Effect of probiotic cultures on the angiotensin converting enzyme inhibitory activity of whey-based fermented beverages

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Abstract Fermented whey-based beverages were elaborated using commercial probiotics cultures (CHR HANSEN): BCT-1®, ABT-4®, ABC where A: *Lactobacillus acidophilus*, B: *Bifidobacterium animalis* subsp. *lactis*, C: *Lactobacillus paracasei* subsp. *paracasei* and T: *Streptococcus thermophiles*. Three treatments were prepared with these probiotics cultures and a treatment without cultures added as a control. All beverage were maintained at 4 °C and evaluated during their shelf life at 0, 7, 14 and 21 days. The variables analyzed were peptide concentration, proteolysis, ACE-inhibitory activity and, peptide and aromatic amino acid profiles. Principal component analysis (PCA) was conducted to assess the relationship between these variables. All whey-based fermented beverages added with probiotics presented ACE inhibitory activity (22–100%).

However, whey-based beverage without probiotics added had the highest ACE inhibitory activity (100%), and it was maintained during its shelf life. Fermented beverage with ABT-4® culture had an ACE inhibitory activity from 80 to 100% during its shelf life. Thus can be attributed to the release of peptides during processing. Meanwhile, significant correlations were observed between variables varying this depending on the treatment, both, in magnitude and in direct or inverse direction (0.684–0.986). The ACE inhibitory activity was correlated to proteolysis and peptide and aromatic amino acids profiles. And, the IC₅₀ was observed in the range of 0.01–0.055 mg/mL.

Keywords Probiotics · Whey · Angiotensin converting enzyme · Fermented beverages

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Introduction

Milk and its derivatives are considered an important source of nutrients since, in addition to providing energy; it contains high quality proteins, minerals and vitamins (Park 2009). The World Health Organization formally defined probiotics as “live microorganism, which, when administered in adequate amounts confer a health benefit on the host”. The health effects attributed to the use of probiotics are numerous, including modulating the number and diversity of beneficial gut microbiota and reducing blood pressure and hypertension (Khalesi et al. 2019). A fermented milk beverage can be defined as a mixture of milk with microbial cultures and other dairy products. The main dairy foods added with probiotics are fermented beverages and cheeses (Castro et al. 2009). Caseins and whey proteins are the two main milk proteins; of these, whey proteins stand out over casein with various biological properties (Pandey et al. 2018). Whey proteins, compared to other food proteins, are of higher quality (Devries and Phillips 2015). Proteolysis is the degradation of proteins, and the lactic acid bacteria (BAL) proteolytic system can carry out this process and produce small peptides and free amino acids (Smit et al. 2005). For this, during milk fermentation, the starter cultures proteolytic system plays a key role. Then, proteolysis is of paramount importance for several aspects: it can determine the survival of the starter cultures, contributes to the formation of flavor compounds and smell, confers rheological properties, and allows the formation of bioactive peptides (Serra et al. 2009). Those that, coupled with their nutritional function, are capable of exerting specific beneficial biological effects, are called bioactive peptides or biopeptides (Costa et al. 2007; Dziuba et al. 2009). Milk proteins are considered some of the main sources of bioactive peptides (Plaisancié et al. 2013; Sallidere and Oner 2011), which have positive effects on consumer health (Hajirostamloo 2010; Miguel et al. 2007). The antihypertensive activity of some biopeptides is given by the blocking of angiotensin converting enzyme (ACE) (Peptidyl-dipeptidase A o kininase II—EC 3.4.15.1) which is a dipeptide ectoenzyme with two catalytic sites, located in different tissues, which releases an exopeptidase associated with the regulation of blood pressure, as it participates in the renin angiotensin system by the conversion of angiotensin-I to angiotensin-II (Ahn et al. 2009; Puchalska et al. 2014; Qureshi et al. 2013). By blocking this enzyme, an antihypertensive effect is achieved and, in turn, benefits the consumer's immune and nervous system as it favors the state of homeostasis (Minervini et al. 2009; Otte et al. 2011). The characteristics of the peptides that block it are the following: they contain hydrophobic aromatic amino acids, such as Trp or Phe in one of the last three positions

