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ARTICLE

Biochemical, Microbiological, and Sensory Characteristics of Stirred Yogurt Containing Red or Green Pepper (*Capsicum annuum* cv. Chungyang) Juice

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Abstract Hot pepper has anti-obesity effects by controlling appetite and reducing blood fat level. To reduce the pungency of capsaicin, red or green hot pepper juice was fermented with *Bacillus licheniformis* SK1230. Fermented hot pepper juice was then added into yogurt at different ratios. The pH of yogurt added with hot pepper juice was decreased from 4.61 to 4.48. Titratable acidity and counts of lactic acid bacteria were increased with increasing amount of pepper juice added. However, the viscosity was decreased significantly compared to the control. On chromaticity test, when more pepper juice was added, L*-value was decreased whereas a*- and b*- values were increased significantly (p<0.05). The spectrum of antimicrobial activity of yogurt was slightly changed compared to using pepper juice. Total polyphenol contents and antioxidant activity were increased with increasing amount of pepper juice added. Stirred yogurt added with fermented red pepper juice at 3% or green pepper juice at 1% showed high scores in flavor, appearance, texture, and overall acceptance in sensory test. Yogurt added with fermented pepper juice with reduced pungency showed also good palatability during storage at 4℃. Yogurt with added hot pepper juice can be play an important role in functional food relative to anti-obesity.

Keywords red pepper juice, green pepper juice, stirred yogurt, capsaicin, antioxidant activity

Introduction

Pepper (Chilli) (*Capsicum* spp.) is consumed as a spice, vegetable, and fruit in many ethnic diets. It contains in various bioactive constituents such as capsaicin, carotenes, ascorbic acids, polyphenols, flavonoids, mineral maters, sugars, protein, and fat. It has a variety of shapes, sizes, and colors. Its pungency and phytochemical levels depend on the species, its maturity, and drying conditions (Howard et al., 2000; Sanatombi and Sharma 2008; Troconis - Torres et al., 2012; Vega-Galvez et al., 2009). Capsaicin, the main ingredient of hot chilli pepper, possesses anticarcinogenic effect on various cancer cells through multiple mechanisms (Lin et al., 2013). Chilli could treat neuropathic and nociceptive musculoskeletal pain such as diabetic neuropathy, postsurgical pain, osteoarthritis, and rheumatic arthritis (Baranidharan et al., 2013). Recently, it has been shown that capsaicin can trigger apoptosis of pancreatic cancer cells via mitochondria-mediated apoptotic pathway (Zhang et al., 2008). Furthermore, peppers can modify fat and energy metabolism in human with anti-obesity effect by controlling appetite, reducing fat concentration in the blood, and restricting the generation of white fat cells (Hsu and Yen, 2007). Capsaicin can also decrease hepatic total-lipid level in mice fed high-fat diet added with fermented pepper powder (Yeon et al., 2013).

Polyphenols are able to carry protons and electrons, which means that they easily undergo oxidation (Cieślik et al., 2006). Polyphenols also possess modulatory actions as anticancer agents, cardioprotectants, and inhibitors of neurogenerator (Tsao, 2010). Polyphenols in red pepper [*Capsicum annuum* var. *aviculare* (Tepin)] exhibit protective effects on lipid peroxidation induced by some pro-oxidants in the brain and liver (Oboh and Rocha, 2007). It has been found that *trans*-*p*-sinapoyl-β-D-glucopyranoside and quercetin 3-*O*-α-L-rhamnopyranoside are main phenolic compounds isolated from hot pepper fruit. They are responsible for the high antioxidant activity of hot pepper (Materska and Perucka, 2005).

