Inhibition of the Angiotensin I Converting Enzyme (ACE) and proteolysis of non-fat probiotic yogurt

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Cite as: Rezaei, A., Amirdivani, S., Asl, A. K., Malekinejad, H., Zomorodi, S., & Hosseinmardi, F. (2019). Inhibition of the Angiotensin I Converting Enzyme (ACE) and proteolysis of non-fat probiotic yogurt. Brazilian Journal of Food Technology, 22, e2018234. https://doi.org/10.1590/1981-6723.23418

Abstract

Yogurt is an important source of many biologically active peptides with specific health benefits. The majority of the bioactive peptides produced during yogurt manufacture are related to angiotensin converting enzyme inhibitory (ACE-I) peptides. The present study evaluated the proteolysis and angiotensin converting enzyme (ACE) inhibitory activities of non-fat probiotic yogurt supplemented with sodium caseinate (0 to 4%), and Mentha piperita (peppermint) extract (0 to 0.4%) during 20 days of storage. Good correlation (R = 0.90) was found between the growth of Lactobacillus casei LFTI® L26 and ACE inhibition in all samples during the initial stages of storage, as compared to the control yogurt, with a significant (p < 0.05) decrease after storage. The results showed that the addition of sodium caseinate and peppermint extract had a significant (p < 0.05) effect on proteolysis and the viability of L. casei LFTI® L26, enhancing the ACE activity. The IC50 values of the sample containing 0.4% of peppermint and of the sample containing 4% of sodium caseinate were 0.12 and 0.02 mg/mL respectively. The results showed that the use of 4% of sodium caseinate and 0.4% of peppermint extract could provide higher probiotic viability (1.3×10^7 cfu/g) on the 20th day of storage.

Keywords: Probiotic; Sodium caseinate; Peppermint extract; Proteolysis; Angiotensin converting enzyme; Yogurt.
Resumo
O iogurte é uma importante fonte de muitos peptídeos biologicamente ativos que podem apresentar benefícios específicos à saúde. A maioria dos peptídeos bioativos produzidos durante a produção de iogurte está relacionada aos peptídeos inibidores da enzima conversora da angiotensina (ECA-I). O presente estudo avaliou a proteólise e as atividades inibitórias da enzima conversora de angiotensina (ECA) de iogurte probiótico desnatado, suplementado com caseinato de sódio (0 a 4%) e extrato de Mentha piperita (0 a 0,4%), durante 20 dias de armazenamento. Foi encontrada uma boa correlação entre o crescimento de Lactobacillus casei LFTI® L26 e a inibição da ECA em todas as amostras (R = 0,90). Todos os iogurtes suplementados mostraram boa atividade inibitória da ECA durante os estágios iniciais de armazenamento em comparação com o iogurte controle, com significativa diminuição (p < 0,05) após o armazenamento. Os resultados mostraram que a adição de caseinato de sódio e extrato de Mentha piperita teve efeito significativo (p < 0,05) na proteólise e na viabilidade de L. casei LFTI® L26, aumentando a atividade da ECA. O valor de IC50 da amostra contendo 0,4% de hortelã-pimenta e a amostra contendo 4% de caseinato de sódio foi de 0,12 e 0,02 mg/mL, respectivamente. Os resultados mostraram que a utilização de 4% de caseinato de sódio e 0,4% de extrato de hortelã-pimenta poderia proporcionar maior viabilidade probiótica (1,3 × 10⁷ UFC/g) aos 20 dias de armazenamento.

Palavras-chave: Probiotic; Caseinato de sódio; Extrato de hortelã-pimenta; Proteólise; Enzima conversora de angiotensina; Iogurte.

