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Novel β-casein derived antioxidant and ACE-inhibitory active peptide from camel milk fermented by *Leuconostoc lactis* PTCC1899: Identification and molecular docking

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ABSTRACT

Antioxidant and angiotensin-I converting enzyme inhibitory (ACE-I) activities of bioactive peptide fractions from camel milk fermented by *Leuconostoc lactis* PTCC 1899 were assessed. The fraction <3 kDa obtained from ultrafiltration showed ACE-I (IC_{50} = 1.61 ± 0.18 mg mL^{-1}) and ABTS radical scavenging (1883.39 μM TE mg^{-1} protein) activities and was purified through RP-HPLC. The active peptide, MVPYPQR, with antioxidant (8933.05 μM TE mg^{-1} peptide) and ACE-I (IC_{50} = 30 μM) activities was identified. To investigate the ACE-I mechanism of the purified peptide the docking study was performed. The presence of hydrogen bond between Gln 162 (S'1 pocket of ACE) and Arg in the C terminal of the peptide was identified and the peptide could distort the Zn^{2+} tetrahedral geometry of the enzyme. This study showed the ability of *Leuc. lactis* to produce a novel and safe functional food by hydrolysing the milk proteins during the fermentation.

1. Introduction

Parallel to the modern lifestyle of people, especially in developed countries, the prevalence of diseases such as obesity, cancer, hypertension, and diabetes is increasing. Dietary factors are strongly linked to these types of health problems around the world. (Hernandez-Ledesma, del Mar Contreras, & Recio, 2011). In this regard, a great deal of interest has been expressed to use functional foods for preventing disease and improving health (Hafeez et al., 2014). Dietary proteins with encrypted bioactive peptides (BAPs) in their sequence could be used for developing functional foods (Korhonen & Pihlanto, 2006). Milk-derived peptides may functionalise it to act as a regulatory substance with antioxidant, hypotensive, immunomodulatory or other different bioactivities (Ibrahim, Isono, & Miyata, 2018; Osuntoki & Korie, 2010; Solanki & Hati, 2018; Virtanen, Pihlanto, Akkanen, & Korhonen, 2007; Xue et al., 2018). One of the main processes for producing BAPs is microbial fermentation of the protein sources such as milk through the proteolytic starter and non-starter cultures (Korhonen, 2009). In this regard, Nakamura, Yamamoto, Sakai, and Takano (1995) identified two antihypertensive active peptides Val-Pro-Pro and Ile-Pro-Pro from a sour milk product.

Camel milk is a potential substitute for bovine milk especially in the arid areas of the world such as Africa and Middle-East (Khalesi, Salami, Moslehishad, Winterburn, & Moosavi-Movahedi, 2017). In contrast to bovine milk, β-lactoglobulin as not present in camel milk; Therefore, it is suggested to use as infant feeding to prevent allergy caused by bovine milk (Homayouni-Tabrizi, Shabestarin, Asoodeh, & Soltani, 2016; Salami et al., 2009). Proteolytic microorganisms such as lactic acid bacteria (LAB) could hydrolyse milk proteins mostly caseins into BAPs (Hernandez-Ledesma et al., 2011). Despite the fact of the lower ratio of casein to whey proteins in camel milk, caseins are a promising source for producing BAPs from camel milk (Elsayed & Agamy, 2009; Khalesi et al., 2017). Homayouni-Tabrizi et al. (2016) hydrolysed camel milk by digestive proteases and reported antioxidant peptides in the hydrolysate. Camel milk-derived peptides could be considered as prominent supplements in developing health-promoting functional foods and producing commercial products with specific bioactivities.

In our previous study, camel milk was fermented by lactic acid bacteria isolated from several samples of traditional fermented camel milk (Chal) produced in Turkman Sahra, Iran (Soleymanzadeh, Mirdamadi, & Kianirad, 2016). The present study assessed the contribution of *Leuconostoc lactis* PTCC1899, the strain

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isolated from Chal to develop antioxidant and angiotensin-1 converting enzyme inhibitory (ACE-I) activities in controlled fermented camel milk.

2. Materials and methods

2.1. Materials

Camel milk used in the study was obtained from Turkman Sahra, Golestan Province, Iran. All chemicals used for assessing the ACE-I and antioxidant activities were purchased from Sigma–Aldrich Chemie GmbH (Munich, Germany) and others were from Merck, Darmstadt, Germany.

2.2. Analysis of camel milk composition

Chemical composition of camel milk was analysed. Titratable acidity (TA) was calculated using the titrimetric method of AOAC No. 947.05 and pH was measured with a potentiometer. Crude protein (CP) was determined according to AOAC method No. 998.05. A mid-infrared analyser (Milko-Scan S50; Foss Electric, Hillerød, Denmark) was used to analyse milk fat and lactose (AOAC No. 972.16). Total solids (TS) was calculated (fat + solid not fat; SNF) (AOAC, 2002).

2.3. Microorganism and camel milk fermentation

Pre-culture of *Leuc. lactis* PTCC 1899 was prepared by adding overnight culture to sterile MRS broth (Biolife, Italy) and incubated at 37 °C for 24 h. The bacteria were separated by centrifuging the pre-culture at 5000× *g* for 15 min. The camel milk was pasteurised at 70 °C for 20 min and was inoculated by the bacterial biomass. The final population of bacteria in milk was approximately 10^7 cfu mL^{-1}. Inoculated milk samples were incubated at 37 °C for 24 h. After fermentation, the pH of the fermented sample and unfermented camel milk as a control was adjusted at 4.6 and centrifuged at 20,000× *g* for 20 min. The supernatant (whey fraction) was kept at −20 °C for further analysis. Fresh camel milk was used as a control.

