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Original article

Production of angiotensin-converting enzyme inhibitory peptides in Iranian ultrafiltered white cheese prepared with *Lactobacillus brevis* KX572382

Leila Yousefi,[1] Mohammad Bagher Habibi Najafi,[1*] Mohammad Reza Edalatian Dovom[1] & Amir Mohammad Mortazavian[2]

1 Department of Food Science and Technology, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad 91775-1163, Iran
2 Department of Food Science and Technology, National Nutrition and Food Technology Research Institute, Faculty of Nutrition Sciences, Food Science and Technology, Shahid Beheshti University of Medical Sciences, Tehran 19395-4741, Iran

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Abstract In this study, ultrafiltered (UF) Iranian white cheese made with adjunct cultures including six Lactobacillus isolates (*Lactobacillus brevis*, *L. casei* and *L. plantarum*) from traditional Iranian Motal cheese. The peptide extract (<5 kDa) of cheese samples were assessed for angiotensin-converting enzyme (ACE)-inhibitory activity during ripening (5 °C). Among the strains used, *L. brevis* KX572382 (M8) was selected because of the greater increase in (ACE)-inhibitory activity in the cheese ($P < 0.05$). The highest activity of M8 extract was observed on the 28th (71.72%) day of ripening ($P < 0.05$). Proteolytic activity assessment and RP-HPLC peptide profile of M8 water-soluble extracts (WSEs) indicated the effect of M8 on further protein degradation due to secondary proteolysis. A total of 7 different peptide sequences, previously known in the literature for their ACE-inhibitory activity, were tentatively identified by LC/ESI-MS in 28-day M8 peptide extract. Although the effect of M8 on pH and the proteolysis development in cheese was significant, no adverse effect was observed on the sensory properties. In conclusion, M8 strain can enhance the functional properties of Iranian UF white cheese.

Keywords ACE-inhibitory activity, adjunct culture, bioactive peptide, Iranian UF white cheese, *L. brevis* KX572382.

Introduction

The demand for functional foods has been steadily increasing in recent years, mainly due to the increasing consumer health awareness of the benefits that are linked to such products. Functional foods are defined as foods that, in addition to their conventional nutritional properties, have a health-promoting effect on the consumer and reduce the risk of developing chronic diseases (Bersi *et al.*, 2018). Bioactive peptides have been proposed as health-promoting compounds. These peptides comprising 2 to 20 amino acids with functional and biological properties such as antihypertensive, antioxidant, antimicrobial, anticancer, opioid and dipeptidyl peptidase IV (DPP IV) inhibitory activities. (Homayouni-Tabrizi *et al.*, 2016; Karami & Akbari-adergani, 2019; Zhu *et al.*, 2019). Among these features, special attention has been paid to the antihypertensive activity of bioactive peptides that exert ACE inhibition (Hernández-Galan *et al.*, 2017).

Hypertension or high blood pressure is one of the main risk factors for cardiovascular diseases. The renin-angiotensin system is a hormone system whose role in regulating blood pressure has been well established. Angiotensin-converting enzyme (EC 3.4.15.1) or ACE increases blood pressure by converting the hormone angiotensin I to angiotensin II, an active vasoconstrictor. In recent years, ACE inhibitors have been used to treat high blood pressure (Kaplan, 2018).

Milk proteins are the precursors of many biologically active peptides. So far, many of these peptides have been identified in milk and fermented dairy products, including different kinds of cheeses (Taha *et al.*, 2017; Sultan *et al.*, 2018; Soleymanzadeh *et al.*, 2019).

Proteolysis in cheese occurs mainly due to the activity of starter and non-starter lactic acid bacteria (SLAB and NSLAB) during ripening, which besides affecting the sensory properties of cheese, bioactive peptides can also be generated (Habibi Najafi, 2016;

*Correspondent: E-mail: habibi@um.ac.ir

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López-Expósito *et al*., 2017). The type of cheese, and its characteristics, such as the type of milk, SLAB, NSLAB and the conditions of ripening are among factors affecting the production of bioactive peptides in cheese (Erkaya & Sengul, 2015; Albenzio *et al*., 2017).

