HF-X-MS/MS.

Figure Captions

Fig. 1 Analysis of difference in peptides between UHP-FM and FM. (A: comparison of the total number of identified peptide segments in UHP-FM versus FM; B: the number of various molecular weight ranges for peptides in UHP-FM versus FM; C: number of various pI ranges for peptides in UHP-FM and FM. D: protein source analysis of peptides in UHP-FM and FM; E: relative abundance of the C-and N-terminal amino acid classes in peptides in UHP-FM and FM; F: scatter plot of molecular weight and pI of peptides in UHP-FM and FM. A(Ala): Alanine; F(Phe): Phenylalanine; G(Gly): Glycine; K(Lys): Lysine; L(Leu): Leucine; P(Pro): proline; R(Arg): arginine; V(Val): valine; W(Trp): tryptophan; Y(Tyr): tyrosine; C(Cys): cysteine; D(Asp): Aspartic acid; E(Glu): glutamic acid; H(His): histidine; I(Ile): isoleucine; M(Met): Methionine; N(Asn): asparagine; Q(Gln): glutamine; S(Ser): serine; T(Thr): threonine.

Fig. 2 Significantly different peptide segments in UHP-FM and FM (A: volcano map; B: clustering).

Fig. 3 pI, charge, and hydrophilicity of small peptides with AA residues ≤ 6 in UHP-FM and FM.

Fig.4 Analysis of small peptides in UHP-FM and FM. (A: comparison of the total number of small peptides; B and C: comparison of the peptide segments at the N- and C-terminals of small peptides and amino acids with potentially high ACE inhibitory activity; D: the number of small peptides with various pI ranges in UHP-FM and FM; E: the number of small peptides with potentially high ACE inhibitory activity at N- and C-terminals. F: a scatter plot of the molecular weight and pI of small peptides. G: The relative abundance of the amino acid classes at N- and C-terminal of small peptides).

Fig.5 Gene ontology and pathway analysis of the precursor proteins of the differentially small peptides in UHP-FM and FM. (A: Canonical signaling pathways. B: The biological process categories. C: The cellular component categories. D: The molecular function Categories.)

Fig.6 KEGG enrichment analysis of proteins corresponding to the differential small peptides of UHP-FM and FM. (A: KEGG analysis of FM and UHP-FM;B:

KEGG analysis of FM and UHP-FM by *P* value.)

Fig.7 The ACE inhibition by peptides. (All samples were 5 mg/mL).

Table 1 Determination of ACE inhibitory activity

Reagent	Sample (μL)	
	Aa	Ab
Whey	0	40
ACE (0.1 U/mL)	40	40
H_2O	40	0
Reaction conditions	37℃ constant temperature water bath for 5 min	
HHL (5 mmol/L)	80	80
Reaction conditions	37℃ constant temperature water bath for 60 min	
HCL (1 M)	200	200

Table.2 ACE Inhibitory Activities of Crude Fractions from Fermented Milk before and after Ultrahigh Pressure Treatment

	Whey	MW<10 kDa	MW<3 kDa
UHP-FM*	58.058 ± 0.98[a]	62.40 ± 0.94[a]	81.71 ± 0.61[a]
FM*	49.70 ± 1.13[b]	59.33 ± 1.30[b]	69.71 ± 0.51[b]
UHP-FM#	91.64 ± 0.64[a]	95.18 ± 0.9[a]	98.95 ± 0.65[a]
FM#	79.83 ± 0.21[b]	83.57 ± 0.36[b]	89.46 ± 0.79[b]

[a,b] in a row indicate significant difference ($P < 0.05$). * represents the same concentration (0.9 mg/mL); # represents the same processing conditions. UHP-FM means ultra-high-pressure-treated fermented milk; FM means fermented milk; MW means molecular weight.