2.4. Characterisation of whey fraction

The Lowry method (Hartree, 1972) with some modifications and a spectrophotometric method for measuring proteins by Layne (1957) were used to measure the protein content of the peptide fractions. The OPA method according to Church, Swaisgood, Porter, and Catignani (1983) was used to assess the protein hydrolysis after fermentation. L-Leucine served as a standard. One millilitre OPA reagent was added to 20 μL sample and vigorously vortexed. The absorbance was detected spectrophotometrically at 340 nm after 2 min incubation at ambient temperature.

2.5. Determination of ACE-I activity

Rabbit lung acetone powder was obtained according to Lossow, Migliorini, Brot, and Chaikoff (1964). ACE-I activity was evaluated through the spectrophotometric method using rabbit lung acetone extract containing ACE-I by Vermeirssen, Van Camp, and Verstraete (2002) with some modifications. Peptide fractions (25 μL) were added to 75 μL FAPGG (5 mM) in 50 mM Tris–HCl buffer (pH: 8.3) containing 400 mM NaCl in ELISA plate wells. The control was distilled water instead of peptide fraction. Then, 10 μL enzyme extract was added to each well to start the enzymatic reaction. The absorbance of the samples was measured at 340 nm every 1 min over 30 min of time interval by an ELISA reader Expert 96 (Bio Tek, Winooski, USA). The ACE-I activity was calculated using the formula (equation (1)):

$$\text{ACE} - \text{I activity}(\%) = \left(1 - \frac{\Delta A\ sample}{\Delta A\ control}\right) * 100 \quad (1)$$

where ΔA sample and ΔA control are the decrease in absorbance of samples and control, respectively. The concentration (mg mL^{-1}) of a sample that inhibited 50% of ACE activity was considered as an IC_{50} value.

2.6. Determination of antioxidant activity

2.6.1. DPPH radical inhibition

The method developed by Son and Lewis (2002) was used for the evaluation of DPPH free radical inhibition. For this purpose, 900 μL of a stock solution (0.002 g DPPH in 100 mL 96% ethanol) was mixed with 100 μL of the sample and was kept for 30 min in the dark. The spectrophotometric read was done at 517 nm (PG Instruments Ltd., model T80+). Ethanol served as the blank. The following equation (2) was used to calculate the percentage of antioxidant activity:

$$\%\text{Activity} = \frac{\text{Absorbance 517 Blank} - \text{Absorbance 517 sample}}{absorbance\ 517\ blank} \times 100 \quad (2)$$

The antioxidant activity was expressed as Trolox equivalent antioxidant capacity (TEAC) using Trolox as a standard antioxidant.

2.6.2. ABTS radical inhibition

The ability to inhibit ABTS free radicals was evaluated according to (Re et al., 1999). One millilitre of the ABTS free radical solution (diluted with phosphate buffer, pH 7.4) was mixed with 25 μL of each sample. The absorbance was read at 734 nm after 2 min. Distilled water served as the blank. The following equation (3) was used to calculate the percentage of antioxidant activity:

$$\%\text{Activity} = \frac{\text{Absorbance 734 Blank} - \text{Absorbance 734 sample}}{absorbance\ 734\ blank} \times 100 \quad (3)$$

The antioxidant activity was expressed as Trolox equivalent antioxidant capacity (TEAC) using Trolox as a standard antioxidant.

2.7. Purification of ACE-I and antioxidant active peptide

The whole whey fraction was passed though ultrafilter membranes with cut off of 3, 5 and 10 kDa (Biotech Company, Geottingen, Germany). RP-HPLC (250 × 4.6, 5 μm, 100 Å) was used for further purification of the active fraction. The peptide fraction (20 μL) was eluted with a mixture containing a linear gradient of solvents A (0.1% trifluoroacetic acid in distilled water) and B (0.1% trifluoroacetic in acetonitrile). The peptide fraction was eluted at the flow rate of 0.6 mL min^{-1} for 45 min and monitored at 215 nm. The peptide fraction with a suitable antioxidant and ACE-I activity was identified by MALDI TOF/TOF MS.

2.8. Active peptide identification

The analysis of peptide fraction was performed with MALDI-TOF/TOF/MS on a 5800 proteomics analyser (Proteomics

International Pty Ltd., Nedlands, Western Australia) (Bringans, Kendrick, Lui, & Lipscombe, 2008).

2.9. Analysis of the physicochemical properties of the peptide

Online tools were used to predict the physicochemical properties of the peptide. The peptide property calculator (https://pepcalc.com/) was used to determine isoelectric point (PI), net charge, and water solubility. The ExPASy ProtParam tool (https://web.expasy.org/cgi-bin/protparam/protparam) was used to predict the instability. The instability index estimates the stability of the peptide in a test tube. The instability index below 40 predicts that the peptide might be stable and the protein or peptide whose the instability index is above 40 may be unstable. The hydrophobicity of the peptide was determined using the PEPTIDE 2.0 https://www.peptide2.com.