of their sequence; in addition, Pro residues alone or in combination with other hydrophobic residues by joining the active sites of the ECA block its activity, thus marking its antihypertensive capacity (Shahidi and Zhong 2008). It has also been shown that the consumption of hydrolyzed whey protein exerts a significant reduction of blood pressure in hypertensive individuals (FitzGerald et al. 2004; Martínez and Martínez 2006). Meanwhile, the use of different probiotics (alone or in combination), recognized as *Lactobacillus* spp and *Bifidobacterium* spp have positives effects on cardiovascular disease risk (Khalesi et al. 2019) and particularly to ferment milk hydrolyze proteins producing peptides with inhibitory ACE activity (Otte et al. 2011; Quirós et al. 2005). Therefore, the objective of the present study was to evaluate and correlate the effect of different probiotics cultures on the ACE inhibitory activity, proteolysis, peptides, and aromatic amino acids profiles of a fermented whey-based beverage.

Materials and methods

Beverages formulation

First, whey from Chihuahua cheese process was obtained; this is a popular Mexican cheese. Next, the mixture of whey and whole milk (80/20% v/v) was made and pasteurized at 95 °C for 20 min to promote protein denaturation which enhances water absorption capacity and stability from milk separation. Then it was homogenized for two minutes according to Nielsen et al. (2009), it has been shown that homogenization after pasteurization favors better consistency in the final product (Chandan and Kilara 2013), after that the mixture was cooled to 37 °C. Then, it was separated in four equal parts to elaborate treatments with three replicates each. Beverage formulations were: whey-milk (80/20% v/v), 0.5% w/v of freeze-dried lyophilized probiotics, 0.5% w/v Avicel®, 9% w/v saccharose, and 0.05% w/v artificial flavour. Probiotic cultures used to elaborate the different treatments were: BCT-1®, ABT-4®, ABC; where A: *Lactobacillus acidophilus*, B: *Bifidobacterium lactis*, C: *L. paracasei* subsp. *paracasei* and T: *Streptococcus thermophiles*. All cultures were acquired from CHR HANSEN™. In addition to these treatment a without probiotics added were elaborate as a control. All beverages were kept refrigerated for three weeks at 4 °C since, generally, fermented dairy beverages remain acceptable for 2–3 weeks under refrigerated storage (Patidar and Prajapati 1998; Salvador and Fiszman 2004). Because of this, the samples were taken at 0, 7, 14 and 21 days (where zero day was the day of beverages elaboration). Total peptide concentration, proteolytic activity,

ACE inhibitory activity, peptide, and aromatic amino acids profiles were determined. Samples were prepared according to Donkor et al. (2007); briefly, 2.5 mL of each treatment were taken and mixed with 5 mL of trichloroacetic acid (ATC) at 0.75%, then mixtures were passed through Whatman paper no. 1 (150 mm Ø) to obtain beverage filtrates (FB), which were then frozen at -20°C until being analyzed. For ACE inhibitory activity and peptide and aromatic amino acid profiles analyses, FBs were lyophilized in a 2.5 plus freeze-dryer (LABCONCO) for 48 h to obtain the freeze-dried beverage filtrates (FBL); these were weighed before and after lyophilization to obtain, by weight difference, the samples' concentration.