Table 3 Screening of small peptides in all samples based on hydrophobicity and activity

UniProt AC	Protein	peptides sequence	Mass	Predicted activity score	Hydrophobicity value (kJ/mol)	PI	Sample source	Previously reported sequences
A0A3Q1LJ66	Uncharacterized protein	YRPF	581.30	0.96	9.98	8.75	##	
A0A3Q1LML7	PKHD1 ciliary IPT domain containing fibrocystin/polyductin	PFRA	489.27	0.89	7.97	10.18	##	
		RFFP	565.30	0.99	9.65	9.75	#O	
		YFPK	553.29	0.84	11.18	8.59	##	
A0A3Q1LRK9	Dynein axonemal heavy chain 11	PHF	438.21	0.94	8.56	7.17	O#	LPHF(P. Zhou, Yang, Ren, Wang, & Tian, 2013)
		WFK	399.19	0.98	11.09	8.75	##	
		WVP	479.25	0.87	11.63	5.52	#O	WVPSV(J.-H. Wang et al., 2013)
		YSGL	400.21	0.52	8.04	5.52	##	
A0A3Q1M2W7	Dynein axonemal	FPWL	539.27	0.99	12.44	5.52	#O	
		VFFO	561.30	0.77	8.22	5.49	O#	

	heavy chain 6							
		DERFF	670.31	0.85	6.79	4.37	##	
		FFSD	712.32	0.87	7.74	3.80	##	
		FFSDK	672.36	0.72	7.63	5.84	O#	
		PHLSF	642.30	0.71	7.95	7.17	##	HPHPHLSF(Hernández-Ledesma, del Mar Contreras, & Recio, 2011)
		QWQVL	514.21	0.58	6.84	5.52	##	
A0A3Q1M5U9	Kappa-casein	RFFSD	599.31	0.76	6.73	5.84	##	
		WQVL	544.30	0.62	8.56	5.52	##	WQVLPNAVPAK(Hernández-Ledesma et al., 2011)
		YPY	441.19	0.74	12.72	5.52	##	YPYY(Segura-Campos, Peralta-González, Castellanos-Ruelas, Chel-Guerrero, & Betancur-Ancona, 2013)
		YPYY	604.25	0.76	12.89	5.52	##	YPYY(Segura-Campos et al., 2013)
	Dynein axonemal	LHML	512.28	0.66	7.40	6.74	##	
A0A3Q1M7D1	heavy chain 12	RFWL	620.34	0.98	10.08	9.75	##	
A0A3Q1MC60	Nebulin	HPLH	502.27	0.52	6.45	6.92	##	
		TNWM	550.22	0.76	6.52	5.19	#O	

		AAPP	383.16	0.67	8.66	5.57	##	
		FFR	422.23	0.99	8.96	9.75	##	
		LWAL	431.22	0.86	10.96	5.52	O#	
		MLPM	490.23	0.93	8.59	5.28	##	
		PAPL	354.19	0.80	10.41	5.96	#O	
A0A3Q1MMV3	Ryanodine receptor 1	PHLG	418.23	0.56	6.90	7.17	O#	HPHPHLSF (Hernández-Ledesma et al., 2011)
		PMH	468.25	0.82	6.26	7.17	##	ALPMH (Sagardia, Roa-Ureta, & Bald, 2013)
		PPTP	459.25	0.69	9.76	5.96	O#	
		RFP	410.22	0.98	8.67	9.75	O#	
		VGWA	396.24	0.70	8.50	5.49	##	
		VGWV	501.30	0.52	9.19	5.49	##	
A0A3Q1MZ31	Dynein axonemal heavy chain 11	DQPL	471.23	0.51	6.01	3.80	#O	
A0A452DHW7	Beta-casein	PSLL	428.26	0.59	9.19	5.96	##	

E1BBB8	Transcriptional repressor GATA binding 1	PGLPN	496.26	0.64	7.75	5.96	##
		FAK	422.23	0.62	8.30	8.75	O#
		FALL	399.19	0.85	10.30	5.52	O#
		FFAT	399.19	0.87	8.58	5.52	##
		FFLL	488.25	0.99	11.74	5.52	##
		FPFP	392.22	0.99	12.22	5.52	##
		LFKF	394.20	0.96	10.68	8.75	##
		LHM	394.20	0.61	6.17	6.74	O#
E1BC24	Midasin	LHPF	477.26	0.91	9.27	6.74	##
		LQAF	477.26	0.67	7.06	5.52	##
		MLH	431.23	0.67	6.17	6.49	O#
		MLW	422.16	0.98	10.49	5.28	O#
		PFK	364.21	0.86	10.28	9.18	#O
		PLDP	368.22	0.61	9.43	3.80	##
		QLAF	493.29	0.74	7.06	5.52	O#
		RFA	415.22	0.87	6.72	9.75	#O
		RFT	484.23	0.76	6.12	9.75	##