2.10. Molecular docking studies

Human ACE-inhibitor (lisinopril) crystal complex (PDB ID108A) was obtained from the RCSB Protein Data Bank (http://www.rcsb.org/pdb/home/home.do//). All the water molecules and inhibitor were removed in the protein. The docking studies were carried out using HADDOCK software at the presence of cofactors zinc and chloride atoms (van Zundert et al., 2016). The molecular structure of the peptide MVPYPQR was prepared and energy minimized by Hyperchem software.

The values of energy binding and scores were used to consider the best ranked docking pose of the peptide MVPYPQR. The interactions between the peptide and ACE were detected using the discovery studio 2016 software. The ability of the peptide to distort the Zn (II) tetrahedral geometry of the enzyme was investigated using the ligplot viewer.

2.11. Statistical analysis

SPSS 16.0 software (SPSS Inc., Chicago, IL, USA) was used for data analysis through ANOVA test. The Tukey's HSD multiple comparison tests were used to compare the individual samples and the level of significance was 0.05.

3. Results and discussion

3.1. Composition of camel milk

Camel milk was analysed in terms of chemical composition. The value of crude protein and fat were obtained as 2.84 ± 0.12 and 4.00 ± 0.20 g 100 g^{-1}, respectively. Lactose content was 3.44 ± 0.08 g 100 g^{-1}. Mean values for the pH and titratable acidity were 6.47 ± 0.04 and 19 ± 0.09 (°D), respectively.

3.2. Preparation of fermented camel milk whey fraction

In the previous study, several LAB strains were isolated from Chal samples. The impact of single LAB strains on antioxidant activity of camel and bovine milk after fermentation was investigated. Both camel and bovine milk samples fermented by the strain *Leuc. lactis* showed significant antioxidant activity as well as acceptable sensory properties (Soleymanzadeh et al., 2016). Considering the mentioned results, *Leuc. lactis* was selected and used as a starter culture for controlled fermentation of camel milk in the present study (Fig. 1). The pH of the fermented sample was measured as 4.3, which was adjusted at 4.6 to separate casein (PI: 4.6) and whey fractions. During fermentation, camel milk proteins were hydrolysed by bacterial enzymes. The increase of free NH_2 groups from 1.25 ± 0.097 mg mL^{-1} in control (whey fraction from unfermented camel milk) to 4.52 ± 0.00 mg mL^{-1} in whey fraction obtained from fermented camel milk was an indication of the proteolytic activity of *Leuc. lactis* (Fig. 2).

Following separation of caseins, the supernatant (whey fraction) was tested for antioxidant and ACE-I activities. An increase in antioxidant activity of the fermented sample was observed based on both ABTS and DPPH radical scavenging methods, in comparison with the control. As indicated in Fig. 3, the antioxidant activity based on DPPH radical scavenging method was increased from 7.37 to 8.35 μM TE mg^{-1} protein. Also, antioxidant activity increased from 454.57 in control to 899.34 μM TE mg^{-1} protein in fermented whey fraction based on the ABTS radical scavenging method. This observation indicates the ability of *Leuc. lactis* in releasing antioxidant peptides during the fermentation through proteolytic activity. Previously, other researchers reported the increase of antioxidant activity in different milk samples fermented by LAB including *Pediococcus pentosaceus* and *Lactobacillus rhamnosus* comparing with the respective unfermented controls (Balakrishnan & Agrawal, 2014; Moslehishad et al., 2013). The observed activity may be a result of the hydrolysis progress and specific activity of proteolytic enzymes produced by *Leuc. lactis*, because the antioxidant activity is related to the peptide profile in the product than the amount of hydrolysis (Virtanen et al., 2007). The ACE-I activity (IC_{50}) was reported as the concentration (mg mL^{-1}) of whey fraction needed to inhibit the activity of ACE by 50%. Due to the proteolytic activity of *Leu. Lactis* during the fermentation, the ACE-I peptides were produced, and the IC_{50} value was evaluated as 3.48 ± 0.12 mg mL^{-1}, while the control sample did not show any inhibitory activity (Fig. 4).

3.3. Purification of ACE-I and antioxidant active peptide

The whey fraction obtained from fermented camel milk was passed through ultrafilter membranes with cut-off of 3, 5 and 10 kDa. Protein contents and free NH_2 groups were determined in permeates, and the antioxidant and ACE-I activities were tested. The most effective fraction for the DPPH radical scavenging activity was the <3 kDa fraction (16.78 μM TE mg^{-1} protein) (Fig. 3A), while the highest ABTS radical scavenging activity (2151.58 μM TE mg^{-1} protein) was observed for the 5–10 kDa fraction (Fig. 3B). The difference observed in DPPH and ABTS radicals scavenging activities may be due to the different properties of DPPH and ABTS radicals. This is consistent with the results stated by other researchers. In the study by Mirzaei, Mirdamadi, Ehsani, and Aminlari (2018), the DPPH and ABTS radical scavenging activities for *Kluyveromyces marxianus* protein hydrolysate were reported as 118.53 and 489.53 μM TE mg^{-1} protein, respectively. As was stated in the studies by Floegel, Kim, Chung, Koo, and Chun (2011) the antioxidant value assayed by ABTS radical scavenging method was significantly higher compared with the DPPH assay. ABTS assay could be used for assessing the hydrophilic and lipophilic antioxidant systems while the DPPH method is better for only the peptides with hydrophobic properties. In another research conducted by our group the fraction 5–10 kDa from fermented camel milk was reported to have the most considerable ABTS radical scavenging activity which is consistent with the present study (Moslehishad et al., 2013). In another study by Mirzaei, Mirdamadi, Ehsani, Aminlari, and Hosseini (2015) on the hydrolysates from *Saccharomyces cerevisiae*, the 5–10 kDa fraction in the autolysis sample showed the best ABTS radical scavenging activity.

