

ACE-inhibitory Effect and Physicochemical Characteristics of Yogurt Beverage Fortified with Whey Protein Hydrolysates

Sung-Min Lim, Na-Kyoung Lee, Keun-Kyu Park, Yoh Chang Yoon, and Hyun-Dong Paik*

Division of Animal Life Science and Bio/Molecular Informatics Center, Konkuk University, Seoul 143-701, Korea

Abstract

This study investigated the ACE-inhibitory effect of yogurt beverage fortified with hydrolysates as well as the suitability of hydrolysates as a nutraceutical additive to yogurt beverage. Three whey protein hydrolysates hydrolyzed by alcalase, protamex, and trypsin were each added to yogurt beverage at concentrations of 1.25, 2.5, and 5 mg/mL. Yogurt beverage fortified with 2.5 mg/mL of hydrolysates had 61-69% ACE-inhibitory activity, whereas yogurt beverage fortified with 5 mg/mL of hydrolysates showed 74% ACE-inhibitory activity. There were no significant differences in ACE-inhibitory activity between the alcalase or protamex hydrolysates during storage; however, trypsin hydrolysate exhibited significant differences. On the other hand, physicochemical characteristics such as pH (3.47-3.77), titratable acidity (0.81-0.84%), colority, viable cell count, and sensory qualities were not significantly different among the tested yogurt beverage samples during storage. These results showed that yogurt beverage fortified with whey protein hydrolysates maintained antihypertensive activity and underwent no unfavorable changes in physicochemical characteristics regardless of enzyme type.

Key words: angiotensin-converting enzyme, whey protein hydrolysate, yogurt beverage

Introduction

Angiotensin-converting enzyme (ACE) is a transmembrane zinc metallopeptidase that cleaves dipeptides from the C-terminus of a peptide substrate. ACE is a regulatory factor in the renin-angiotensin system (RAS), which plays an important role in cardiovascular homeostasis, blood pressure regulation, and fluid and salt balance. In the RAS, rennin cleaves the liver-derived precursor angiotensinogen into a decapeptide, angiotensin I, which is then cleaved by ACE to form angiotensin II (a vasoconstrictor). In addition, ACE inactivates the vasodilative peptides bradykinin and kallidin and stimulates the release of aldosterone, which increases blood pressure (Campbell, 2003).

Several lactic acid bacteria are known to produce inhibitors of ACE during fermentation. These inhibitors are formed by proteinase in lactic acid bacteria during the hydrolysis of milk proteins, mainly casein, into peptides, which can then be used as nitrogen sources for bacterial growth. The total activity and specificity of proteolysis

plays an important role in this process since the resulting inhibitors are peptides (FitzGerald and Murray, 2006; Korhonen and Pihlanto, 2006; López-Fandino *et al.*, 2006). Some of these inhibitor peptides have also been shown to lower blood pressure in hypertensive rats (Fuglsang *et al.*, 2002).

Peptides with ACE-inhibitory activity have already been isolated from various food proteins (Ariyoshi, 1993; Jang and Lee, 2005; Yamamoto *et al.*, 1994). Especially, fermented milk products are known as an excellent source of bioactive peptides (Yamamoto, 1997). Such peptides possess antibacterial, anticancer, immunomodulatory, mineral-binding, and antihypertensive properties (Minervini *et al.*, 2003; Shah, 2000; Silvia and Malcata, 2005). Extracellular proteinases are involved in the initial degradation of caseins, yielding a large number of oligopeptides. Consequently, further breakdown by intracellular peptidases is critical to making available essential and growth-stimulating amino acids and peptides (Christensen *et al.*, 1999; Donkor *et al.*, 2007; Kunji *et al.*, 1996). Bioactive peptide fragments originating from whey proteins should be taken into account as components that have a positive effect on cardiovascular health (Clare and Swaisgood, 2000; Pihlanto-Leppala *et al.*, 2000; Seppo *et al.*, 2003; Yusuf *et al.*, 1999). Bovine milk

*Corresponding author: Hyun-Dong Paik, Division of Animal Life Science and Bio/Molecular Informatics Center, Konkuk University, Seoul 143-701, Korea. Tel: 82-2-2049-6011, Fax: 82-2-455-3082, E-mail: hdpaik@konkuk.ac.kr

whey is considered one of the main raw materials in the production of functional ingredients. Several authors reported that whey protein hydrolysates show ACE-inhibitory activity as well as hypotensive activity in animals and humans (Fujita *et al.*, 2001; Van der Ven *et al.*, 2002; Vermeirssen *et al.*, 2003).