1 Introduction
The prevalence of hypertension is a global concern, reaching about 40% of the worldwide adult population (Mendis et al., 2011) and 20.8% of Brazilians (Selem et al., 2014). It increased dramatically in Brazil, by about tenfold, over the last four decades (Ruílope et al., 2018), such that it is now responsible for more than a third of all deaths in this country (Pimenta & Assunção, 2015). These high rates have occurred despite the availability of advanced blood pressure control drugs, so it appears that lifestyle habits are mainly effective in this case (Selem et al., 2014; Ribeiro et al., 2018). Thus diet was identified as the single most important factor to prevent and control hypertension, which is easier for the public to interpret (Selem et al., 2014). Hence fermented milk products are becoming increasingly popular because of the numerous health benefits they provide, associated with highly digestible nutrients especially for those with lactose intolerance, and also large amounts of bioactive peptides (Amirdivani, 2015; Chen et al., 2015). Many peptides with bioactive characteristics have been isolated from different fermented dairy products, including fermented milk, cheese and yogurt. These peptides have many benefits in inhibiting diseases such as cancer and hypertension, and are already used in commercial products.

The inhibition of the angiotensin I-converting enzyme (ACE) is considered to be a useful therapeutic approach in the treatment of high blood pressure in both diabetic and non-diabetic patients (Omedì et al., 2016; Chen et al., 2015; Ibrahim et al., 2016). Most of the milk-derived peptides with multiple functions are involved in the production of bioactive substances (Smacchi & Gobbetti, 2000; Rojas-Ronquillo et al., 2012). The in vitro ACE-I activity could be related to the liberation of the peptide due to casein degradation (Donkor et al., 2007) as a result of proteolysis by L. casei. On the other hand, with respect to the activity of the lactic acid bacteria, proteolysis is the most important biochemical process occurring in sour milk products during fermentation and storage (Wang et al., 2015, 2016), and improvements in the ACE inhibitory activity during fermentation depend on the degree of hydrolysis (Nejati et al., 2013). L. casei is known to be specific in the production of ACE inhibitors during fermentation. (Donkor et al., 2007; González-Olivares et al., 2014; Pihlanto et al., 2010).
The study determined the ACE inhibitory activities of *L. casei* as a commercial probiotic organism for the hydrolysis of sodium caseinate in yogurt. Lactic acid bacteria (LAB) are known to be probiotic organisms which are important for food fermentations (Wang et al., 2015; González-Olivares et al., 2014; Pihlanto et al., 2010). The incorporation of probiotic organisms such as *L. casei* in yogurt provides the potential to improve the quality of the product and of the consumer’s health status. In addition, peppermint is one of the oldest medicinal plants, is rich in polyphenolic compounds and flavonoids, and is widely used in the people’s diet. Recent studies have shown that phenolic compounds have high antioxidant activity and therapeutic properties, including the prevention of diabetes and hypertension. In addition, plants rich in flavonoids have ACE-I activities (Sweetie et al., 2007; Amirdivani & Baba, 2011).

Thus this is a worthwhile issue to study, and by doing so, the ability to prevent high blood pressure could be greatly improved. However, the inhibitors present in food are not as powerful as the drugs used to treat high blood pressure, but they also have mild activities so it can be viewed as a natural functional food and placed in the daily diet.

### 2 Material and methods

#### 2.1 Materials

Skim milk powder (SMP) was purchased from the Kaleh Dairy Company (Urmia, Iran). A DVS (Direct Vat Set) freeze-dried yogurt culture (YC-350: mixed cultures of *L. delbrueckii* subsp. *Bulgaricus* and *S. thermophilus*) was purchased from Chris Hansen, Denmark. *L. casei* LAFTI® L26 was obtained from DSM Food Specialties (Moorebank, NSW, Australia). Sodium caseinate with 78.95% protein (Milad Khorasan, Iran), water and an alcoholic extract of peppermint (Zardband Iran) were also used. Freeze-dried rabbit lung angiotensin-converting enzyme, enalapril, O-phthaldialdehyde and the ACE substrate FAPGG (N-(3-(2-Furyl) acryloyl)-L-phenylalananyl-glycyl-glycine), were purchased from Sigma-Aldrich (St. Louis, MO, USA).