Kocak *et al*. (2020) used a mixed starter culture with three adjunct cultures, namely *L. casei*, *L. plantarum* and *L. bulgaricus* in white brined goat-milk cheese. They showed that the use of adjunct cultures in this type of cheese increases ACE-inhibitory activity until the 60th day of ripening. Dimitrov *et al*. (2015) used different adjunct cultures in Bulgarian cheese to investigate ACE-inhibitory activity. They found that most inhibitory activity generated by *L. helveticus* A1, which releases the peptide Ala-Leu-Pro-Met as primary ACE inhibitor.

Ultrafiltered white cheese is classified under a soft cheese group produced from UF and pasteurised milk with mesophilic or thermophilic starter cultures, and commercial microbial rennet (Rashidi *et al*., 2015; Jalilzadeh *et al*., 2018). The use of ultrafiltration technology is one of the most common methods of cheese production. In this method, milk is concentrated using membrane filters, which causes higher efficiency and preservation of more protein compounds than the traditional method of cheese production (McSweeney, 2007).

Ultrafiltered cheese is one of the most widely used types of cheese in Iran, so its production as functional product can help to improving the consumer's health.

To the best of our knowledge, there are no published studies on the effect of adjunct cultures in Iranian UF white cheese to increase bioactivity. This study aimed to investigate the effects of different Lactobacillus strains isolated from traditional Iranian Motal cheese on ACE-inhibitory activity during 56 days of UF white cheese ripening. The proteolysis rate, peptide profile and some physicochemical characteristics of the Iranian UF white cheese elaborated with *L. brevis* KX572382 were also investigated.

Materials and methods

Materials

The retentate of UF cow's milk was obtained from Iran Dairy Industry Inc., Pegah Co. (Mashhad, Iran). Rennet (Fromase®TL) and mesophilic starter composed of *Lactococcus lactis* subsp. lactis and *Lactococcus lactis* subsp. Cremoris were purchased from DSM Co. (The Netherlands) and Christian Hansen Co. (Denmark), respectively. De Man, Rogosa, and Sharpe (MRS) were obtained from HiMedia (India). ACE (EC 3.4.15.1) and N-[3-(2-Furyl) acryloyl]-L-phenylalanylglycyl-glycine (FAPGG) were purchased from Sigma Chemical Co. (St. Louis, MO).

Bacterial cultures and growth conditions

In this research, six Lactobacillus strains consisted of *L. brevis* KX572376 (M2), *L. brevis* KX572378 (M4), *L. brevis* KX572382 (M8), *L. brevis* KX572386 (M12), *L. casei* KX572389(M15) and *L. plantarum* KX572390 (M16), that had previously been isolated and fully identified from Iranian raw milk Motal cheese (Azizi *et al*., 2017), were selected as the adjunct cultures. Pre-inoculum for each adjunct culture was prepared in MRS medium and overnight incubation at 30 °C for *L. brevis*, and 37 °C for *L. plantarum* and *L. casei*.

Cheesemaking

Experimental UF white cheese was made according to the method applied in Iran Dairy Industry Inc., Pegah Co. (Mashhad, Iran). The ultrafiltration process with inlet pressure of 5.3 and outlet pressure of 1.7 bar was performed in 3 consecutive loops so that in loops 1, 2 and 3 the milk was concentrated to brix 16, 21 and 28, respectively. The concentration factor was 4.5 kg milk to 1 kg retentate.

Adjunct-treated and control cheese (without adjunct culture) in a volume of 100 grams were prepared by adding 1 mg kg^{-1} rennet and 0.001% starter to UF milk. Adjunct culture was added along with the starter culture to achieve a final population of 10^6 CFU per mL and was then left to coagulate at 32 °C for 20 min. A parchment paper was placed on the top of the coagulum and 1.5% (w/w) dry salt was then added on the parchment paper. The containers were sealed with aluminium foil. All samples were kept at 30 °C in the pre-ripening stage and were then stored at 5 °C until the day of the experiment (14, 28, 42 and 56 days).

Preparation of WSEs

Water-soluble extract (WSE) is a fraction that contains proteins, peptides and amino acids. WSEs of the cheese samples were prepared using the method of Kuchroo & Fox (1982). The resulting WSEs were passed through an ultrafiltration membrane with a molecular weight cut-off of 5 kDa (Sartorius, Melbourne, Australia) by centrifugation at 13 000***g***, 4 °C for 30 min (Sigma, Germany). The filtered solutions, and WSEs were stored lyophilised for further analyses.