Peptide concentration

This analysis was determined using Bradford (1976) methodology. Briefly, FB samples (0.1 mL) or standards were taken and mixed with 1 mL of Bradford reagent, stirred once by immersion, and allowed to react in the dark at room temperature for 5 min. The absorbance of each sample at 595 nm was measured spectrophotometrically (UV-1800, Shimadzu). To perform the calibration curve, five standards of bovine serum albumin (BSA) were prepared at 0.1, 0.008, 0.006, 0.003, and 0.001 mg/mL, in 0.15 M saline solution. The curve was plotted and the regression equation ($y = 0.1526x - 0.0597$, $R^2 = 0.9973$) was obtained. The absorbance obtained was linearized in the regression equation of the calibration curve, and the peptide concentration of each of the FBs was determined. The results of the peptides quantification were expressed in natural logarithm (Ln). All measures were made by triplicate.

Proteolytic activity

The proteolysis of each FBs was determined according to Church et al. (1983), for which 50 μL of each FB were taken and mixed with 1 mL of OPA reagent. After 2 min of incubation at room temperature, the absorbance was read on a UV-1800 (Shimadzu) spectrophotometer at a wavelength of 340 nm. Thus, the degree of proteolysis was obtained according to Donkor et al. (2007). This was determined by the difference between the proteolytic activities of the control sample (beverage without probiotics) and those of the treatments. All samples were read in triplicate.

ACE inhibitory activity

Samples were analyzed by reverse phase high-resolution liquid chromatography (RP-HPLC), as described by Pritchard et al. (2010) and Ghassem et al. (2011), with

some modifications. One unit of commercial ACE (Sigma-Aldrich) was diluted in 50 mM Tris-HCl (pH = 7.5) with 0.3 M NaCl to obtain a concentration of 100 mU/mL. Then, 1 mL aliquots were stored at -20°C until use. To carry out the reaction, samples were taken from the FBL and resuspended in HPLC grade water to a concentration of 50 mg/mL. Then, 50 μL of them were taken from samples or HPLC grade water in the case of the control (MC), and 200 μL of the 2.5 mM solution of Hipuril-L-Histidyl-L-Leucine (HHL) were added. The reaction was stopped by adding 250 μL of 1 M HCl. The solution was then filtered through Whatman nylon filters (30 mm /0.20 μm) to separate the reaction product and the hippuric acid (HA) from the HHL and injected into a high resolution liquid chromatograph (HPLC) coupled with a UV-visible detector (Shimadzu), using a 25 cm $\times$ 4.6 mm Discovery C18 column and 5 μm particle size (SUPELCO), which was eluted with 50–50% (v/v) water-methanol plus 0.1% (v/v) trifluoroacetic acid (TFA) at a flow rate of 0.4 mL/min and an absorbance of 228 nm. All samples were analyzed by triplicate. The evaluation of ACE inhibition was performed based on the comparison between concentrations of HA in the absence of the inhibitor (MC) and with it. For each peak, the area under the curve was determined according to the HA obtained. The mean value of the two determinations was measured to calculate the percentage inhibition of ACE using Eq. (1)

$$\text{Inhibition of ACE (\%)} = \frac{(B - A)}{(B - C)} \times 100 \quad (1)$$

where A is the relative area of the peak generated by HA in the presence of the ACE inhibitor, B is the relative area of HA obtained in the absence of the inhibitor, and C is the relative area of HA generated without ACE (corresponding to autolysis of HHL in the course of the reaction). FBs contained a certain degree of HA since it is naturally found in milk, so the HA concentrations of each sample were quantified to subtract them from the HA generated by the reaction and to calculate the ACE inhibition percentage.

Additionally, peptide concentration and ACE-inhibitory activity (IC₅₀) were estimated for each sample according to Quirós et al. (2005).

Peptide and aromatic amino acids profiles

Samples, 50 μL of each FBL or water HPLC grade (blank) were analyzed by RP-HPLC, as described by Nielsen et al. (2009) and Rodríguez-Figueroa et al. (2012), with some modifications. Peptides were separated at 40°C with a 200 mm $\times$ 4.6 mm Pinnacle II C18 column, and 5 μm particle size (RESTEK) equilibrated with solvent A (TFA at 0.04% v/v). Then these were eluted with a gradient

increase of solvent B (acetonitrile at 0.03% v/v) in solvent A from 0 to 45% for 60 min at 0.5 mL/min. The wavelengths for detection were 220 nm for peptide profiles and 280 nm for aromatic amino acids (Pro, His, Phe, Tyr, and Trp). All samples were analyzed by triplicate. Areas under the curve were integrated in arbitrary units for each of the samples for the following retention times: 6.9–26.5 min. The total area obtained in the blank sample was subtracted. This process was carried out in an HPLC coupled with a UV–visible detector (Shimadzu).