The IC_{50} value for ACE-I activity for the fractions was determined, and the <3 kDa fraction showed the highest activity (IC_{50} = 1.61 ± 0.18 mg mL^{-1}), and there was no significant difference between 5 and 10, 3–5 and <3 kDa fractions (Fig. 4).

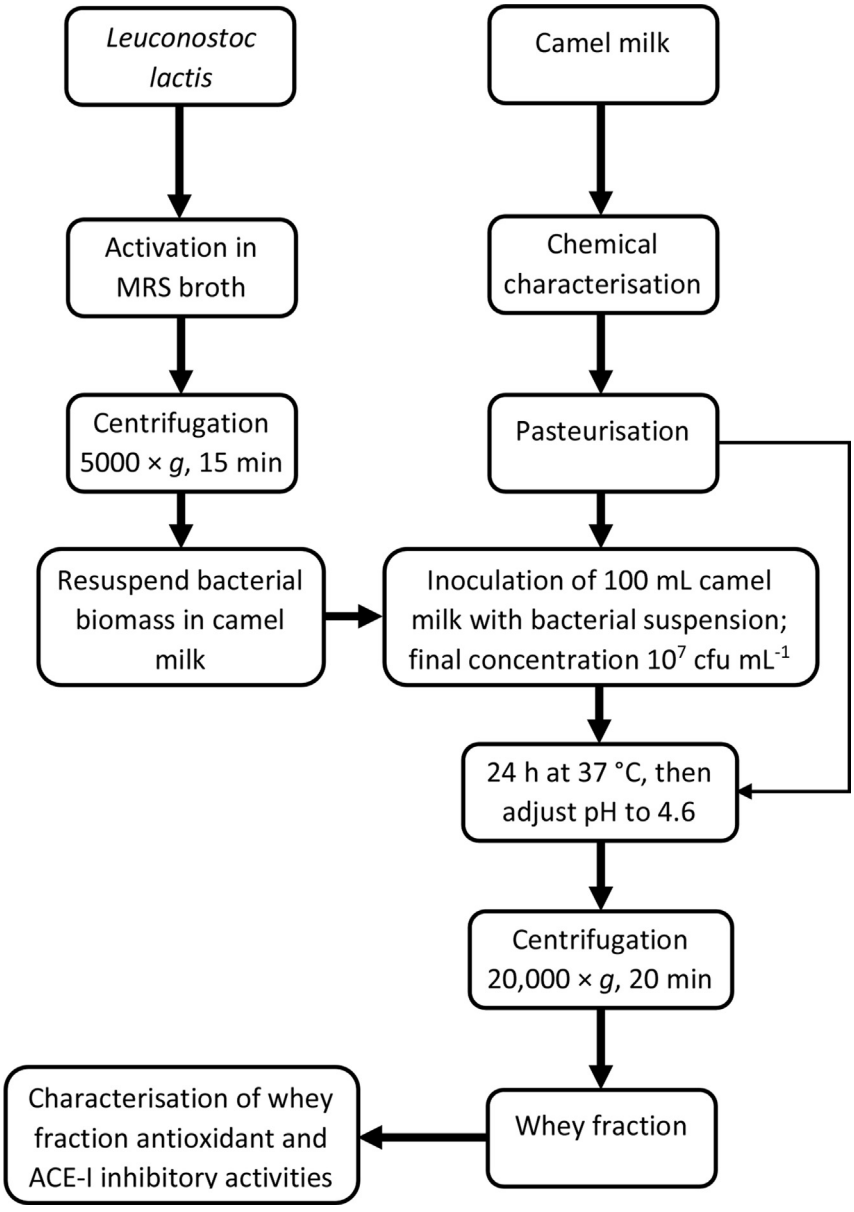

Fig. 1. Camel milk fermentation.

Moslehishad et al. (2013) reported the IC_{50} value of 1.45 ± 0.015 mg mL^{-1} for the <3 kDa fraction obtained from camel milk fermented by *Lb. rhamnosus*, which is in agreement with the result of this study. This result indicates that smaller peptides show the highest ACE-I activity. Pihlanto-Leppala, Koskinen, Piilola, Tupasela, and Korhonen (2000) indicated that peptides < 1 kDa show higher ACE-I activity. The small size peptides have better accessibility to the active site of the enzyme due to the protection of two active sites in N and C-terminal positions of ACE by an N terminus lid (Norris & FitzGerald, 2013).

The <3 kDa fraction was chosen and subjected to RP-C_{18} column with gradient elution and fractionated to further fractions (Fig. 5). Antioxidant and ACE-I assays were performed for the HPLC fractions. ABTS radical scavenging assay showed that almost all peptide fractions harbour antioxidant activity. The range of ACE-I and antioxidant activities in HPLC fractions were 2.3–27 μg mL^{-1} (Figs. 4) and 1175.04–12,290 μM TE mg^{-1} peptide (Fig. 6), respectively. Eventually, the F_6 fraction which was a sharper and higher peak showing both ACE-I and antioxidant activities was analysed to determine the sequence of amino acids and molecular mass (Fig. 7). The molecular mass of the peptide was 890.30 Da and consisted of 7 amino acids with the sequence of Met-Val-Pro-Tyr-Pro-Gln-Arg. Homology search showed that the obtained sequence resembled the residues 193–199 of β-casein in *Camelus dromedarius*. The sequence also showed high similarity with the residues 192–198 of β-casein in *Bos Taurus* (AVPYPQR) which is because of the high homology of camel and bovine casein proteins. It has been demonstrated that during the milk fermentation BAPs could be produced by the starter cultures with proteolytic activity (Jäkälä & Vapaatalo, 2010). Until now, about 200 bioactive peptides have been reported from camel milk proteins in which 15–20 percent have been identified as antioxidant and ACE-I active peptides (Khalesi et al., 2017). The peptide sequence obtained in this study contains four hydrophobic, two polar and one charged amino acids and the hydrophobicity of the peptide was estimated as 57.14% which shows moderate hydrophobicity of the peptide. Other