Protein assay

The protein concentration in WSEs was determined by Bradford assay with bovine serum albumin (BSA) as standard (Marshall & Williams, 1993).

Proteolytic activity assessment

A spectrophotometric assay using OPA was carried out to assess the proteolytic activity in cheese samples,

according to Church *et al.* (1983). Serine served as a standard, and free amino groups were calculated using the standard curve.

ACE-inhibitory activity assay

The method of Holmquist *et al.* (1979) with slight modifications was used to determine the ACE-inhibitory activity of the samples. Briefly, 30 μL ACE (0.1 U mL^{-1}), 50 μL peptide extract (0.01 mg mL^{-1}) or water (for control), 160 μL ACE buffer [50 mM Tris-HCl (pH 7.5), 0.3 M NaCl and 1 mM ZnCl2] were added to each well of ELISA plate and pre-incubated for 5 min at 37 °C and then 65 μL FAPGG (N-[3-(2-Furyl) acryloyl]-L-phenylalanyl-glycyl-glycine) 0.5 mM as a substrate for ACE was added to the mixture. The reaction was performed for 30 min at 37 °C. The absorbance was measured at 340 nm. The ACE-inhibitory activity was calculated as follows:

$$\text{ACE inhibition}\,(\%) = [1 - (\Delta A_{\text{inhibitor}}/\Delta A_{\text{control}})] \times 100.$$

RP-HPLC analysis of peptide profile

RP-HPLC was performed to investigate the peptide profile of cheese samples at the highest inhibitory activity, as described by Hayaloglu *et al.* (2004) using Alliance 2695 HPLC system (Waters, Milford, MA). Peptide solution (20 μL) was injected into an analytical C18 column (PerfectSil Target, ODS-3, 250 × 4.6, 5 μm, 300 Å). Eluent A was 0.1% trifloroacetic acid (TFA) in distilled water and Eluent B was 0.1% TFA in acetonitrile. The elution was conducted using a linear gradient of 0–100% eluent B at a flow rate of 0.75 mL min^{-1} for 85 min. The eluate was monitored at 214 nm using a UV detector for 85 min.

Peptide identification by LC/ESI-MS

Peptide extract of M8 with the highest inhibitory activity was selected, and its peptides were identified by LC/ESI-MS technique. Five μL of the extract was injected on an Atlantis T3 C18 column (100 × 2.1 mm, 3 μm) at the operating temperature of 35 °C. Eluent A was 0.1% formic acid in the water, and eluent B 0.1% formic acid in acetonitrile. The components were separated using a flow rate of 0.2 mL min^{-1} and a linear gradient of 10–90% eluent B for 20 min. The mass spectrometer (Micromass Quattro micro API, Waters, Milford, MA) was operated in a positive mode, nitrogen flow of 200 L h^{-1} and a desolvation temperature of 300 °C. Mass spectra were recorded in the scan mode 50–2000 m/z with the target mass set to 1521 m/z. All data were acquired and processed by the software MassLynx 4.1 (Waters, Milford, MA). The molecular mass of the peptides in the extract was determined using their mass to charge ratio, as described by Fenn *et al.* (1989). The General Protein Mass Analysis for Windows software (GPMAW, Lighthouse data, Odense, Denmark) was used to assign peptide masses to particular sequences of the caseins. Proposed peptides bioactivity with a detailed survey on similar experiments performed in literature and a BIOPEP database search (database of bioactive peptides available at http://www.uwm.edu.pl/biochemia/index.php/pl/biopep) were determined.

Physicochemical and sensory evaluation of cheese treatment

For the physicochemical evaluation of cheese, the pH was determined according to the Iranian National Standard No. 2852 by pH metre (Metrohm 827, Herisau, Switzerland) at 14, 28, 42 and 56-day M8 and the control stored cheeses (ISIRI, 2005). The sensory evaluation of M8 and the control cheeses was performed at the end of ripening time, according to the Iranian National Standard No. 4691 (ISIRI, 1998). Sensory parameters including taste, texture, colour and overall acceptability were performed based on a hedonic scale from 0 to 5 by ten well-trained food science and technology students.

Statistical analysis

Data were analysed by analysis of variance (ANOVA) using SPSS software version 25. Results were expressed as mean ± standard deviations (SD) of triplicate. Tukey comparison test was used to determine the statistically significant differences between means.