Statistical analysis

All analyses were performed in SAS package (2006, version 9.1.3; SAS Institute Inc, Cary, NC, USA). For analyzing linear association between variables (peptide concentration, proteolytic activity, inhibitory ACE activity, peptide profile, and aromatic amino acids), a correlation analysis was performed using the CORR procedure. For the global study of the variability structure, a principal component analysis was performed with the measured variables, using the PRINCOMP procedure. In addition to evaluate the effects of the same variables during shelf life, an analysis was performed using the MIXED procedure, using a randomized block design with repeated measurements over time, considering the covariate the measurement at time zero of each of the variables. Significant differences were compared ($P < 0.05$). Additionally, to determine the differences between them, a contrastive analysis was performed with the ESTIMATE syntax. The following model was considered: $y_{ijkl} = \mu + \tau_i + D_j + (\tau D)_{ij} + \beta_k + \rho(ij) + \lambda + \varepsilon_{ijkl}$. Where: y_{ijkl} = response variable measured over time, μ = general mean, τ_i = fixed effect of i -th treatment, D_j = effect of j -th day (7, 14, 21), $(\tau D)_{ij}$ = fixed effect of the interaction between the i -th treatment and the j -th day, β_k = random effect of the k -th block, $\rho(ij)$ = random effect of the i -th experimental unit, where the j -th treatment was nested in the experimental unit, λ = effect of the covariate of each variables studied at day zero, ε_{ijkl} = random error distributed in normal form, with zero mean and variance σ^2 . For the analysis of the peptide concentration required to inhibit the activity of the enzyme by 50% (IC_{50}), an analysis of variance was performed with GLM procedure using LSMEANS syntax, and it was conducted for each of the probiotics separately and based on the following model: $y_{ij} = \mu + \tau_i + \varepsilon_{ij}$. Where: y_{ij} = response variable in the i -th repetition of the i -th treatment, μ = general mean, τ_i = fixed effect of the i -th treatment, ε_{ij} = random error distributed normally with zero mean and variance σ^2 .

Results and discussions

Peptide concentration

Peptides quantification is expressed in Ln (Fig. 1). Results showed no significant difference in peptides concentration as a function of treatments ($P > 0.05$) or shelf life ($P > 0.05$). The measurement at zero day was taken as covariate, and no effect on the interaction between them ($P > 0.05$) was observed.

Proteolytic activity

Regarding proteolysis (Fig. 2), the effect by treatments ($P < 0.05$) and shelf life ($P < 0.05$) had significant differences on this variable, although the interaction was not significant ($P > 0.05$). A difference was observed at day 7 between the treatments. It should be noted that the ABT-4 beverage presented strong and constant proteolytic activity in shelf life. This activity may be due to several causes; proteolysis is generated during the obtaining of whey, during beverages preparation, or through the effect of thermophilic microorganisms (Walsh et al. 2012), since it is known that milk pasteurization is not sufficient to eliminate all microorganisms present in the milk. This last effect influences the beverages' shelf life due to the enzymatic activity of proteases (Ribeiro et al. 2018).