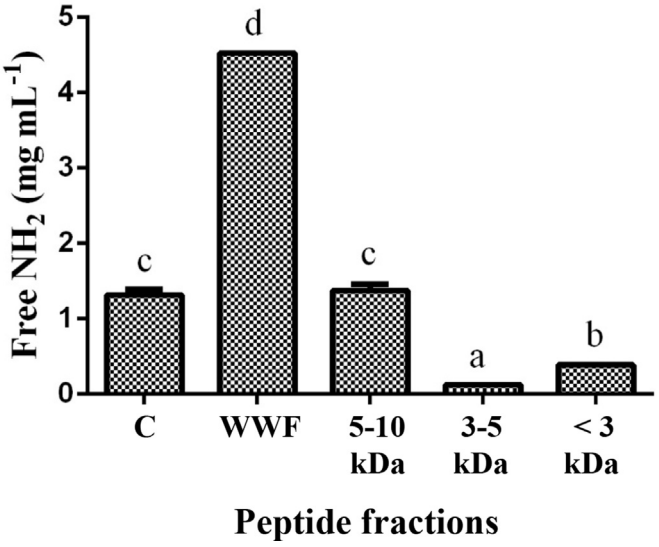

Fig. 2. Amount of free NH_2 groups in control (unfermented whey fraction), whole whey fraction (WWF) obtained from camel milk fermented by *Leuconostoc lactis* and distribution of them among different molecular mass fractions (5–10 kDa, 3–5 kDa and <3 kDa). The experiments were done in three replicates; significantly different values are shown with different letters ($P < 0.05$).

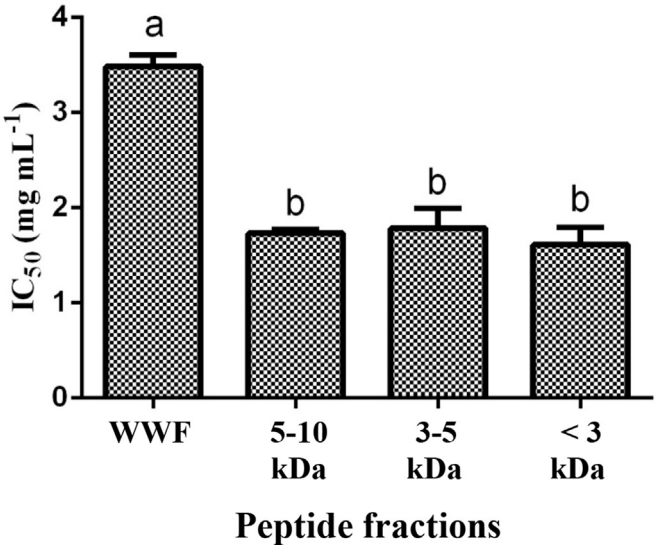

Fig. 4. The ACE-I activity of whey obtained from fermented camel milk (WWF) and its different molecular mass fractions (5–10 kDa, 3–5 kDa and <3 kDa). The experiments were done in three replicates; significantly different values are shown with different letters ($P < 0.05$).

physical properties were: a net charge of 1; theoretical pI 9.59; instability index 81.23 (i.e., unstable); aliphatic index 41.43, GRAVY index −0.914; water solubility, poor).

The presence of amino acids with hydrophobic properties in the sequence has a significant impact on the antioxidant activity, which is due to solubility in lipid. Tyr and Met are amino acids which are considered to possess antioxidant activity. Aromatic amino acids such as Tyr could quench electron deficient radicals by donating electron (Asoodeh, Homayouni-Tabrizi, Shabestarian, Emtenani, & Emtenani, 2016; Saiga, Tanabe, & Nishimura, 2003; Shabestarian, Asoodeh, Homayouni-Tabrizi, & Hossein-Nejad-Ariani, 2017). There are two Pro and one Val within the sequence, and there are reports on contributing these amino acids in antioxidant activity (Chen, Muramoto, & Yamauchi, 1995; Mirzaei et al., 2018). Rival, Boeriu, and Wichers (2001) worked on several peptides from bovine casein and reported the peptide AVPYPQR that showed the highest antioxidant activity among the others. They attributed the activity of the peptide to the presence of Tyr in the sequence apart from hydrophobicity.