		RPF	418.23	0.98	8.67	9.75	#O	RPF (X. Wang et al., 2011)
		RPP	450.26	0.86	8.30	9.75	##	RPP (J. Wu, R. E. Aluko, & S. Nakai, 2006)
		SKFL	450.26	0.69	8.30	8.47	O#	
		VLF	434.23	0.73	10.57	5.49	##	TTENVLFG (Kang et al., 2013)
		VLFF	512.27	0.94	11.10	5.49	#O	
		WAR	440.23	0.90	7.81	9.75	#O	
		WRQ	390.23	0.83	6.21	9.75	##	
		WSM	462.28	0.95	8.04	5.52	O#	
		YGR	448.21	0.68	6.87	8.75	##	
		YLR	377.23	0.56	8.96	8.75	##	DYLRSC (Anne & Sari, 2013)
		YRG	553.33	0.67	6.87	8.75	#O	YRGGLEPINF (Martínez-Maqueda, Miralles, Recio, & Hernández-Ledesma, 2012)
		YRL	524.30	0.64	8.96	8.75	##	KDYRL (García, Puchalska, Esteve, & Marina, 2013)
		YRP	538.32	0.77	9.14	8.75	##	YRPY (Puchalska, Marina Alegre, & García López, 2015)
		YWL	506.25	0.95	12.63	5.52	##	INYWL (Tânia & Malcata, 2013)
E1BHN4	RING-type domain-	FLR	480.24	0.95	8.50	9.75	#O	RLPSEFDLSAFLRA (Kang et al., 2013)
		FPH	450.22	0.94	8.56	6.74	O#	TFPHGP (He, Liu, & Ma, 2013)

	containing	FQYQ	378.20	0.54	6.65	5.52	##	
	protein	FRE	562.32	0.60	6.00	6.00	##	FREGD (Gu, Majumder, & Wu, 2011)
		PHL	584.26	0.61	7.98	7.17	O#	HPHPHLSF (Hernández-Ledesma et al., 2011)
		PLPN	477.27	0.64	8.76	5.96	##	
		PYR	496.24	0.73	9.14	9.18	##	
		QFLR	365.21	0.81	6.46	9.75	O#	
		RFG	434.26	0.96	6.25	9.75	##	
		RFGV	399.19	0.65	6.69	9.75	#O	
		VTFM	439.24	0.55	7.44	5.49	O#	
		YVF	434.23	0.71	11.32	5.52	#O	
	Vacuolar	VGTF	427.21	0.54	6.82	5.49	#O	
E1BIF6	protein sorting 13 homolog D	WMG	422.22	0.99	8.61	5.52	#O	
E1BLT3	Spectrin beta, non-erythrocytic 4	WLPH	392.15	0.91	9.94	6.74	O#	
E1BMG2	Dynein axonemal	LPNP	551.29	0.64	8.76	5.52	O#	
		LPPL	439.24	0.86	11.82	5.52	##	

	heavy chain 5	WALL	573.36	0.89	10.96	5.52	##	
		WKLK	501.30	0.50	9.99	10.00	##	
E1BNY9	HECT-type E3 ubiquitin transferase	WVDL	438.28	0.58	9.45	3.80	##	
E1BPZ7	F-box protein 38	YLPL	531.27	0.74	12.08	5.52	O#	
F1MCG9	Diacylglycerol kinase	FLRKF	504.29	0.93	9.08	11.00	##	
		KFPK	709.43	0.65	9.51	10.00	#O	
		LAQF	518.32	0.63	7.06	5.52	#O	
		LFH	418.24	0.85	8.39	6.74	O#	
	Spectrin repeat	LHF	477.26	0.85	8.39	6.74	##	
		LHPL	582.29	0.59	8.87	6.74	##	
F1MF78	containing nuclear	LLF	392.21	0.94	11.47	5.52	#O	LLF (Hernández-Ledesma, Miguel, Amigo, Aleixandre, & Recio, 2007)
	envelope	LLQW	406.22	0.70	9.24	5.52	#O	
	protein 2	LPPA	390.19	0.70	10.41	5.52	O#	
		LQF	488.29	0.81	7.63	5.52	O#	
		LRY	415.22	0.51	8.96	8.75	##	LRY (J. Wu et al., 2006)