The purpose of this study was to investigate the ACE-inhibitory effect of yogurt beverage fortified with hydrolysates as well as whether or not hydrolysates are suitable as a nutraceutical additive to yogurt beverage. The results would determine the potential of whey protein hydrolysate as an ingredient for the production of advanced anti-hypertensive functional dairy foods.

Materials and Methods

Enzymatic hydrolysis of whey protein concentrate

Whey protein concentrate (WPC, 70% proteins) was supplied by Hilmar Cheese Company (USA). The enzymes used in this study were alcalase, protamex, and trypsin (Novo Nordisk Biochem. Inc., Denmark). WPC was solubilized in deionized water (10% (w/w) protein basis). The enzyme preparation was then added to the homogenate at concentration of 1.25% (w/v). The optimized hydrolysis conditions used for these enzymes were as follows: alcalase (50°C, pH 6.5, 3 h), protamex (50°C, pH 7.0, 5 h), and trypsin (37°C, pH 8.0, 2 h). Hydrolysis was performed in a shaking incubator at 180 rpm (Kim *et al.*, 2007; Nicholas and Eric, 1996; Sinha *et al.*, 2007).

After the reactions, the whey protein hydrolysates were placed in a water bath (20 min, 120°C) for enzyme inactivation followed by cooling. The hydrolysates were centrifuged at 5,200×g for 15 min, after which the supernatant was collected and frozen at -70°C for 24 h before freeze-drying in a chemical-free freeze drier (FDCF-12012, Operon, Korea).

Sample preparation

All yogurt beverage (Namyang yogurt beverage, Namyang Dairy Products Co., Korea) samples were obtained from a local shop in Seoul. The hydrolysates were added at concentrations of 1.25, 2.5, and 5 mg/mL of yogurt beverage. For storage tests, the samples were placed in a 4°C refrigerator for up to 7 d. The day after the designated expiration date was fixed as 0 d of storage, and samples were taken at 1 or 2 d intervals for analytical and microbial testing.

In vitro assay for ACE inhibitory activity

ACE-inhibitory activity was measured using a modified method (Cushman and Cheung, 1971; Van der Ven *et al.*, 2002; Vermeirssen *et al.*, 2003). For each assay, a mixture containing 50 µL of sample, 100 µL of 0.1 M sodium borate buffer (pH 8.3), and 50 µL of ACE (5 mU) solution was incubated at 37°C for 5 min. Then, 50 µL of Hip-His-Leu solution was added and incubated for 30 min. The enzymatic reaction was stopped by adding 250 µL of 0.5 N HCl. Hippuric acid formed by the action of the ACE on HHL was extracted from the acidified solution into 1.5 mL of ethyl acetate by vortexing for 15 s. Then, centrifugation was carried out at 3,290 g for 10 min at 4°C, after which a 1 mL aliquot of each ethyl acetate layer was transferred into a clean tube and evaporated by heating at 90°C for 60 min in a water bath. The hippuric acid was redissolved in 3 mL of deionized water, and the amount formed was determined by its absorbance at 228 nm. The activity of each sample was tested in triplicate. ACE-inhibitory activity was calculated according to the following formula:

$$\text{ACE-inhibitory activity (\%)} = \frac{\text{absorbance of control} - \text{absorbance of sample}}{\text{absorbance of control}} \times 100$$

pH, titratable acidity, and color evaluation

The pH levels of the samples were determined using a pH meter (Model 340, Mettler-Toledo GmbH, Schwerzenbach, Switzerland). The pH values of the yogurt beverage samples were each measured in 30 mL of sample. The titratable acidity was determined according to the Association of Official Analytical Chemists (AOAC, 1995) method.

Instrumental color analysis of yogurt was performed as follows. The yogurt beverage sample was measured (40 mL) in Petri dishes (90×15 mm) for color analysis, and color measurements were taken using a colorimeter (Chroma meter CR-400, Konica-Minolta, Japan), which contained an 8 mm diameter measuring area and a 50 mm diameter illumination area. Color values (CIE L*, a*, and b*) were measured on the surfaces of the samples, and the results are in the mean of triplicate measurements.