ACE inhibitory activity

In the ACE inhibitory activity (Fig. 3), both treatments and shelf life and their interaction showed a significant effect ($P < 0.05$). Therefore, the contrasts analysis showed differences between treatments during shelf life (7, 14, and 21 days). It was observed that the control beverage

Fig. 1 Peptide concentration (LN mg/mL) of whey-based fermented beverages added with different probiotics (BCT-1, ABC, ABT-4 and a control without probiotics). Where: A-*Lactobacillus acidophilus*, B-*Bifidobacterium animalis* subsp. *lactis*, C-*Lactobacillus paracasei* subsp. *paracasei*, T-*Streptococcus thermophilus*. Results are expressed as the mean $\pm$ standard deviation. No significant differences identified ($P > 0.05$)

Fig. 2 Proteolytic activity of whey-based fermented beverages added with different probiotics (BCT-1, ABC, ABT-4 and control without probiotics). Where: A-*Lactobacillus acidophilus*, B-*Bifidobacterium animalis* subsp. *lactis*, C-*Lactobacillus paracasei* subsp. *paracasei*, T-*Streptococcus thermophilus*. Different literals indicate significant difference ($P < 0.05$) due to the effect of monitoring day

maintained its ACE inhibitory activity at 100% throughout its shelf life. This result could be attributed to the fact that the peptides formed were kept constant, and there was no subsequent hydrolysis, this may be due to the fact that probiotics, that could metabolize and modify them, unlike the treatments. Many bioactivities presented on the whey proteins are encrypted within their native protein sequences; these could be liberated by different ways, such as during food processing (Dullius et al. 2018). Pre-treatment of whey proteins with heat, as in the case of the elaborated beverages, could lead α -helices to refold into β -sheets or random coils, this improves endoproteases access and as a consequence hydrolysis improve (Ngarize et al., 2004). Meanwhile, the ABT-4 treatment remained close to 100% activity during its shelf life. This result confirms that the effect of the peptides with this activity is attributed more to the nature of the proteins present in the beverages and secondary to the probiotics that metabolize them. For the

Fig. 3 ACE inhibitory activity of whey-based fermented beverages added with different probiotics (BCT-1, ABC, ABT-4 and control without probiotics). Where: A-*Lactobacillus acidophilus*, B-*Bifidobacterium animalis* subsp. *lactis*, C-*Lactobacillus paracasei* subsp. *paracasei*, T-*Streptococcus thermophilus*. Different literals indicate significant difference ($P < 0.05$) due to the effect of monitoring day

other treatments, during their shelf life, greater variations were observed these can be attributed to the proteolytic activity of the probiotics. Vermeirssen et al. (2003) analyzed the degree of proteolysis and ACE inhibitory activity of whey protein fermented with *Lactobacillus helveticus* and *Saccharomyces cerevisiae*, observing significant changes in the degree of proteolysis during fermentation, depending on the culture and type of protein employed. They observed that increasing fermentation increased the percentage of ACE inhibitory activity further, and the increment was higher using lactobacilli alone than when combined with yeasts. Meanwhile, Regazzo et al. (2010) elaborated fermented milk with *Enterococcus faecalis* and *Lactobacillus delbrueckii* subsp. *bulgaricus*, noting that milk fermented with *enterococci* showed a significantly higher ACE inhibitory activity ($69.43 \pm 3.12\%$) than milk fermented with *lactobacilli* ($60.86 \pm 1.01\%$). Additionally, when measuring peptide concentration, the result was greater (14.78 mg / mL) in milk fermented with *enterococci*, as opposed to that fermented with *lactobacilli* (4.89 mg / mL), suggesting that the ability to produce small peptides is directly involved with the ACE inhibitory activity.