Results and discussion

ACE-inhibitory activity

In this study, ACE-inhibitory activity of the UF cheeses containing *L. brevis* KX572376 (M2), *L. brevis* KX572378 (M4), *L. brevis* KX572382 (M8), *L. brevis* KX572386 (M12), *L.casei* KX572389 (M15) and *L. plantarum* KX572390 (M16) as adjunct cultures and control cheese (without adjunct culture) were evaluated during 14, 28, 42 and 56 days of ripening. Since ACE-inhibitory peptides are generally low molecular weight, WSEs of samples were passed through the 5 kDa cut-off diafiltration membrane. The ACE-inhibitory activity of the peptide extract (<5 kDa) of cheese samples during the ripening period is shown in Fig. 1. A significant difference was observed between the ACE-inhibitory activity of the peptide extract of cheese samples during ripening ($P < 0.05$). There was also a significant difference in cheese sample and ripening

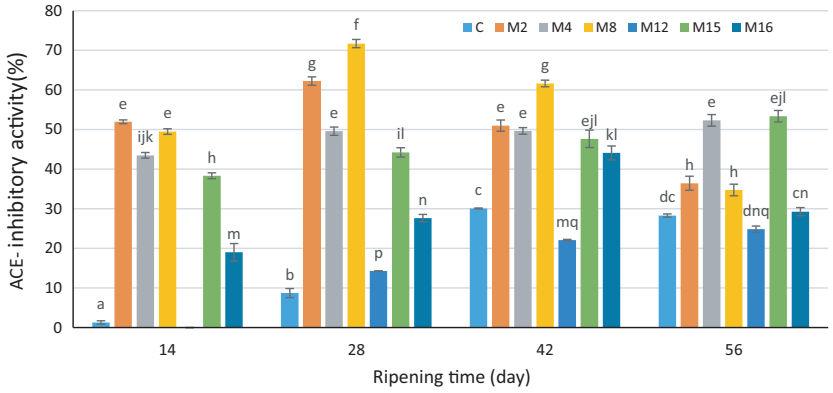

Figure 1 ACE-inhibitory activity of peptide extract (<5 kDa) of UF cheeses containing *L. brevis* KX572376 (M2), *L. brevis* KX572378 (M4), *L. brevis* KX572382 (M8), *L. brevis* KX572386 (M12), *L. casei* KX572389 (M15) and *L. plantarum* KX572390 (M16) as adjunct cultures and control cheese (without adjunct culture) during ripening time (14, 28, 42 and 56 day). Error bars denote standard deviations. Different letters above the bars indicate significant differences ($P < 0.05$) between cheese samples.

time interaction ($P < 0.05$), indicating different proteolysis patterns in the samples.

According to previous studies, the production of bioactive peptides in cheese is affected by proteolytic enzymes of SLAB and NSLAB of cheese during ripening, which is species and/or strain specific (Santiago-Lopez *et al.*, 2017; Raveschot *et al.*, 2018).

Among six adjunct-treated UF cheeses, M8 strain displayed the highest ACE-inhibitory activity, so it was selected for further studies. The ACE-inhibitory activity of M8 cheese samples was significantly different during ripening time ($P < 0.05$; Fig. 2). The activity increased during the first month, and then decreased with increasing proteolysis in the second month. The highest and lowest activity was observed on the 28th (71.72%) and 56th (34.74%) days of ripening. The results of this study showed that inhibitory activity increased to a certain level of proteolysis and then decreased, indicating the breakdown of active peptides with ACE-inhibitory into lower molecular weight peptides and amino acids with lower inhibitory activity. Indeed, the higher rate of degradation of bioactive peptides can reduce the ACE inhibition activity during ripening (Meira *et al.*, 2012; Gupta *et al.*, 2013).

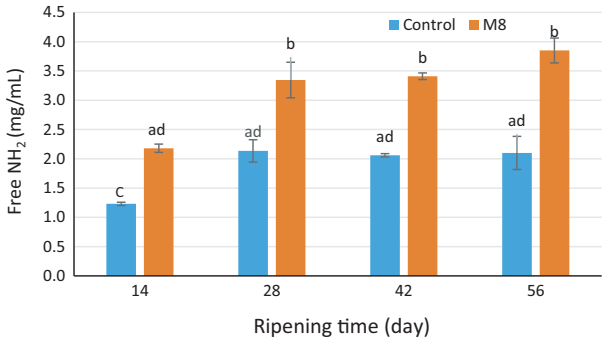

Figure 2 ACE-inhibitory activity of peptide extract <5 kDa of M8 (containing *L. brevis* KX572382 as an adjunct culture) and control cheese samples (without adjunct culture) during ripening time (14, 28, 42 and 56 day). Error bars denote standard deviations. Different letters above the bars indicate significant differences ($P < 0.05$) between cheese samples.