#### 2.2 Methods

**2.2.1 Yogurt preparation**

Low-fat yogurt was prepared using skimmed milk (Kaleh, Urmia, Iran) standardized at 12, 14 and 16 g/100mL total solids with skim milk powder and sodium caseinate (0 to 4%). According to Table 1, sodium caseinate and peppermint extract were added to the pre-warmed (41 °C) non-fat milk and treated at 85 °C for 15 minutes, followed by cooling. The DVS freeze-dried yogurt culture (YC-350: mixed cultures of *L. delbrueckii* subsp. *Bulgaricus* and *S. thermophilus*) was added at 1% (w/v) and *L. casei* as the probiotic culture at 10 g/100kg of milk at a temperature of (43 ± 1 °C) (Shah & Yogurt, 2003), and 250 mL aliquots of the mixture filled into disposable plastic containers. The plain yogurt was essentially prepared in the same manner. The yogurts were then incubated at (43 ± 1 °C) until the pH was reduced to 4.5, followed by refrigeration (5 ± 1 °C) for up to 20 days.
Table 1. Coded and real values for the independent variables used in the central composite design with three responses: sodium caseinate content ($X_1$), peppermint extract content ($X_2$), and storage time ($X_3$).

| run | $X_1$ [%w/w] | $X_2$ [%v/v] | $X_3$ [day] | $X_1$ [%w/w] | $X_2$ [%v/v] | $X_3$ [day] |
|-----|--------------|--------------|-------------|--------------|--------------|-------------|
| 1   | -1           | -1           | -1          | 0            | 0            | 4           |
| 2   | +1           | +1           | +1          | 0            | 0            | 20          |
| 3   | +1           | +1           | -1          | 0            | 0.4          | 4           |
| 4   | +1           | +1           | +1          | 0            | 0.4          | 20          |
| 5   | +1           | -1           | -1          | 4            | 0            | 4           |
| 6   | +1           | -1           | +1          | 4            | 0            | 20          |
| 7   | +1           | +1           | -1          | 4            | 0.4          | 4           |
| 8   | +1           | +1           | +1          | 4            | 0.4          | 20          |
| 9   | 0            | 0            | 0           | 0            | 0.2          | 12          |
| 10  | -1           | 0            | 0           | 4            | 0.2          | 12          |
| 11  | 0            | -1           | 0           | 2            | 0            | 12          |
| 12  | 0            | +1           | 0           | 2            | 0.4          | 12          |
| 13  | 0            | 0            | -1          | 2            | 0.2          | 4           |
| 14  | 0            | 0            | +1          | 2            | 0.2          | 20          |
| 15  | 0            | 0            | 0           | 2            | 0.2          | 12          |
| 16  | 0            | 0            | 0           | 2            | 0.2          | 12          |
| 17  | 0            | 0            | 0           | 2            | 0.2          | 12          |
| 18  | 0            | 0            | 0           | 2            | 0.2          | 12          |
| 19  | 0            | 0            | 0           | 2            | 0.2          | 12          |
| 20  | 0            | 0            | 0           | 2            | 0.2          | 12          |

2.2.2 Determination of the pH value and TA (Titratable Acid)

The pH value was determined after calibrating a digital pH meter using standard buffers at pH values of 4 and 7. The electrode of the pH meter was immersed directly in the yogurt samples and the pH recorded. The acidity was determined by titrating with 0.1 mol L⁻¹ NaOH in the presence of 2 drops of phenolphthalein indicator until the appearance of an amethystine color, according to the Official Methods of the Association of Official Analytical Chemists (1997). The acidity of the samples was calculated according to Equation 1.

$$ \text{Acidity} = \frac{V \times 10^{-m}}{m} $$

where $V$ represents the volume of NaOH solution used and $m$ the sample weight.