Microbiological analysis

Viable cells were counted using the spread plate method with MRS agar. The plates were incubated at 35°C for 48 h.

Sensory evaluation

Sensory evaluation of yogurt samples was carried out at 0, 3, and 7 d using a five-point hedonic scale ranging from “dislike extremely” to “like extremely”. Ten panelists (5 females, 5 males) were selected by screening tests conducted with dairy product based on interest, availability, and performance, and these who confirmed consuming yogurt at least once a week were chosen for this study.

Statistical analysis

All experiments were performed at least three times under each experimental condition and mean values were reported. Analysis of variance (ANOVA) was performed on all of the variables measured using the General Linear Model (GLM) procedure of the SAS statistical package (SAS Institute, Inc., 1999). Duncan’s multiple range test ($p < 0.05$) was used to determine differences between treatment means.

Results and Discussion

ACE inhibitory activity of yogurt fortified with hydrolysates

Numerous studies have focused on the production and isolation of ACE-inhibitory materials (Fujita *et al.*, 2001; Van der Ven *et al.*, 2002; Vermeirssen *et al.*, 2003). Fig. 1 shows that the ACE-inhibitory activity of yogurt beverage ranged from 47 to 50%. Japanese Calpis yogurt reportedly has similar ACE-inhibitory activity, but it is dependent on the seed culture (Otte *et al.*, 2003). Therefore, this study studied yogurt beverage fortified with WPH according to enzyme type. Yogurt beverage fortified with 1.25 mg/mL of protamex hydrolysates possessed approximately 57–64% ACE-inhibitory activity. Yogurt beverage fortified with 2.5 mg/mL of alcalase hydrolysates had approximately 61–69% ACE-inhibitory activity. Finally, yogurt beverage fortified with 2.5 mg/mL of trypsin hydrolysates had similar ACE-inhibitory activity. Yogurt beverage fortified with 5 mg/mL of protamex hydrolysates had a maximum ACE-inhibitory activity of 74%. Yogurt beverage fortified with hydrolysates did not significant during storage ($R^2=0.94$).

pH and titratable acidity

The pH values for yogurt samples at 1 d ranged from 3.53 to 3.73 (Table 1). No significant differences were noted between the samples during storage. Changes in titratable acidity were not significantly different among