Proteolytic activity

Proteolysis in cheese samples investigated by determining free NH2 groups. Free NH2 groups content of samples containing M8 strain was higher than the control during ripening ($P < 0.05$). The increase in free NH2 groups in M8 samples compared to the control was an indication of intense proteolytic activity of M8 strain. This result was consistent with those reported by Sahingil *et al.* (2014) and Kocak *et al.* (2020). They demonstrated that the presence of adjunct culture enhances the proteolysis in white cheese during ripening. Differences in the content of free NH2 groups between cheese samples were statistically significant during ripening ($P < 0.05$). No significant difference was observed in the cheese sample and ripening time interaction ($P > 0.05$).

RP-HPLC peptide profile

Most inhibition activity of M8 peptide extract was observed at 28 days of ripening, therefore, to investigate differences between the peptide profiles of WSEs of M8, and the control cheeses, RP-HPLC was performed and the results are shown in Fig. 3. M8 sample displayed a profile with a higher total area than the control. There were notable differences between M8 and the control chromatograms in the region with retention time (RT) of 50–80 min. Unlike the control sample, many peaks were observed in the hydrophilic region at retention time between 50 and 70 min in M8 sample chromatogram. The height and number of peaks also varied in the hydrophobic region (70–80 min) of the two chromatograms.