2.2.3 Enumeration of the Viable Cell Count (VCC)

The $L$. casei count was determined on MRS-agar (Amyl media, Dandenong, Australia) containing filter sterilized vancomycin in a final concentration of 0.35 mol L⁻¹, which was added to the liquid after autoclaving the MRS-agar (DSM Co. catalogue). For the microbiological analyses, 5g of a yogurt sample was suspended in 45 mL sterile peptone water (0.1%) and serially diluted 10-fold (10⁴ to 10⁸). Using the pour plate technique, 1 mL of each of the appropriate dilutions was cultured and the plates incubated at 37 °C for 72 h (Donkor et al., 2007). Plates showing 30-300 colonies were counted, and the results expressed as colony forming units per gram (cfu/g) of the sample.

2.2.4 Proteolysis activity using the o-Phthaladehyde (OPA) reagent

0.15 mL of yogurt extract was added to 3 mL of OPA reagent (based on β-mercaptoethanol) (Papadimitriou et al., 2007; Nielsen et al., 2009) in a 5 mL quartz cuvette, mixed for 5 seconds, held for 2 minutes at room temperature, and the absorption at 340 nm read in a spectrometer (Beckman, America). The blank was prepared by substituting the yogurt sample with distilled water. The degree of proteolysis was
expressed based on the peptides measured and absorption by free amino acids (Amirdivani & Baba, 2011). The peptide concentration was assessed against a tryptone standard.

2.2.5 Determination of ACE inhibition Activity

The ACE-inhibition activity of the yogurt was determined following the protocol described by Pihlanto (Pihlanto et al., 2010). According to this method, 0.125 mL of FAPGG reagent were added to 0.125 mL of yogurt samples in the cuvette. The mixture was stirred thoroughly, incubated in a water bath at 37 °C for 15 min and rabbit lung extract (0.3 mL in 50 mmol L⁻¹ Tris-HCl) added. The absorption was measured at 340 nm and the plate then incubated at 37 °C for 10 min before reading the final absorption. Enalapril was used as the standard. ACE inhibition was determined in duplicate and the inhibitory activity calculated (Amirdivani & Baba, 2011) using Equations 2 and 3:

\[
\% A_{\text{ACE inhibitory activity}} = 100 \left(1 - \frac{\Delta A_{\text{sample}}}{\Delta A_{\text{control}}}\right) \times 100
\]

\[
\Delta A_{\text{sample}} = A_0 - A_{10}, \quad \Delta A_{\text{control}} = A_0 - A_{10}
\]

2.3 Experimental design and statistical analysis

A centred central composite design was used, the results submitted to an ANOVA using SAS (version 9.1, SAS Institute Inc.), and the graphs drawn using Matlab software (version 7.10.0449, The MathWorks Inc.). Response surface methodology (RSM) is a collection of mathematical and statistical techniques used to design experiments, modelling, and for investigating the effects of some factors on one or more dependent variables (Bitaraf et al., 2012). The independent variables here were sodium caseinate, peppermint extract and the storage time. Table 1 shows the real and coded values of the factors. Twenty yogurt samples were evaluated according to the design, with three variables and three levels for each variable. The data were fitted to the second-order polynomial according to Equation 4.

\[
Y = \beta_0 + \sum_{i=1}^{3} \beta_i X_i + \sum_{i=1}^{3} \beta_{ij} X_i^2 + \sum_{i=1}^{3} \sum_{j=i+1}^{3} \beta_{ij} X_i X_j
\]

where \(Y\) represented the predicted response; \(\beta_0\) the constant term; \(\beta_i\) the linear coefficient; \(\beta_{ii}\) the squared coefficient and \(\beta_{ij}\) the interaction coefficient, the \(X\) being the independent coded variables.