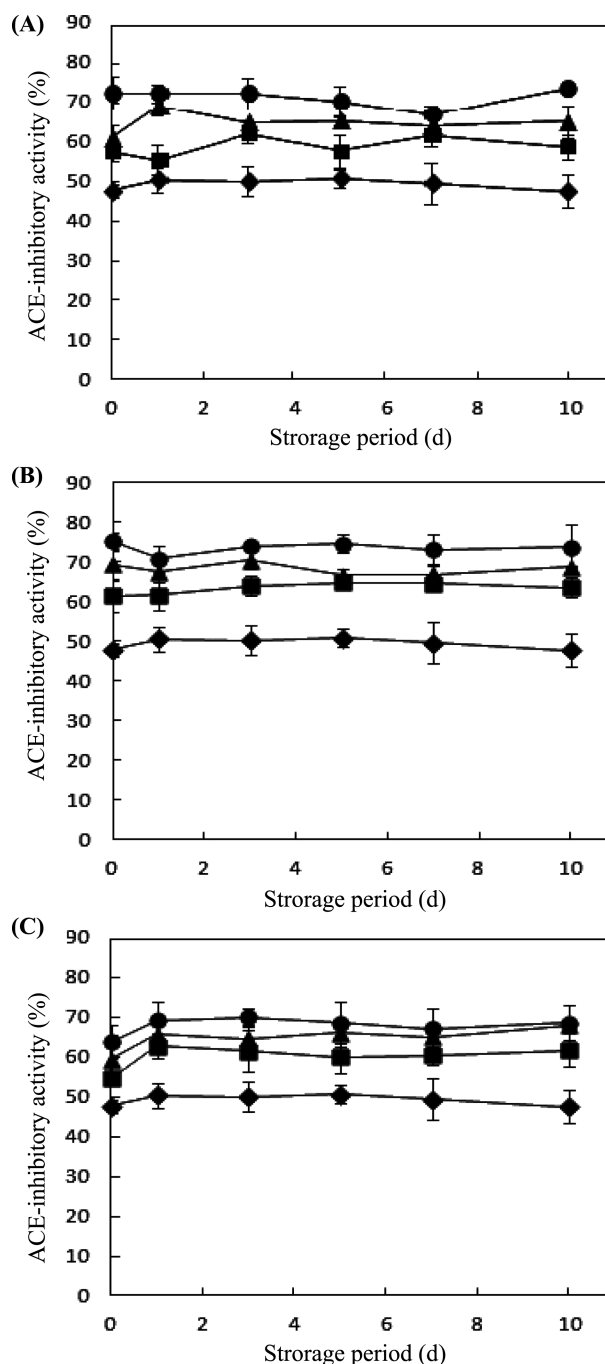


Fig. 1. Angiotensin-converting enzyme (ACE)-inhibitory activity of yogurt beverage fortified with whey protein hydrolysates. Hydrolysates concentrations were adjusted to 1.25 mg/mL (■), 2.5 mg/mL (▲), 5.0 mg/mL (●), or 0 mg/mL (◆). (A) alcalase, (B) protamex, and (C) trypsin

the hydrolysates during storage, and titratable acidity values ranged from 0.81% to 0.83% at 7 d (Table 2).

Color evaluation

In the color evaluation, L^* value (lightness) was not significantly different among the enzymatic hydrolysate-treated samples, whereas a^* (redness) and b^* (yellowness)

Table 1. Changes in pH value of yogurt beverage fortified with whey protein hydrolysates

Enzyme	Hydrolysate conc. (mg/mL)	Storage period (d)				
		0	1	3	5	7
Control	0	3.63±0.02	3.53±0.02	3.55±0.01	3.54±0.02	3.64±0.01
Alcalase	1.25	3.64±0.03	3.63±0.01	3.53±0.07	3.47±0.12	3.63±0.07
	2.5	3.61±0.02	3.67±0.02	3.56±0.04	3.53±0.07	3.66±0.05
	5.0	3.63±0.01	3.69±0.07	3.65±0.03	3.63±0.01	3.77±0.02
Protamex	1.25	3.49±0.09	3.63±0.02	3.50±0.13	3.59±0.04	3.66±0.02
	2.5	3.57±0.01	3.65±0.05	3.57±0.04	3.60±0.02	3.70±0.02
	5.0	3.60±0.02	3.73±0.02	3.67±0.02	3.62±0.03	3.76±0.01
Trypsin	1.25	3.53±0.01	3.63±0.03	3.57±0.01	3.61±0.05	3.66±0.03
	2.5	3.55±0.03	3.68±0.02	3.61±0.01	3.60±0.02	3.72±0.01
	5.0	3.63±0.01	3.73±0.01	3.64±0.03	3.63±0.01	3.74±0.02

All values are the mean±SD of three replicates.

Table 2. Changes in titratable acidity of yogurt beverage fortified with whey protein hydrolysates

Enzyme	Hydrolysate conc. (mg/mL)	Storage period (d)				
		0	1	3	5	7
Control	0	0.81±0.01	0.81±0.01	0.82±0.01	0.82±0.01	0.83±0.01
Alcalase	1.25	0.81±0.01	0.82±0.01	0.83±0.01	0.82±0.01	0.82±0.01
	2.5	0.83±0.01	0.82±0.01	0.82±0.01	0.83±0.01	0.83±0.01
	5.0	0.84±0.01	0.81±0.01	0.82±0.01	0.83±0.01	0.81±0.01
Protamex	1.25	0.84±0.01	0.83±0.01	0.82±0.01	0.82±0.01	0.82±0.01
	2.5	0.84±0.01	0.81±0.01	0.83±0.01	0.81±0.01	0.81±0.01
	5.0	0.84±0.01	0.82±0.01	0.82±0.01	0.82±0.01	0.81±0.01
Trypsin	1.25	0.81±0.01	0.81±0.01	0.81±0.01	0.81±0.01	0.81±0.01
	2.5	0.82±0.01	0.81±0.01	0.81±0.01	0.82±0.01	0.82±0.01
	5.0	0.82±0.01	0.81±0.01	0.82±0.01	0.82±0.01	0.82±0.01

All values are the mean±SD of three replicates.