Regarding the IC_{50} observed during shelf life (Fig. 4), the results did not present significant differences ($P > 0.05$), that is, peptide concentration required to carry out 50% of ACE inhibition was not influenced by the cultures employed, the monitoring time, or the lot analyzed. These results reinforce what is suggested by the results obtained from the ACE inhibitory activity, that the inhibition is mainly influenced by the nature of the proteins of origin itself and not by the different cuts that can be generated by microbial proteolysis. The detected values of

Fig. 4 Peptide concentration required to inhibit 50% of the ACE enzyme (IC_{50}) of whey-based fermented beverages added with different probiotics (BCT-1, ABC, ABT-4 and control without probiotics). Where: A-*Lactobacillus acidophilus*, B-*Bifidobacterium animalis* subsp. *lactis*, C-*Lactobacillus paracasei* subsp. *paracasei*, T-*Streptococcus thermophilus*. No significant differences identified ($P > 0.05$)

IC₅₀ were in the range of 0.01–0.055 mg/mL, which coincides with the reported IC₅₀ by Vermeirssen et al. (2003) of 0.07–0.2 mg / mL in whey protein and Quirós et al. (2005) of 0.061–1.18 mg / mL in kefir peptides for the ACE inhibitory activity. In another study, Tu et al. (2018) reported an IC₅₀ value of 0.77 mg/mL from a mixture of casein hydrolysates. Therefore, it can be noted that peptides derived from whey present a greater ACE inhibitory activity since it requires less concentration to obtain the IC₅₀ value.

Peptide profiles

In relation to the peptide profile (Fig. 5), treatments, shelf life, and their interaction were significant ($P < 0.05$), observing differences between treatments at day 7, except between control and ABT-4. At day 14, only control and ABC showed differences, and at day 21, no differences were identified between any of the treatments. However, in this graph, it can also be observed that both control and ABT-4 treatments maintained their profile more constantly in the same way that was observed in the ACE inhibitory activity.

Finally, for the amount of aromatic amino acids present (data not showed), both treatments and shelf life and their interaction were significant ($P < 0.05$). The same effect was observed in the analysis of contrasts, in which there were the differences between all treatments for shelf life (7, 14, and 21 days). Meanwhile, significant differences were observed between treatments with respect to the variables analyzed, except for the amount of peptides present, which remained constant between the treatments and shelf life. The effects of inoculated probiotics in the treatments were observed in the beverages during shelf life for proteolysis and peptide profile and therefore for ACE inhibitory

activity. All fermented beverages with different probiotics added showed different percentages of ACE inhibitory activity; however, the control beverage had a 100% inhibition rate, which was maintained throughout its shelf life. This result may be attributed to the fact that, after hydrolyzing the protein, by the heat treatment given to the whey-milk mixture (95 °C for 20 min), there was no additional hydrolytic effect due to the absence of microbial enzymes, thus keeping the peptides formed, unlike the treatments in which, during the fermentation process, the peptides underwent significant changes.

Correlations

Although some variables showed high correlations (Table 1), it was not a constant factor in all treatments. Therefore, it can be said that variables were linearly independent of each other. For treatment BCT-1, the highest correlations were detected (all were inverse): ACE inhibitory activity with peptide profile ($R = -0.986$; $P < 0.0001$) and with aromatic amino acids ($R = -0.913$; $P = 0.0006$) and the peptide profile with the aromatic amino acids ($R = -0.913$; $P = 0.0006$). For the control, the correlation was direct with proteolysis and aromatic amino acids ($R = 0.684$; $P = 0.0006$). Additionally, in the control, the ACE inhibitory activity during shelf life was kept constant at 100%; therefore, in Table 1, the correlations were not calculated with the rest of the variables. The treatment ABC showed a high correlation (direct) between peptide profile and aromatic amino acids ($R = 0.928$; $P = 0.0003$). Finally, the treatment ABT-4 showed correlation, inversely proportional, between ACE inhibitory activity and aromatic amino acids ($R = -0.920$; $P = 0.0004$).