The accessibility of the peptide to the active site of ACE is substantially related to the hydrophobic amino acids that are present in the peptide sequence. ACE-I activity of MR7 peptide could be due to hydrophobicity. Aliphatic chained amino acids like Val in the sequence could enhance ACE-I activity of the peptide. Moreover, the previous researches reported that the presence of Pro in every three positions of the C terminal residues could positively affect the ACE-I activity of the peptide (Girgih, He, & Aluko, 2014; Li, Le, Shi, & Shrestha, 2004). Besides, it has been demonstrated that Pro in the antepenultimate position in the peptide sequence could enhance peptide binding to the enzyme (Xue et al., 2018). Moreover, the presence of positively charged amino acids in C terminal of the peptide such as Arg could contribute in ACE-I activity and cause a substantial increase in inhibitory potency (Ferreira et al., 2007; Li et al., 2004; Solanki & Hati, 2018). The peptide AVPYPQR obtained from tryptic bovine casein with the C terminal identical to the peptide, MVPYPQR, was reported to show hypotensive activity in spontaneously hypertensive rats (Karaki et al., 1990).

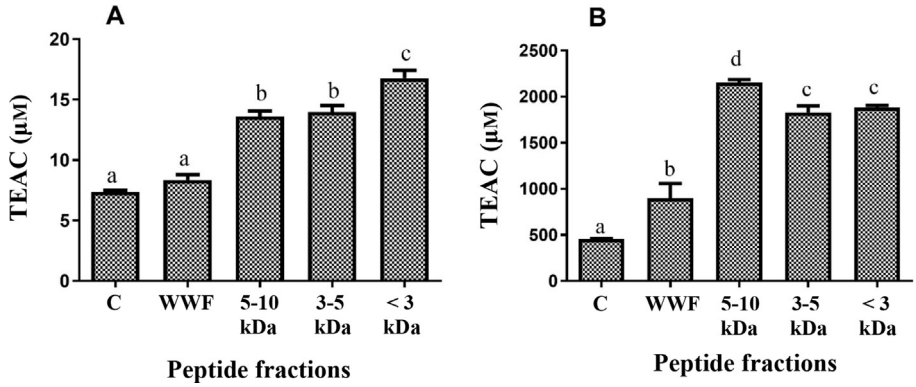

Fig. 3. DPPH (A) and ABTS (B) radical scavenging activities in control (unfermented whey fraction), whole whey fraction (WWF) obtained from camel milk fermented by *Leuconostoc lactis* and distribution of them among different molecular mass fractions (5–10 kDa, 3–5 kDa and <3 kDa). The experiments were done three in replicates; significantly different values are shown with different letters ($P < 0.05$).

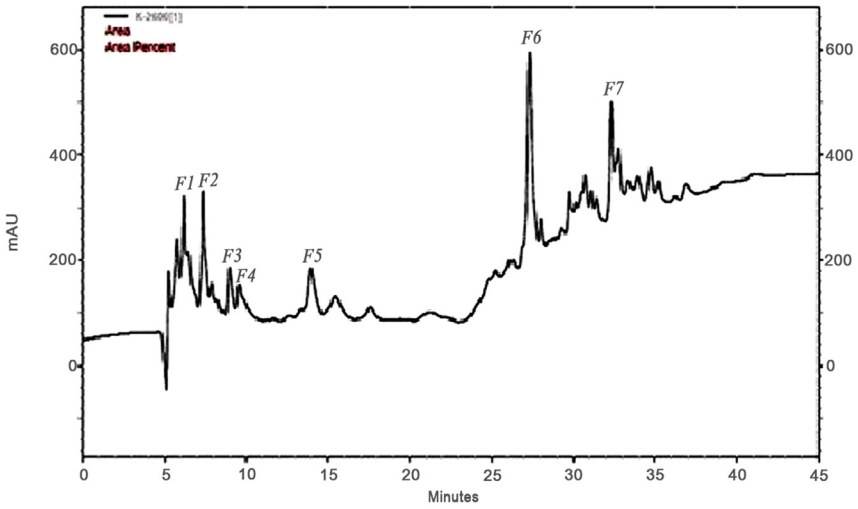

Fig. 5. RP-HPLC chromatogram of whey fraction after fermentation with the size of <3 kDa. The ACE-I activity (IC_{50}) of the peptide fractions was (μg mL^{-1}): F2, 4.9 ± 0.9[b]; F3, 27 ± 0.0[a]; F4, 2.3 ± 0.5[c]; F6, 27 ± 2.3[a] (significantly different values are indicated by the different superscript letters; $P < 0.05$). The experiments were done in three replicates; ACE-I inhibitory activity was not detected in fractions F1, F5 and F7.

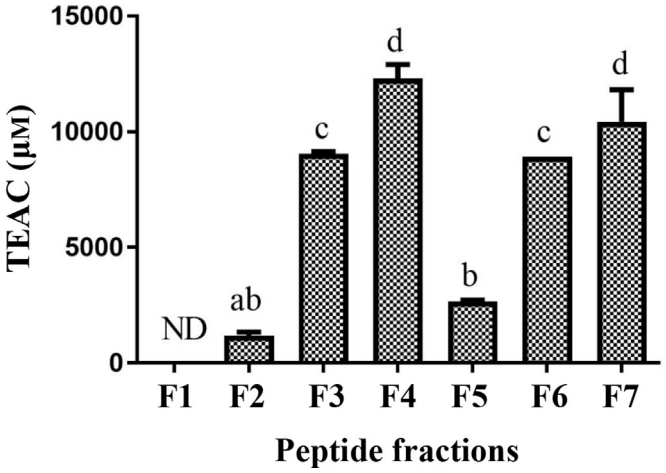

Fig. 6. ABTS radical scavenging activity of purified peptide fractions. The experiments were done in three replicates; significantly different values are shown with different letters ($P < 0.05$).