Table 3. Changes in color values of yogurt beverage fortified with whey protein hydrolysates

Storage period (d)	Control	Alcalase hydrolysate (mg/mL)			Protamex hydrolysate (mg/mL)			Trypsin hydrolysate (mg/mL)			
		1.25	2.5	5.0	1.25	2.5	5.0	1.25	2.5	5.0	
0	CIE L*	66.21±0.01	66.55±0.11	66.13±0.01	66.58±0.02	65.81±0.20	66.24±0.02	66.19±0.13	66.46±0.02	66.50±0.05	66.97±0.13
	CIE a*	1.22±0.01	0.74±0.09	1.42±0.01	0.83±0.03	1.37±0.04	1.18±0.01	0.95±0.03	1.30±0.02	1.18±0.01	1.61±0.06
	CIE b*	23.66±0.02	22.34±0.17	23.69±0.03	22.65±0.06	23.53±0.23	23.59±0.02	22.52±0.01	23.77±0.03	23.76±0.08	24.30±0.19
1	CIE L*	66.09±0.04	65.65±0.52	66.22±0.01	66.50±0.02	65.96±0.01	66.20±0.02	66.42±0.02	66.33±0.02	66.56±0.06	66.69±0.02
	CIE a*	1.19±0.03	0.50±0.06	1.53±0.01	0.83±0.03	1.53±0.03	1.17±0.01	1.06±0.01	1.16±0.01	1.30±0.10	1.39±0.01
	CIE b*	23.82±0.19	19.28±0.63	24.01±0.01	22.88±0.01	23.92±0.01	23.76±0.01	23.39±0.02	23.81±0.02	23.82±0.30	23.96±0.01
3	CIE L*	66.48±0.03	66.80±0.03	66.48±0.02	66.91±0.03	66.43±0.02	66.63±0.03	66.91±0.02	66.72±0.01	66.86±0.01	67.07±0.04
	CIE a*	1.24±0.02	0.71±0.05	1.41±0.02	0.94±0.04	1.63±0.06	1.36±0.03	1.21±0.01	1.20±0.01	1.29±0.01	1.52±0.04
	CIE b*	23.25±0.05	22.00±0.03	23.37±0.04	22.43±0.04	23.59±0.03	23.45±0.05	23.12±0.03	23.32±0.01	23.44±0.01	23.59±0.04
5	CIE L*	66.22±0.01	66.58±0.01	66.37±0.03	66.93±0.03	66.16±0.04	66.56±0.01	66.68±0.08	66.65±0.07	66.89±0.11	67.00±0.01
	CIE a*	1.12±0.02	0.64±0.03	1.54±0.06	1.06±0.01	1.51±0.04	1.48±0.01	1.23±0.08	1.38±0.03	1.45±0.04	1.68±0.01
	CIE b*	23.09±0.01	21.92±0.01	23.41±0.04	22.76±0.02	23.41±0.06	23.61±0.01	23.11±0.10	23.59±0.10	23.73±0.16	23.77±0.01
7	CIE L*	66.14±0.01	66.45±0.02	66.15±0.01	66.62±0.01	66.06±0.03	66.26±0.01	66.49±0.01	66.35±0.02	66.53±0.03	66.70±0.01
	CIE a*	1.18±0.01	0.71±0.03	1.49±0.01	1.04±0.01	1.60±0.01	1.31±0.01	1.18±0.01	1.18±0.02	1.31±0.04	1.43±0.01
	CIE b*	22.83±0.02	21.71±0.03	22.99±0.02	22.26±0.01	23.16±0.04	23.06±0.02	22.75±0.02	22.96±0.02	23.08±0.02	23.18±0.01

All values are the mean±SD of three replicates.

values were higher than that of the control. However, the observed color changes were not significantly among the samples during storage (Table 3).

Microbiological analysis

Viable LAB was counted using the spread plate method with MRS agar. The plates were incubated at 35°C for 48 h. The results imply that the physicochemical characteristics of the hydrolysates are quite adequate for inclusion in yogurt, as no unfavorable changes in yogurt beverage for-

tified with hydrolysates were observed (Table 4).