Yogurt is one of dairy products containing beneficial bacteria. Many researches have reported that yogurt has therapeutic and prophylaxis effects on some diseases such as cancers, infections, gastrointestinal disorders, asthma, and antibiotic-associated diarrhea (Beniwal et al., 2003; Fernandez and Marette, 2017; Karagül-Yüceer and Avsar, 2016; Moghbel and Abbaspour, 2013). Functional yogurts enriched with micronutrients (iron, zinc, iodine and vitamin A) (Sazawal et al., 2013), vitamin D (Shab-Bidar et al., 2011), acacia fiber (Min et al., 2012), sweet pumpkin (Jung et al., 2011), MACA (*Lepidium meyenis*) hot water extract (Chung et al., 2010), black garlic extract (Shin et al., 2010), red ginseng extract (Kim et al., 2008), and spices oleoresins (Illupapalayam et al., 2014) have been reported.

Despite its medicinal benefits, the strong pungency of hot pepper restricts its widespread use. To diminish the pungency of pepper without losing its biological activity, fermentation of pepper juice by *Bacillus licheniformis* SK1230 isolated from Korean traditional pickled pepper (Cho et al., 2014) and *B. subtilis* P3-3 (Lee et al., 2010) has been reported. By fermentation with *B. licheniformis* SK1230 at 37℃ for 5 days, the capsaicinoid content of red or green pepper radically decreased while its phenolic compounds and antioxidant activity were maintained (Cho et al., 2015). *B. licheniformis* SK1230, which can degrade capsaicin in our previous work and reduce the spicy taste of hot pepper juice (Cho et al., 2015). It was isolated from Korean traditional pickled pepper. *B. licheniformis* is a common bacterium found in foods such as Korean traditional soybean paste, Jeot-gal, and Chungkook-Jang (Bae and Yoon, 2012; Hwang et al., 2007; Kim et al., 2004). According to Haramizu et al. (2011), capsiate, a nonpungent capsaicin analog has no spicy taste but has an anti-obesity effect. In this study, we wanted to produce a functional yogurt with anti-obesity effect of capsaicin derivatives produced by *B. licheniformis* SK1230.

Yu et al. (2014) in our lab have reported the preparation of stirred yogurt containing 0.025–0.1% of red pepper powder fermented by *B. licheniformis* SK1230. Unlike the previous report, this study was purposed to explore the yogurt added with squeezed red or green pepper juice for better fermentation. In addition, the yogurts added with red and green pepper juices were first compared in terms of biochemical, microbiological and sensory characteristics.

Materials and Methods

Preparation of fermented hot pepper juice

Red and green peppers (*Capsicum annuum* cv. Chungyang) cultivated in Cheong-Yang, Chungcheong Nam-do province during September were purchased from a local market to prepare juice without adding water using a juicer (DA 5000, Dasung Health Mix, Korea). *B. licheniformis* SK1230 was grown in Luria-Bertani (LB) broth or solid medium (Difco™, BD, USA). The overnight culture was inoculated at 5% into 2 X *Bacillus* minimal medium (BMM, $Na_2HPO_4 \cdot 7H_2O$, 33.5 mM; KH_2PO_4, 22 mM; NaCl, 8.6 mM; NH_4Cl, 18.7 mM; $MgSO_4$, 1 mM; $CaCl_2$, 0.1 mM) without glucose containing pepper juice at 1:1 ratio. The mixture was sterilized and fermented at 37°C for 5 days using published method (Cho et al., 2014). The fermented pepper juice was finally sterilized to kill *B. licheniformis* SK1230. The content of capsaicin in red or green pepper juice was about 300 or 250 ppm, respectively, consistent with previous report (Cho et al., 2015).

Preparation of stirred yogurt

The above fermented red or green pepper juice was added to milk at concentrations of 1–5% and homogenized using a Homogenizer T 25 (Janke and Kunkel type, Ika, Germany). *Streptococcus thermophilus*, *Lactobacillus delbrueckii* ssp. *bulgaricus*, *Lactobacillus acidophilus*, and *Bifidobacterium animalis* ssp. *lactis* (Lyofast YAB 450 AB, Sacco srl., Codaragok, Italy) were inoculated as described previously (Yu et al., 2014). The yogurt was fermented at 37°C for 5 h and stored at 4°C.