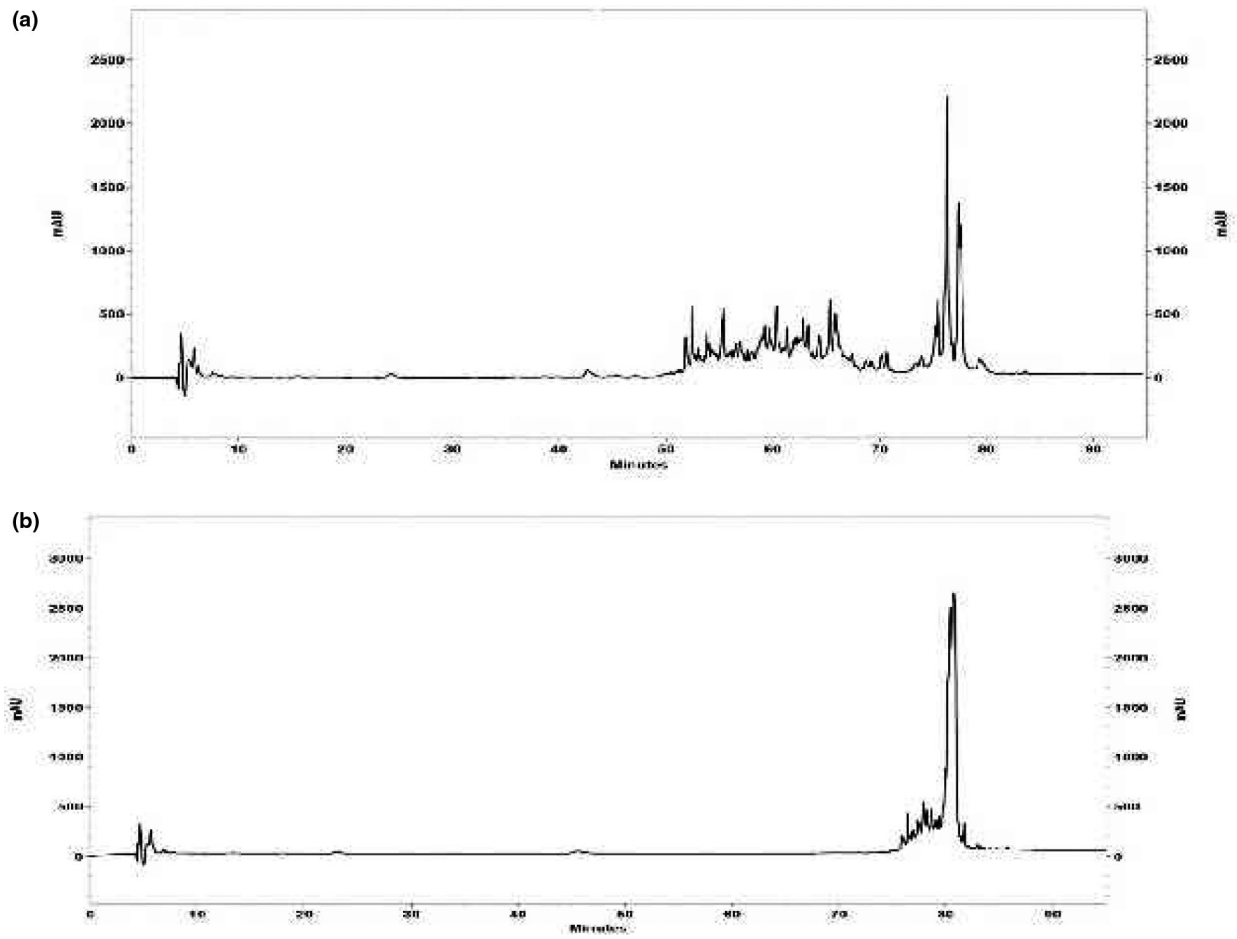

Figure 3 RP-HPLC peptide profile of WSEs of UF white cheese samples at 28 days of ripening. A (Control): without adjunct culture, B: containing *L. brevis* KX572382 (M8) as an adjunct culture.

The early peaks in chromatograms are mostly included low molecular weight hydrophilic peptides and amino acids and the peaks that appear later consist of hydrophobic peptides (Acquah *et al.*, 2019). Differences between the two chromatograms showed that M8 was active on secondary proteolysis and the production of low molecular weight hydrophobic and hydrophilic peptides and amino acids in UF white cheese.

Peptide identification

In a detailed literature survey and BIOPEP database search for the peptides proposed by GPMAW software, seven peptides with ACE-inhibitory activity (Table 1) and peptides with activities other than ACE inhibition as well as peptides for which no activity has been reported in the literature (Table 2) were found.

The sequences of the identified peptides ranged from five to seventeen amino acids residues. Five peptides were derived from β-casein and two from αS1-casein. Fan *et al.* (2019) showed that the effect of *L. helveticus* on fermentation of casein caused the highest productions of bioactive peptides from β-casein. In the study of Galia *et al.* (2009) highly proteolytic strains of *Streptococcus thermophilus* generated the maximum number of bioactive peptides from β-casein. Peptide sequences FPIIV, YPVEPFTE, SLVYPFPGPIHN originated from β-casein obtained from the fermentation of casein by *L. helveticus* (Fan *et al.*, 2019). Sequence MPFPKYPVEP in which FPKYPVEP is enclosed has already been produced in milk fermented by *L. animalis* and introduced as an ACE inhibitor (Hayes *et al.*, 2007; Pritchard, 2012). Sequences RPKHPI and YQEPVLGPVRGPFPIIV which are derived from αS1 and β-casein, respectively, have previously been found in

Table 1 Suggested peptides with ACE-inhibitory activity in M8 peptide extract <5 kDa (containing *L. brevis* KX572382 as an adjunct culture) at 28 days of ripening based on the tentatively identification by LC/ESI-MS

Mass observed $[M + H]^+$	Mass calculated[a]	Peptide charge	Suggested feragment	Sequence	References[b]
589.29	587.37	+1	β-CN (205-209)	FPIIV	Fan *et al.* (2019) Capriotti *et al.* (2016)
746.62	746.46	+1	αS1-CN (1-6)	RPKHPI	Ong *et al.* (2007)
874	875.6	+1	αS1-CN (102-108)	KKYNVPQ	Gómez-Ruiz *et al.* (2002)
976.92	975.51	+1	β-CN (126-133)	FPKYPVEP	Pritchard (2012) Hayes *et al.* (2007)
981.98	980.45	+1	β-CN (114-121)	YPVEPFTE	Fan *et al.* (2019) FitzGerald *et al.* (2004)
1340.85	1339.7	+2	β-CN (57-68)	SLVYPFPGPIHN	Fan *et al.* (2019) Krizkova *et al.* (2014)
1881.72	1880.06	+1	β-CN (193-209)	YQEPVLGPVRGPFPIIV	Ong *et al.* (2007) Birkemo *et al.* (2008)

[a]Monoisotopic mass value

[b]Peptide identification supported with the results of the references in the table.