Sensory evaluation

Sensory evaluation of the yogurt beverage samples was carried out at 0, 3, and 7 d using a five-point hedonic scale ranging from "dislike extremely" to "like extremely". Sensory evaluation was not significantly different at any storage point (0, 3, and 7 d) or any enzymatic hydrolysate concentration (Tables 5-7). However, the value of mouth feel of 0 d and color of 5.0 mg/mL was significant differ-

Table 4. Changes in viable cells (log CFU/mL) in yogurt beverage fortified with whey protein hydrolysates

Enzyme	Hydrolysate conc. (mg/mL)	Storage period (d)				
		0	1	3	5	7
Control	0	8.64±0.04	8.67±0.06	8.68±0.02	8.68±0.06	8.72±0.06
	1.25	8.58±0.04	8.68±0.12	8.70±0.06	8.67±0.02	8.68±0.07
Alcalase	2.5	8.64±0.13	8.70±0.04	8.60±0.02	8.68±0.08	8.66±0.03
	5.0	8.61±0.08	8.57±0.06	8.65±0.04	8.73±0.10	8.67±0.06
	1.25	8.65±0.08	8.62±0.04	8.66±0.07	8.70±0.08	8.66±0.04
Protamex	2.5	8.65±0.11	8.64±0.05	8.62±0.04	8.66±0.06	8.65±0.01
	5.0	8.65±0.06	8.64±0.04	8.65±0.09	8.66±0.05	8.70±0.07
	1.25	8.62±0.06	8.64±0.03	8.63±0.08	8.72±0.10	8.73±0.04
Trypsin	2.5	8.63±0.04	8.61±0.05	8.70±0.10	8.65±0.10	8.70±0.12
	5.0	8.62±0.03	8.64±0.06	8.69±0.07	8.66±0.10	8.67±0.08

All values are the mean±SD of three replicates.

Table 5. Changes in sensory scores of yogurt beverage fortified with alcalase hydrolysate

Sensory parameter	Storage period (d)	Hydrolysate conc. (mg/mL)			
		0	1.25	2.5	5.0
Odor	0	4.1±0.32	4.0±0.47	4.3±0.48	4.3±0.48
	3	4.0±0.67	4.0±0.67	3.9±0.57	3.9±0.57
	7	4.2±0.63	4.2±0.63	4.2±0.63	4.3±0.67
Taste	0	4.1±0.32	4.1±0.32	4.3±0.48	4.1±0.32
	3	4.0±0.67	3.9±0.57	4.1±0.74	4.0±0.67
	7	4.3±0.67	4.4±0.70	4.3±0.67	4.4±0.70
Mouth feel	0	4.3±0.48 ^{ab}	4.1±0.32 ^b	4.2±0.42 ^{ab}	4.6±0.52 ^{Aa}
	3	3.9±0.57	4.0±0.67	4.0±0.67	3.8±0.42 ^B
	7	4.1±0.57	4.4±0.70	4.5±0.71	4.2±0.63 ^{AB}
Color	0	4.2±0.32	4.2±0.42	4.3±0.48	4.5±0.53 ^A
	3	4.0±0.67	4.0±0.67	4.0±0.67	3.9±0.57 ^B
	7	4.1±0.57	4.5±0.71	4.4±0.70	4.2±0.63 ^{AB}
Total	0	4.1±0.57	4.4±0.70	4.2±0.42	4.2±0.42
	3	3.9±0.57	4.0±0.67	4.0±0.67	3.8±0.42
	7	4.2±0.63	4.3±0.67	4.5±0.71	4.3±0.67

All values are the mean±SD of three replicates.

Sensory scores were assessed on a 5 point scale based on 1=extremely bad, 5=extremely good.

^{A,B}Means within columns with different superscripts are significantly different ($p<0.05$).

^{a,b}Means within row with different superscripts are significantly different ($p<0.05$).

Table 6. Changes in sensory scores of yogurt beverage fortified with protamex hydrolysate