3 Results and discussion

3.1 Determination of pH and acidity

According to Figure 1 the pH of the sample decreased from 4.2 to 3.5 and the acidity increased from 1 to 1.2 during the 20 days of storage. It seems that the presence of the Mentha piperita extract caused an increase in the metabolic activity of the yogurt bacteria (Amirdivani & Baba, 2011) and provided more substrates for bacterial proteolysis and cell growth in the presence of phenolic compounds (Shori & Baba, 2011). The number of free amino groups and peptides increased due to proteolysis of the milk protein by bacterial enzymes (Chen et al., 2015). However, by increasing the amount of sodium caseinate, the pH increased and the acidity decreased (Aziznia et al., 2008), probably due to consumption of the carbohydrates by the microorganisms and the production of more organic acids (Ostlie et al., 2003), thereby reducing the acidity and proteolysis, and releasing calcium from the casein micelles. Consequently, elevated free \(-\text{Ca}^{2+}\) concentrations may be a factor in lowering the blood pressure. González-Olivares et al. (2014) and Nielsen et al. (2009) reported that ACE-I was < 50% in the pH range from 4.3 to 4.6. At pH 6 the activity of the cell-envelope proteinases and amino peptidases increased, followed by a decrease at lower pH values.
Pan et al., 2010). González-Olivares et al. (2014) reported that the -Ca²⁺ concentration was correlated with pH and the ACE inhibitory activity. *L. casei* showed the highest ACE-inhibitory activity at low -Ca²⁺ concentrations. The present results were similar to the results found by González-Olivares et al. (2014). The highest ACE inhibition activity was observed when the pH of the yogurt reached 4.1 on the 20th day of storage.

It was observed that the amounts of free amino acids and peptides were lowered when LAB degraded the proteins, a process which is time dependent (Donkor et al., 2007). During fermentation, the microorganisms started using both the proteins and the organic acids (Ruiz et al., 2004; Donkor et al., 2007), causing an increase in pH and decrease in acidity (Jai, 1990; Amirdivani, 2015) throughout the storage period. The yogurt containing 4% sodium caseinate had a strong buffering capacity, explaining why the yogurt supplemented with sodium caseinate showed a weaker capacity for post acidification during the storage time than the others.

**Figure 1.** Response surface plot for pH as a function of (a) percent peppermint extract and storage time for a caseinate content of 2% and (b) percent peppermint extract and sodium caseinate content on the 12th day of storage and (c) response surface plot for acidity as a function of percent peppermint extract and storage time for a sodium caseinate content of 2%.
3.2 Probiotic cell count

Figure 2 shows the change in the viable *L. casei* cell count. During the storage time, the number of *L. casei* cells first decreased and then increased. This study showed that the pH value decreased up to the 4th day of storage, followed by an increase up to the 10th day of storage. In addition, an increase in the amount of sodium caseinate added from 0 to 4% also increased the number of *L. casei* cells, which could be justified by increasing the amount of substrate available for bacterial growth (Pihlanto et al., 2010). Lactic acid bacteria need free amino acids for growth, more than natural milk proteins (Donkor et al., 2007) and therefore the LAB tend to use other proteins. Donkor et al. (2007) reported that proteolysis was dependent on time, and hence bacterial growth was slow in the early stages of fermentation and increased with time. The protein hydrolysis system is the most important factor for LAB survival, and thus the lack of free amino acids should have stimulated the culture to produce the free amino acids using its own proteolytic capability (Wang et al., 2015). Chen et al. (2015) reported a positive correlation between ACE-inhibition and free amino acids in a highly acid environment, which was associated with the bacterial activities. In the present study, the highest viable count was observed at pH 4.42, parallel to the results found by Wang et al. (2015). Oligopeptides were produced by extracellular proteinases in the initial stages of casein hydrolysis during fermentation, but further hydrolysis of the oligopeptides is necessary in order to fulfil the needs of the culture. Amirdivani & Baba (2011) reported that the survival of the starter bacteria was enhanced by increasing the amount of peppermint extract, but due to a lactose limitation for the starter bacteria applied in advance, there was a reduction in the number of *L. casei* cells with time, and therefore its growth was increased by using the sources and the synergistic influence of the starter culture (Samona & Robinson, 2007). González-Olivares et al. (2014) reported that *L. casei* produced the most potent peptides with an IC50 value of 0.47 mg/mL, after 48 hours fermentation. After fermentation, the yogurt containing sodium caseinate and peppermint extract had a pH value of 4.42 and a cell density of 9.1 log cfu/mg.