LSF	434.26	0.83	8.70	5.52	##	HPHPHLSF (Hernández-Ledesma et al., 2011)
LWLA	434.26	0.79	10.96	5.52	O#	
PAAP	360.19	0.54	8.66	5.96	#O	
PFQ	445.23	0.90	7.77	5.96	O#	PNNKPFQ (Kodera & Nio, 2006)
PLY	354.19	0.73	12.18	5.95	##	FNVPLYE (Ketnawa & Rawdkuen, 2013)
RFL	365.20	0.96	8.50	9.75	##	
RLF	478.29	0.95	8.50	9.75	##	
RLM	450.26	0.79	6.39	9.75	##	
VGFA	558.32	0.59	7.48	5.49	##	
WEL	446.22	0.61	9.48	4.00	##	
WKK	460.28	0.51	9.56	10.00	##	
WKKL	573.36	0.58	9.99	10.00	#O	
WLAL	396.24	0.85	10.96	5.52	##	
WLHQ	501.30	0.60	7.14	6.74	##	
WLQ	501.30	0.82	8.60	5.52	#O	
WR	495.25	0.98	8.59	9.75	##	MRWRD (García et al., 2013)
WRK	391.25	0.81	8.22	11.00	##	
YW	391.21	0.97	12.91	5.52	##	YW (J. Wu et al., 2006)
YWK	367.15	0.78	11.42	8.59	##	

F1MJ00	Immunoglobulin superfamily member 3	WLDRD	703.33	0.57	7.14	4.21	#O	
F1MJJ0	Dynein axonemal heavy chain 9	FRF	359.16	1.00	8.96	9.75	#O	
		FRT	422.23	0.78	6.12	9.75	O#	
		HFA	373.18	0.71	6.51	6.74	O#	
		HGF	447.22	0.94	6.00	6.74	##	
		PGFP	401.21	0.97	10.47	5.96	O#	
		PLNP	439.24	0.62	8.76	5.96	#O	
		RLLF	468.25	0.90	9.17	9.75	##	
		VHF	547.35	0.56	7.44	6.71	##	
		WHL	454.23	0.91	9.28	6.74	O#	
		WPH	438.20	0.95	9.47	6.74	##	JKWPHEHPP (Menin et al., 2008)
		WRP	457.24	0.98	9.53	9.75	#O	
		WSR	416.21	0.85	6.96	9.75	#O	
F1MK55	Dynein axonemal heavy chain 2	VGRF	477.27	0.84	6.69	9.72	##	
		YHFA	536.24	0.69	8.59	6.74	##	

F1MR22	Polymeric immunoglobulin receptor	LDPSF	577.27	0.77	8.21	3.80	##	
F1MRU4	Dynein axonemal heavy chain 3	MLFV	672.36	0.79	9.44	5.28	##	
		WQQVL	508.27	0.55	6.84	5.52	##	
F1N0A6	Adhesion G protein-coupled receptor V1	PSFF	457.24	0.98	9.96	5.96	##	
		WLW	496.23	0.99	12.83	5.52	O#	
		WPR	531.25	0.98	9.53	9.75	##	JKWPRP (Menin et al., 2008)
		WVMP	540.29	0.92	10.29	5.52	#O	
		YFLV	503.25	0.66	11.41	5.52	##	
F1N3S5	Ubiquitinyl hydrolase 1	MFTL	510.25	0.86	8.21	5.28	#O	
G5E556	ATP binding cassette subfamily A member 12	AAPW	443.22	0.91	9.86	5.57	O#	
P02453	Collagen alpha-1(I) chain	PGPP	366.19	0.92	10.34	5.96	##	GPPGPP (Oshima, Shimabukuro, & Nagasawa, 1979)