Sensory parameter	Storage period (d)	Hydrolysate conc. (mg/mL)			
		0	1.25	2.5	5.0
Odor	0	4.1±0.32	3.9±0.32 ^B	4.0±0.47 ^B	4.2±0.42
	3	4.0±0.67	4.2±0.63 ^{AB}	4.6±0.70 ^A	4.5±0.53
	7	4.2±0.63	4.4±0.52 ^A	4.4±0.52 ^{AB}	4.2±0.42
Taste	0	4.1±0.32	4.2±0.42	4.1±0.32 ^B	4.1±0.32
	3	4.0±0.67 ^b	4.1±0.57 ^{ab}	4.6±0.52 ^{Aa}	4.5±0.53 ^{ab}
	7	4.3±0.67	4.3±0.48	4.3±0.48 ^{AB}	4.2±0.42
Mouth feel	0	4.3±0.48	4.1±0.32	4.2±0.42	4.1±0.32
	3	3.9±0.57 ^b	4.2±0.63 ^{ab}	4.6±0.52 ^a	4.4±0.52 ^{ab}
	7	4.1±0.57	4.1±0.32	4.2±0.42	4.3±0.48
Color	0	4.2±0.32	4.1±0.32	4.1±0.32	4.1±0.32
	3	4.0±0.67	4.3±0.67	4.4±0.52	4.3±0.48
	7	4.1±0.57	4.2±0.42	4.2±0.42	4.2±0.42
Total	0	4.1±0.57	4.2±0.42	4.1±0.32	4.1±0.32
	3	3.9±0.57	4.3±0.67	4.3±0.48	4.4±0.52
	7	4.2±0.63	4.4±0.52	4.3±0.48	4.2±0.42

All values are the mean±SD of three replicates.

Sensory scores were assessed on a 5 point scale based on 1=extremely bad, 5=extremely good.

^{A,B}Means within columns with different superscripts are significantly different ($p<0.05$).

^{a,b}Means within row with different superscripts are significantly different ($p<0.05$).

Table 7. Changes in sensory scores of yogurt beverage fortified with trypsin hydrolysate

Sensory parameter	Storage period (d)	Hydrolysate conc. (mg/mL)			
		0	1.25	2.5	5.0
Odor	0	4.1±0.32	4.1±0.57	4.3±0.67	4.1±0.57
	3	4.0±0.67 ^b	4.2±0.63 ^{ab}	4.6±0.52 ^a	4.4±0.52 ^{ab}
	7	4.2±0.63	4.2±0.63	4.4±0.70	4.3±0.67
Taste	0	4.1±0.32	4.0±0.47	4.3±0.67	4.1±0.57
	3	4.0±0.67 ^b	4.5±0.71 ^{ab}	4.6±0.52 ^a	4.4±0.52 ^{ab}
	7	4.3±0.67	4.4±0.70	4.5±0.71	4.1±0.57
Mouth feel	0	4.3±0.48	4.1±0.57	4.2±0.63	4.2±0.63
	3	3.9±0.57 ^b	4.4±0.52 ^{ab}	4.3±0.48 ^{ab}	4.5±0.53 ^a
	7	4.1±0.57	4.4±0.70	4.4±0.70	4.0±0.47
Color	0	4.2±0.32	4.0±0.47	4.0±0.47 ^B	4.3±0.67
	3	4.0±0.67 ^b	4.5±0.53 ^{ab}	4.6±0.52 ^{Aa}	4.3±0.48 ^{ab}
	7	4.1±0.57	4.4±0.70	4.4±0.70 ^{AB}	4.1±0.57
Total	0	4.1±0.57	4.4±0.70	4.0±0.47	4.2±0.63
	3	3.9±0.57 ^b	4.5±0.53 ^a	4.3±0.48 ^{ab}	4.4±0.48 ^{ab}
	7	4.2±0.63	4.1±0.57	4.1±0.57	4.4±0.52

All values are the mean±SD of three replicates.

Sensory scores were assessed on a 5 point scale based on 1=extremely bad, 5=extremely good.

^{A,B}Means within columns with different superscripts are significantly different ($p<0.05$).

^{a,b}Means within row with different superscripts are significantly different ($p<0.05$).

ent in yogurt beverage fortified with alcalase hydrolysate. Also, the value of taste of 3 d and mouth feel of 3 d was significant different in yogurt beverage fortified with protamex hydrolysate. And total sensory evaluation was over 4.0 as "like level" in both cases.

The use of these hydrolysates in the manufacture of yogurt did not significantly influence pH, titratable acidity, colority, LAB, or sensory evaluations during storage. Therefore, as stated above, the physicochemical characteristics of the hydrolysates are quite adequate for inclusion in yogurt beverage, as no unfavorable changes in yogurt beverage fortified with hydrolysates were observed. Our results demonstrate that whey protein hydrolysates have potential as an ingredient in the production of anti-hypertensive functional dairy product.

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