![Figure 2](image-url)

**Figure 2.** (a) Response surface plot for the probiotic count as a function of storage time (days) and sodium caseinate content, with a peppermint extract percentage of 0.2%; (b) Response surface plot for the probiotic count as a function of storage time (days) and peppermint extract content, with a sodium caseinate percentage of 2%.

3.3 Evaluation of proteolysis

Proteolysis is the most important biochemical process taking place during fermentation and storage (Amirdivani & Baba, 2013). During fermentation, the milk proteins are hydrolyzed by the LAB exopeptidase, leading to an increase in the free -NH$_2$ groups, which can be determined by the OPA method. According to Table 2 the statistical analyses suggested that the effects of sodium caseinate and storage time and the mutual effect of sodium caseinate and peppermint extract had significant effects on yogurt proteolysis ($p < 0.05$).
According to Figure 3a, proteolysis increased during the storage time and with an increasing level of sodium caseinate. Figure 3b shows that with a low amount of peppermint extract, proteolysis could be increased by increasing the sodium caseinate level, but with a large amount of this extract, an increase in the sodium caseinate level first increased proteolysis and then decreased it. One possible reason for this is that the extract leads to growth in the cell metabolism, consequently increasing the acidity (Ashraf & Shah, 2011). Thus the lactic acid produced influenced the yogurt proteins, leading to protein denaturation and facilitating hydrolysis. However, due to hydrolysis, the peptides and free amino acids produced caused a decrease in the acidity. Similarly, by using up the sugar materials, the bacteria began to consume the organic acids and peptides produced and, as a result, proteolysis decreased (Frazier & Westhoff, 1995). By increasing the amount of sodium caseinate, the growth of \textit{L. casei} increased, due to provision of the requirements for growth, materials available and the production of intra and extracellular enzymes (Ashraf & Shah, 2011; Frazier & Westhoff, 1995). These enzymes can hydrolyse active biological peptides and bradykinin, the enzymes being widely used in the food industry. Proteolysis provides the necessary growth factors to enhance \textit{L. casei} survival, in the form of peptides and amino acids, and thus proteolysis increases due to the materials available for growth.

![Figure 3](image-url)  
\textit{Figure 3.} (a) The effect of sodium caseinate and peppermint extract on proteolysis; (b) The effect of sodium caseinate and storage time on proteolysis.

### 3.4 ACE-inhibitory activity

The ACE enzymatic activities can be transformed by the binding of biomolecules such as polyphenols, flavonoids and bioactive peptides onto the active binding sites of the enzyme (Donkor et al., 2007). The yogurt studied here contained peppermint extract, which is particularly rich in polyphenols and flavonoids (Sweetie et al., 2007), plus sodium caseinate as a source of bioactive peptides (Donkor et al., 2007). According to Figure 4a, the presence of a large amount of sodium caseinate provided plentiful substrate for \textit{L casei} growth, leading to an increase in ACE inhibition, although a decreasing trend was observed with a high level of peppermint extract (Figure 4b) after the first storage period, while showing a reversal of this trend on the 20th day. Compared with similar results for the OPA values, the ACE Inhibitory activity was enhanced as proteolysis developed, although it decreased when proteolysis exceeded a certain level (Fung & Liong, 2010) and by the 20th day of storage the casein-derived bioactive peptides had been used up by the microorganisms. Thus the proteolytic activity of \textit{L. casei} is associated with the production of bioactive compounds and with ACE inhibition (Donkor et al., 2007; Fung & Liong, 2010).