Table 2 Suggested peptides with activities other than ACE inhibition in M8 peptide extract <5 kDa (containing *L. brevis* KX572382 as an adjunct culture) at 28 days of ripening based on the tentatively identification by LC/ESI-MS

Mass observed $[M + H]^+$	Mass calculated[a]	Peptide charge	Suggested feragment	Sequence	Reported bioactivity	References[b]
426.54	427.26	+1	β-CN(199-202)	GPVR	–	Rasmusson (2012)
571.92	571.34	+1	β-CN(84-88)	VPPFL	–	Jin *et al.* (2016)
643.115	642.33	+1	β-CN(9-14)	PGEIVE	–	Fan *et al.* (2019)
659.47	658.34	+1	αS1-CN(40-45)	VAPFPE	DPP IV inhibitory	Gutiez *et al.* (2014) Fan *et al.* (2019)
671.86	670.41	+1	β-CN(83-88)	VVPPFL	–	Fan *et al.* (2019)
740.16	739.45	+1	αS2-CN(132-138)	VPITPTL	DPP IV inhibitory	Shanmugam *et al.* (2014) Fan *et al.* (2019)
877.82	876.45	+1	β-CN(124-131)	SLTLTDVE	–	Fan *et al.* (2019)
897.83	897.45	+1	κ-CN(174-181)	IESPPEIN	–	Fan *et al.* (2019)
939.79	940.45	+1	αS1-CN(181-189)	DIPNPIGSE	–	Fan *et al.* (2019)
995.68	996.51	+1	κ-CN(173-181)	VIESPPEIN	–	Fan *et al.* (2019)
1119.04	1119.56	+2	αS1-CN(26-35)	APFPEVFGKE	–	Jin *et al.* (2016)
1050.09	1051.62	+2	αS1-CN(17-24)	NENLLRFF	–	Jin *et al.* (2016)

[a]Monoisotopic mass value.

[b]Peptide identification supported with the results of the references in the table.

Cheddar cheese made with the addition of a probiotic strain of *L. casei* (Ong *et al.*, 2007). Sequence KKYNVPQL originated from αS1-casein shares four amino acid residues at the C-terminal positions with YKVPQL, which has previously been shown to lower blood pressure in hypertensive rats (Maeno *et al.*, 1996; Gómez-Ruiz *et al.*, 2002).

It is well established that the primary activity of ACE is to cleave the C-terminal dipeptide of substrates, and therefore, the C-terminal sequence of ACE-inhibitory peptide as well as hydrophilic-hydrophobic ratio is effective in their inhibitory activity. Most ACE-inhibitory peptides are usually short-chain peptides with aromatic amino acids, the imino acid proline and positively charged amino acids, such as lysine and arginine at one of the three C-terminal positions (Hayes *et al.*, 2007; Homayouni-Tabrizi *et al.*, 2016; Manoharan *et al.*, 2017). However, finding peptides that are not in accordance with the results of structure-activity correlation studies on ACE-inhibitory peptides revealed that the mechanism of ACE inhibition by these peptides is not understood and their promise is mainly supported by in vitro and in vivo ACE inhibition data (Pan *et al.*, 2011).

Physicochemical and sensory properties

The pH of M8 and control cheese samples during ripening is shown in Fig. 4. The effect of M8 adjunct culture on the pH of UF cheese during ripening was found to be significant ($P < 0.05$). In the M8 sample, pH increased during the first 14 days and remained almost constant up to 28 days and then dropped to the end of the ripening time. These results were partly consistent with those found by Kocak *et al.* (2020). The ANOVA results showed that the interaction between cheese sample and ripening time was significant ($P < 0.05$).

SLAB ferment lactose and decrease pH by producing lactic acid during cheese production. During ripening, SLAB and NSLAB interact with each other and compete for the residual lactose (Blaya *et al.*, 2018). On the other hand, the proteolysis process during the ripening time can lead to the production of metabolites contribute significantly to increase the pH (Pagthinathan & Mohamed Nafees, 2017).