		APFPE	507.23	0.72	8.88	4.00	##	VAPFPEVF (Sagardia et al., 2013)
		APSFS	709.39	0.58	6.70	5.57	##	LGTQYTDAPSFSDIPNPIGSENSEK (Minervini et al., 2003)
		FPEVF	507.28	0.80	9.56	4.00	##	FPEVFGK (Sagardia et al., 2013)
		FRQFY	400.28	0.92	8.31	8.75	##	FRQFYQL (Contreras et al., 2009)
		FYPE	684.31	0.71	10.12	4.00	##	FYPEL (Gu et al., 2011)
		FYPEL	759.37	0.79	10.39	4.00	##	FYPEL (Gu et al., 2011)
		FYQLD	434.26	0.60	8.15	3.80	##	
		LFR	434.26	0.94	8.50	9.75	O#	LFRQ (Mine & Shahidi)
P02662	Alpha-S1-casein	LFRQF	513.36	0.92	7.70	9.75	#O	
		LLRF	567.32	0.90	9.17	9.75	##	LLRF (Gútiez et al., 2013)
		LLRL	559.26	0.55	8.80	9.75	##	
		LRF	468.25	0.95	8.50	9.75	#O	LRF (J. Wu et al., 2006)
		LRFF	450.26	0.98	9.50	9.75	##	NENLLRFFVAPFPE (Robert et al., 2004)
		LRL	547.35	0.56	7.97	9.75	##	
		PFPE	581.33	0.74	9.61	4.00	##	PFPE (Kopf-Bolanz et al., 2014)
		PFPEVF	637.31	0.82	10.01	4.00	##	VAPFPEVF (Contreras et al., 2009)
		PLW	646.31	0.98	12.44	5.96	#O	PLW (J. Wu et al., 2006)
		RFF	488.23	0.99	8.96	9.75	##	NENLLRFFVAPFPE (Robert et al., 2004)
		RFFV	432.24	0.92	8.85	9.75	##	NENLLRFFVAPFPE (Robert et al., 2004)

		RYL	351.18	0.56	8.96	8.75	#O	VRYL (Murray & FitzGerald, 2007)
		RYLG	734.36	0.52	7.96	8.75	##	RYLGY (Puchalska et al., 2015)
		TMPLW	554.24	0.91	9.57	5.19	##	TTMPLW (Hernández-Ledesma et al., 2011)
		VAPF	529.29	0.78	9.70	5.49	##	FVAPFP (Gútiez et al., 2013)
		VAPFP	667.32	0.82	10.31	5.49	##	FVAPFP (Gútiez et al., 2013)
		YFYPE	667.32	0.68	10.85	4.00	##	AYFYPE (Yamamoto, Akino, & Takano, 1994)
		YLG	717.30	0.64	9.98	5.52	O#	RYLGY (Hernández-Ledesma et al., 2011)
		YLGY	514.24	0.61	11.04	5.52	##	RYLGY (Hernández-Ledesma et al., 2011)
		YLGYL	627.33	0.74	11.17	5.52	##	
		YPELF	414.23	0.77	10.39	4.00	O#	
		FPQYL	518.29	0.91	8.99	5.52	##	FPQYLQY (Sagardia et al., 2013)
		KFPQ	392.21	0.67	7.62	8.75	##	YQKFPQY (Hernández-Ledesma et al., 2011)
P02663	Alpha-S2-casein	KFPQY	666.34	0.68	8.99	8.59	#O	YQKFPQY (Hernández-Ledesma et al., 2011)
		LNPW	681.35	0.92	9.75	5.52	##	
		MKPW	415.19	0.93	9.91	8.50	##	AMKPW (Sagardia et al., 2013)
		NFL	528.27	0.89	8.04	5.52	##	