In general, it was observed that correlations between variables varied greatly depending on the treatment both in magnitude and in the direct or inverse direction. These results are understood since the probiotic mixtures of each culture used were different, and the characteristics of each beverage were outlined. The results coincide with Nielsen et al. (2009), who found differences between the correlation of peptide profile and ACE inhibitory activity due to the different probiotics used in the fermentation of each treatment. These authors argued that, even in similar studies, correlations or the detected correlations have been very low (around $R = 0.25$) among the same variables analyzed. It should also be noted that, in all treatments, there were significant correlations with aromatic amino acids, showing that they are key in the development of peptide development with ACE inhibition activity which have shown the following characteristics: they contain hydrophobic aromatic amino acids such as Trp, Tyr, or Phe

Fig. 5 Peptide profile of whey-based fermented beverages added with different probiotics (BCT-1, ABC, ABT-4 and control without probiotics). Where: A-*Lactobacillus acidophilus*, B-*Bifidobacterium animalis* subsp. *lactis*, C-*Lactobacillus paracasei* subsp. *paracasei*, T-*Streptococcus thermophilus*. Different literals indicate significant difference ($P < 0.05$) due to the effect of monitoring day

Table 1 Pearson Correlations Coefficients (R) of peptide concentration, proteolysis, ACE inhibitory activity, peptide profile and aromatic amino acids of whey-based fermented beverages

	PC	PRO	ACE IA	PP	AAS
BCT-1^a					
PC	1				
PRO	0.017	1			
ACE IA	0.039	− 0.471	1		
PP	− 0.00	0.413	− 0.986^d	1	
AAS	− 0.156	0.522	− 0.913^d	0.919^d	1
Control (without probiotics added)					
PC	1				
PRO	− 0.750^c	1			
ACE IA	−	−	1		
PP	0.417	0.120	−	1	
AAS	− 0.233	0.684^c	−	0.652	1
ABC^a					
PC	1				
PRO	0.053	1			
ACE IA	0.462	0.317	1		
PP	− 0.444	− 0.239	− 0.048	1	
AAS	− 0.368	− 0.416	0.074	0.928^d	1
ABT-4^a					
PC	1				
PRO	− 0.549	1			
ACE IA	0.384	0.406	1		
PP	− 0.561	0.458	− 0.469	1	
AAS	− 0.485	− 0.330	− 0.920^d	0.438	1

^aA, *Lactobacillus acidophilus*; B, *Bifidobacterium animalis* subsp. *lactis*; C, *Lactobacillus paracasei* subsp. *paracasei*; T, *Streptococcus thermophilus*

^bPC Peptide concentration expressed as mg/mL, PRO proteolysis expressed as absorbance at 340 nm, PP peptide profile expressed as arbitrary units, AAS aromatic amino acids expressed as arbitrary units, ACE-IA ACE inhibitory activity expressed as percentage

^cThe correlation coefficient is significant with an interval of confidence of 95% (P < 0.05)

^dThe correlation coefficient is significant with an interval of confidence of 99% (P < 0.01)

in one of the last three positions of their sequence, and Pro residues alone or in combination with other hydrophobic residues by joining the active sites of the ECA block its activity, thus marking its antihypertensive capacity (Shahidi and Zhong, 2008).

Conclusion

All whey-based fermented beverages showed ACE inhibitory activity, which it was found to be related to proteolysis and peptide and aromatic amino acids profiles.

However, whey-based beverage without probiotics added had the highest ACE inhibitory activity (100%), and it was maintained during its shelf life. Fermented beverage with the ABT-4® culture had an ACE inhibitory activity from 88 to almost 100% during its shelf life, additionally this culture presented the strongest and most constant proteolytic activity which shows a synergistic effect among the strains that compose it. Moreover, fermented beverage added with BCT-1 culture had the lowest ACE inhibitory activities at the day 14 of the shelf life (40%). These findings are important for further development of whey-based fermented beverages added with probiotics and aimed to have ACE inhibition activity. Therefore, an additional study is recommended to carry out the identification of the peptides responsible for the ACE inhibitory activity found in the whey-based fermented beverages and, finally, to conduct an in vivo evaluation of these beverages.

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Compliance with ethical standards

Conflict of interests The authors declare no conflict of interest regarding the publication of this article.

Ethic statement This research did not include any human subjects and animal experiments.

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