3.4. Molecular docking studies

The flexible tool of HADDOCK software was used to study the mechanism of interactions between MR7 and ACE. The Fig. 8 shows the best pose of MR7 within the ACE active site in the presence of Zn^{2+} with the Z score of −1.6. Pan, Cao, Guo, and Zhao (2012) stated that among the different interaction forces between the inhibitor and ACE, the hydrogen bonds have an essential role in stabilising the docking complex. As indicated in Fig. 8, there were three predicted hydrogen bonds between the Arg in the C terminal of the peptide and the residues Glu 162, Glu 376, and Asn 167 of ACE. Another hydrogen bond was observed between the second Pro in the peptide sequence and Lys 343 of the ACE structure that indicates the substantial effect of Arg in the C terminal on the interaction of the MR-7 and ACE. However, the other interaction forces including van der Waals, salt bridge and carbon hydrogen-bonds are involved in the interaction of the peptide and ACE. The molecular docking study revealed that there was a hydrogen bond between the Arg of the MR-7 and Glu 162 of

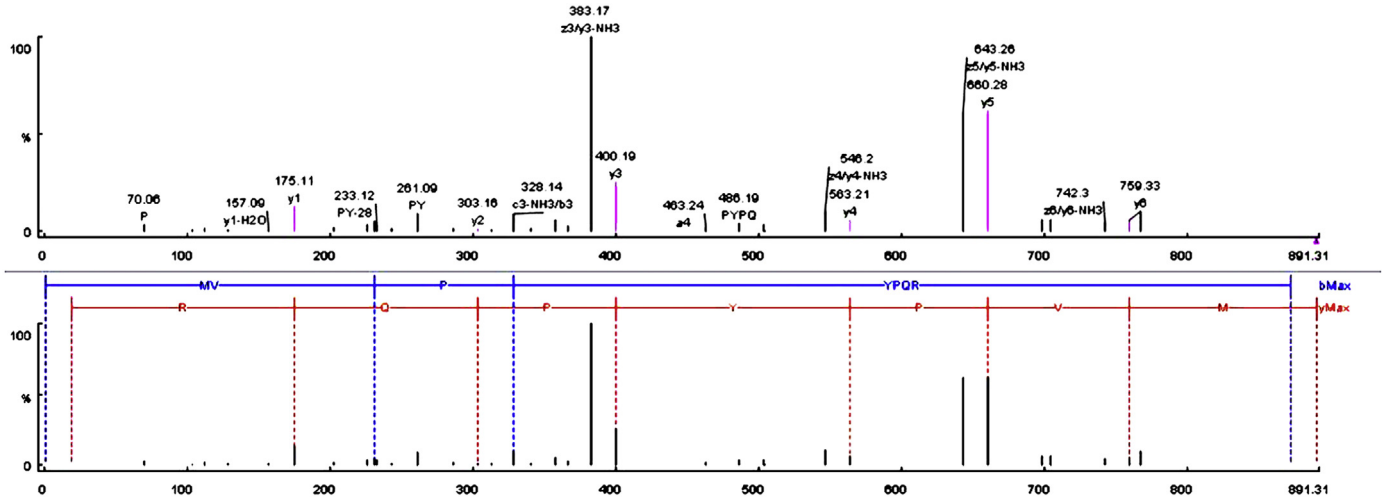

Fig. 7. Determination of the molecular mass and identification of the peptide with de novo spectra for the peptide MVPYPQR.