		NPW	560.28	0.95	9.20	5.52	##	NPW (Jianping Wu, Rotimi E. Aluko, & Shuryo Nakai, 2006)
		YLQYL	698.36	0.53	9.95	5.52	O#	
		YLY	457.22	0.54	12.42	5.52	##	YLYEIA (Sagardia et al., 2013)
		AFL	550.22	0.93	9.83	5.57	#O	AFL (He et al., 2013)
		DKIHPF	421.23	0.69	8.30	6.74	O#	DKIHPF (Sagardia et al., 2013)
		FPPQ	663.31	0.93	8.81	5.52	##	FPPQSVL (Contreras et al., 2009)
		FPPQS	477.26	0.71	7.51	5.52	##	FPPQSVL (Contreras et al., 2009)
		HLP	532.26	0.58	7.98	6.74	O#	LHLPLP (Hernández-Ledesma et al., 2011)
		HLPL	574.28	0.63	8.87	6.74	##	HLPLP (Martínez-Maqueda et al., 2012)
		HLPLP	659.34	0.75	9.68	6.74	##	HLPLP (Martínez-Maqueda et al., 2012)
		HPF	365.21	0.95	8.56	6.74	##	HPFA (P. Zhou et al., 2013)
P02666	Beta-casein	KFQ	422.16	0.52	6.38	8.75	##	
		LLY	618.28	0.50	11.87	5.52	##	LLYQEP (Gútiez et al., 2013)
		LPLL	717.35	0.72	11.55	5.52	##	PLPLL (Puchalska et al., 2015)
		LPLP	755.40	0.79	11.82	5.52	##	LPLP (Phelan & Kerins, 2011)
		LPPT	399.19	0.59	9.55	5.52	O#	QSWMHQPHQPLPPTVM (Contreras et al., 2009)
		LQSW	487.24	0.59	7.23	5.52	O#	LQSW (Hernández-Ledesma et al., 2011)
		LQSWM	478.29	0.79	7.02	5.52	##	

MFPPQ	503.24	0.92	8.26	5.28	#O	
PLPL	587.30	0.84	11.82	5.96	##	PLPLL (Puchalska et al., 2015)
PLPLL	426.25	0.84	11.80	5.96	##	PLPLL (Puchalska et al., 2015)
PPF	575.34	0.99	12.13	5.96	##	PPF (X. Wang et al., 2011)
PVEPF	349.20	0.60	9.40	4.00	##	YPVEPF (Abd El-Salam & El-Shibiny, 2012)
PYPQ	557.32	0.64	9.22	5.95	##	AVPYPQ (Robert et al., 2004)
PYPQR	579.27	0.65	7.84	9.18	##	AVPYPQR (Hernández-Ledesma et al., 2011)
QAFL	458.25	0.79	7.06	5.52	##	
QSWM	454.32	0.87	6.05	5.52	##	QSWMHQPHQPLPPTVM (Contreras et al., 2009)
SWM	524.26	0.96	8.04	5.24	##	QSWMHQPHQPLPPTVM (Contreras et al., 2009)
VMFPPQ	551.37	0.69	8.27	5.49	O#	
VPPF	438.28	0.93	11.31	5.49	#O	VVVPPF (Gútiez et al., 2013)
VVPPF	438.28	0.75	10.79	5.49	##	VVVPPF (Gútiez et al., 2013)
VYPF	407.24	0.87	11.63	5.49	##	VYPFPG (Martínez-Maqueda et al., 2012)
YPFPG	359.18	0.96	11.28	5.52	##	VYPFPG (Martínez-Maqueda et al., 2012)

P80195	Glycosylation-dependent cell adhesion molecule 1	FSHAF	607.28	0.91	7.04	6.74	#O
		LKSLF	606.37	0.55	8.94	8.75	O#

"#" indicates that peptide is detected in the sample, and "O" indicates that peptide is not detected in the sample.

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CRediT authorship contribution statement

Nan Wu: Conceptualization, Data curation, Formal analysis, Methodology, Project administration, Resources, Writing - original draft. **Fengmei Zhang:** Conceptualization, Resources, Project administration. **Quan Shuang:** Conceptualization, Resources, Project administration.

Highlights

ACE inhibitory peptides in fermented milk were screened by peptidomics.

UHP enhanced ACE inhibitory activity and changed peptide sequence in fermented milk.

130 peptides with potential ACE inhibitory activity were reported.

Eleven novel ACE inhibitory peptides were reported.

VAPFP had the highest ACE inhibitory activity with inhibition rate 10.56 μmol\/L.

Declaration of interests

☒ 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.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Graphical Abstract