The difference between pH changes in the control and M8 samples can be due to the effect of M8 strain on proteolysis and competition with the starter for residual lactose consumption.

Sensory properties of cheese containing M8 adjunct culture and the control sample at the end of ripening are shown in Table 3. There was no significant difference between the sensory characteristics of both samples ($P > 0.05$). The strain M8 had no adverse effect on the sensory properties of UF cheese. No cracks were found in M8 cheese. Unexpectedly, there was no bitterness in the experimental cheese.

LAB play a significant role in the basic taste of cheese by producing free amino acids from casein and peptides. The activity of these bacteria is affected by temperature, so the main factor affecting the sensory properties in cheese is the temperature of ripening (Blaya *et al.*, 2018). Vinderola *et al.* (2009) found that storing Argentine cheese containing probiotic culture at 5 °C had no adverse effect on the sensory properties of cheeses, while higher temperatures produced an unpleasant taste in the experimental cheeses.

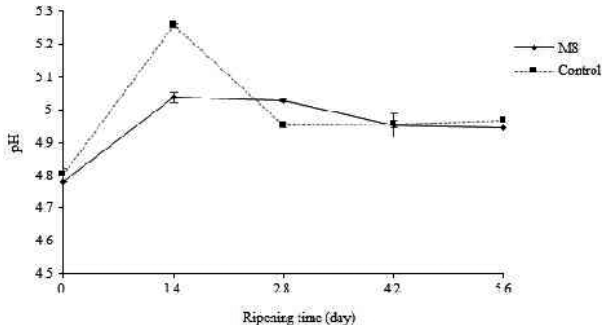

Figure 4 The pH of M8 (containing *L. brevis* KX572382 as an adjunct culture) and control cheese (without adjunct culture) samples during ripening (14, 28, 42 and 56 days) with their respective standard errors.

Table 3 Sensory properties of cheese containing M8 adjunct culture (*L. brevis* KX572382) and control sample at the end of ripening

	Sensory properties			
Sample	Taste	Texture	colour	Overall acceptability
M8	3.60 ± 0.52[a]	4.70 ± 0.48[a]	4.80 ± 0.42[a]	4.10 ± 0.31[a]
Control	3.90 ± 0.56[a]	4.80 ± 0.42[a]	4.90 ± 0.31[a]	4.40 ± 0.51[a]

Values are expressed as mean ± SD.
Values with the same lower case letters in a column are not statistically significant by the ANOVA test ($P > 0.05$).

Conclusions

This study showed that the use of *L. brevis* KX572382 (M8) isolated from traditional Iranian Motal cheese as an adjunct culture in Iranian UF white cheese was able to significantly increase ACE-inhibitory activity over a ripening period of 56 days. The highest inhibition was observed in the middle of the ripening time. Comparison between RP-HPLC chromatograms of 28-day WSEs of M8 and control sample indicated the effect of M8 on secondary proteolysis and the production of low molecular weight hydrophobic and hydrophilic peptides and amino acids in UF cheese.

LC/ESI-MS analysis of peptides confirmed the presence of ACE-inhibitory peptides in M8 cheese. These peptides often originated from β-casein. There were also peptides for which no biological activity has been reported so far and may contribute to ACE-inhibitory activity. In this study, low temperature during ripening (5 °C) prevented the bitterness and off-flavour that has been reported in some literature for *L. brevis*.

Since traditional dairy products can be a good source of NSLAB, studying them in terms of the presence of bioactive peptide-producing strains can be a practical step in the production and development of functional food.

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Ethical guidelines

Ethics approval was not required for this research.

Conflict of interest

The authors declare that they have no competing interests.

Author contribution

leila yousefi: Data curation (lead); Formal analysis (equal); Investigation (equal); Writing-original draft (lead). **Mohammad B. Habibi Najafi:** Conceptualization (lead); Funding acquisition (lead); Project administration (lead); Supervision (lead); Writing-review & editing (lead). **Mohammad R. Edalatian:** Funding acquisition (lead); Project administration (supporting); Supervision (supporting). **A. M. MORTAZAVIAN:** Investigation (supporting); Supervision (supporting); Validation (supporting); Visualization (supporting).

Peer Review

The peer review history for this article is available at https://publons.com/publon/10.1111/ijfs.14891.

Data availability statement

Research data are not shared.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Data S1. Molecular docking studies.