These results showed that increasing levels of sodium caseinate and peppermint extract led to increases in bacterial growth. With respect to proteolytic activity, up to a certain level the amount of bioactive peptides and the rate of ACE inhibition increased significantly according to Table 2 ($p < 0.05$). However, as the
bioactive peptides were used up by the microorganisms, so the rate of ACE inhibition was cut back. In the present study the IC₅₀ values were from 0.035 mg/mL to 0.12 mg/mL, which agrees with the results found in Leclerc et al. (2002). The highest IC₅₀ value was observed in the yogurt supplemented with 4% peppermint extract (0.12 mg/mL).

Figure 4. (a) Response surface plot for percent of ACE-inhibitory activity as a function of storage time (days) and sodium caseinate content, with a percent peppermint extract of 0.2%; (b) Response surface plot for ACE-inhibitory activity as a function of storage time (days) and peppermint extract content with a sodium caseinate percent of 2%.

Table 2. Analysis of variance for the responses of the dependent variables.

| Source of variation | df | pH Mean square | F value | Acidity Mean square | F value | Proteolysis Mean square | F value | Log probiotic count Mean square | F value | ACE-inhibitory activity Mean square | F value |
|---------------------|----|----------------|---------|---------------------|---------|-------------------------|---------|-------------------------------|---------|-------------------------------|---------|
| Regression          | 9  | 0.034579       | 6.013** | 0.0273             | 6.049** | 351.118                | 7.204** | 0.207                         | 6.2885** | 5.324.606                     | 9.627***|
| Linear              | 3  | 0.074049       | 12.876***| 0.0553             | 12.249***| 311.020                | 41.244**| 0.342                         | 10.396** | 8410.47                       | 15.206***|
| Square              | 3  | 0.015295       | 26.595  | 0.0068             | 1.519   | 279.308                | 7.176*  | 0.187                         | 5.687*   | 1.079.964                     | 19.526  |
| Interaction         | 3  | 0.014395       | 2.503   | 0.0197             | 4.381   | 463.026                | 1.013***| 0.0915                        | 2.873    | 6.483.385                     | 11.72252**|
| Lack of fit         | 5  | 0.001195       | 0.1159  | 0.007              | 3.659   | 12.474                | 3.541   | 0.05                          | 3.184    | 906.888                       | 45.514  |
| Pure error          | 5  | 0.010307       | 0.0019  | 84.997             | 0.0157  | 1.992.533             |         |     |                               |         |
| Total               | 19 |               |         |                    |         |                        |         |     |                               |         |

R²: 0.844
R² (adj): 0.703

4 Conclusion

The proteolytic activity of *L. casei* in the supplemented yogurt could have given rise to ACE inhibitory activity. While extensive research has centred on the bioactive peptides resulting from the addition of sodium caseinate or *Mentha piperita* extract separately, this study was carried out using them both together. To our knowledge, this is the first report on the use of probiotic yogurt containing sodium caseinate and *Mentha piperita* extract with an *in vitro* antihypertensive effect. Yogurt is a great alternative which can be included in the diet and is thus suitable for those with high blood pressure. The effects of other plant extracts necessitate further studies to develop a novel antihypertensive supplement. The present results showed that traditional products containing probiotics and bioactive compounds may possess multifunctional health benefits, but nevertheless, further research is needed to indicate these properties via *in vivo* and clinical studies.
Acknowledgements

The authors are grateful to Prof. Khalil Farhadi, Prof. Minervini and Fatemeh Nejati, the Engineering and Agricultural Research Centre of West Azerbaijan and the Pharmacology and Toxicology Division of Urmia University, for their support during this study. This research was sponsored by the University of Urmia.

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Funding: The work has received funding from Urmia University.

Received: Feb. 05, 2018; Accepted: June 03, 2019