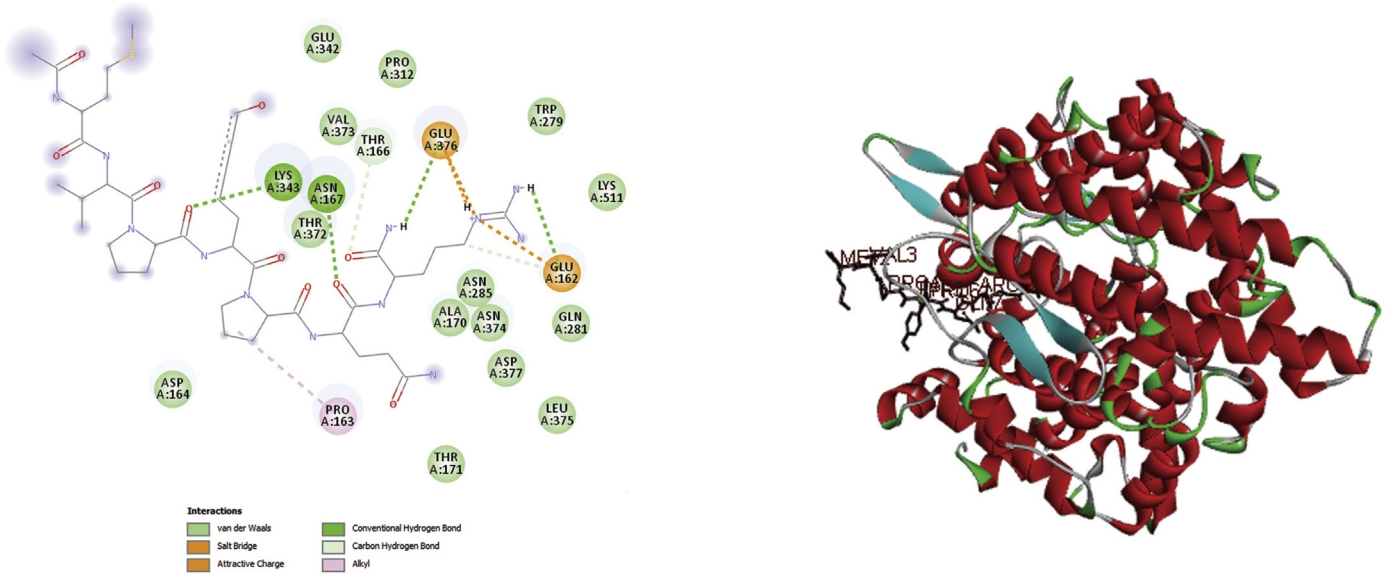

Fig. 8. The best docking pose of MVPYPQR at ACE catalytic site (PDB: 1O8A). Two-dimensional diagram and general overview. Accelrys DS Visualizer software was used to create the image.

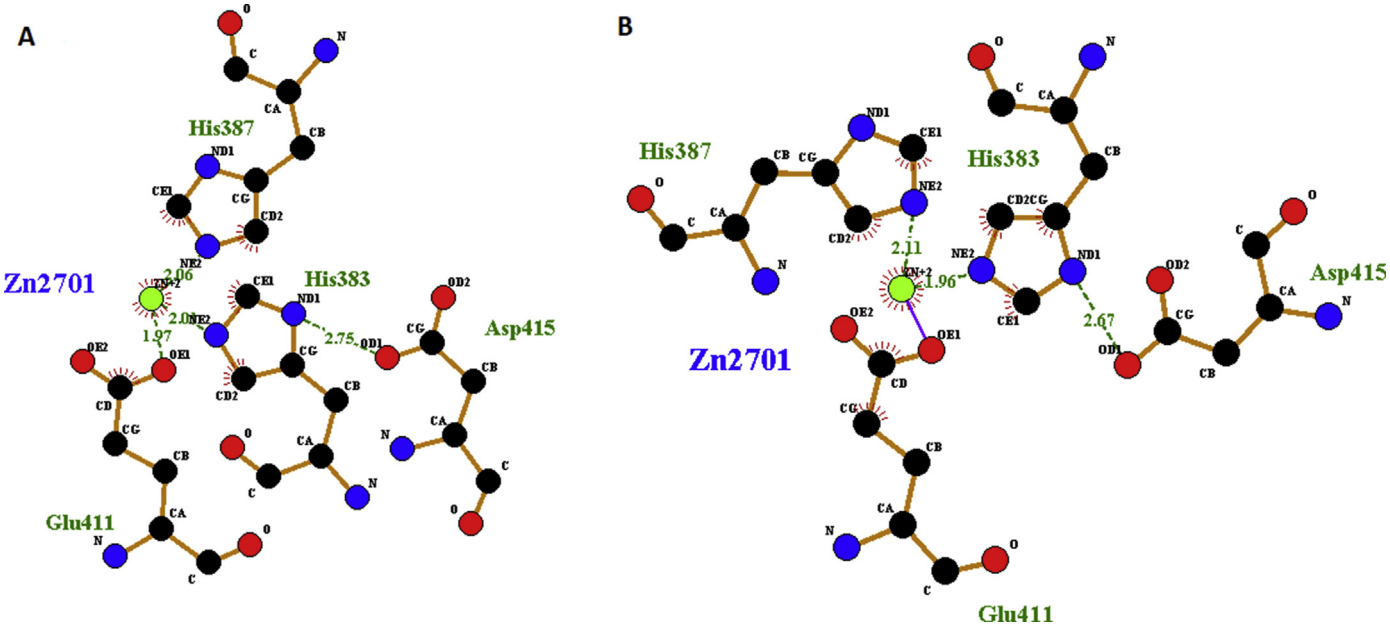

Fig. 9. Tetrahedral coordination of Zn (II) (green) with ACE residues as affected by docking with MVPYPQR (A: the original form of the enzyme, B: the enzyme form after docking). Formation of hydrogen bonds are demonstrated in green dotted lines. Purple and brown lines indicate formation of ligand and non-ligand bonds, respectively. Ligplot version v.1.4.5 software was used to create the image. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

the ACE active site (S'1 pocket). These results indicate that the peptide MVPYPQR have a productive interaction with the catalytic site of the enzyme and could lead to the inactivation of ACE (Wu, Jia, Yan, Du, & Gui, 2015).

According to the results of the docking study, the hydrogen bond between Zn and the residue Glu 411 was lost and led to the distortion of the tetrahedral geometry of the enzyme (Fig. 9). Zn is bound to three ACE residues (Glu 411, His 387, and His 383) and the interaction between Zn^{2+} and ACE-I peptides at the enzyme active site plays a significant role in the mechanism of the inhibition (Girgih et al., 2014; He, Aluko, & Ju, 2014).

4. Conclusion

This study is suggesting that antioxidant and ACE-I activities of the camel milk could be highly affected by the fermentation with LAB. *Leuc. lactis* showed the ability to release an antioxidant and ACE-I active peptide (MVPYPQR) from camel casein structure during the fermentation. The molecular docking study revealed the substantial effect of Arg in C terminal of the peptide to establish hydrogen bonds with ACE and distort the tetrahedral geometry of the enzyme. The active peptide, MVPYPQR, could be used as an ingredient in nutraceuticals and functional foods. Further animal

and stability studies are needed for usage of the peptide in food and pharmaceuticals industries.

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