Proximate composition

Moisture and total ash were analyzed by the method of Korean Food Standards Codex (2011). Contents of protein, crude fat, lactose, and total solids were determined using Milkoscan (Milkoscan Minor 78110, Foss Co., Denmark).

pH and titratable acidity

The pH of homogenized yogurt was determined using a digital pH meter (ISTEC 735P, Korea). Titratable acidity (TA) was determined by titration with 0.1N NaOH. Yogurt sample (3 mL) was transferred into an Erlenmeyer flask containing 27 mL of dH_2O. Three to five drops of 0.1% phenolphthalein as pH indicator were added. The yogurt mixture was then titrated with 0.1N NaOH with continuous stirring until a stable pink color was achieved. The amount of acid produced during fermentation was calculated as follows:

$$\text{TA (\% Lactic acid)} = \text{Dilution factor} \times V_{\text{NaOH}} \times 0.1\text{N} \times 0.009 \times 100\%$$

Where V_{NaOH} was the volume of NaOH required to neutralize the acid. A dilution factor of 10 was used.

Enumeration of lactic acid bacteria in yogurt

Lactic acid bacteria was counted on MRS agar (Difco Laboratories, USA) plate after incubating for 48 h at 37°C and expressed as CFU/mL.

Measurement of viscosity

The viscosity of yogurt stored at 4°C was measured using a Brookfield-Viscometer (Model LVDV 1+, Brookfield

Engineering Laboratories, Inc., USA) with spindle No. 63 at 100 rpm for 5 to 8 min.

Measurement of color

Color of yogurt was measured using Hunter Lab color meter (Minolta chromameter CR-210, Japan) and expressed as L*, a*, and b* values to represent lightness, redness, and yellowness, respectively. L*, a*, and b* values on a standard color plate were 97.83, –0.45, and 2.00, respectively.

Antimicrobial activity

The pathogens used for antimicrobial activity were provided by the Gyeonggi Institute of Health and Environment (Korea). The pathogens cultured in LB broth were spread on the same agar plate. The wells with 6 mm diameter were prepared using a Pasteur pipette on the plate. 50 μL of hot pepper juice or the supernatant of yogurt with 5% hot pepper juice was added to each well. The plates were incubated at 37°C. The diameter (mm) of clear zone formed around the well after incubation was measured to confirm the antimicrobial activity. The experiment was repeated three times.

Total polyphenol content assay

Water extract from yogurt containing red or green pepper juice (1 mL) was mixed with methanol [9 mL, 95% (v/v)] and 9 mL of dH_2O. Folin-Ciocalteu reagent (1 mL, 1N) was added to each supernatant and thoroughly mixed. The solution was incubated at room temperature stand for 3 min. Na_2CO_3 (300 μL, 1N) was then added and absorbance at 725 nm (UV-1601, Shimadzu, Japan) was measured after incubation for further 90 min at room temperature (Wei, 2011). Known concentrations of gallic acid (Sigma-Aldrich, Germany; 5–60 μg/mL in ethanol) were treated in the same manner as water extract of yogurt. Regression line of gallic acid standard was used to determine total phenolic content of yogurt water extract to [μg gallic acid equivalent (μg GAE)/mL].

Determination of antioxidant activity using 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) inhibition assay

DPPH inhibition was determined as described previously (Apostolidis et al., 2007). Briefly, 1 mL of 0.15 mM DPPH (Sigma-Aldrich, Germany) was mixed with 4 mL of yogurt extract or 4 mL of water which served as a control. The mixture was shaken thoroughly and allowed to stand at room temperature for 30 min. Constant absorbance readings at 517 nm were then recorded using a spectrophotometer (UV-1601, Shimadzu, Japan) at 5 min intervals. Inhibition of DPPH oxidation (%) was calculated as follows (Apostolidis et al., 2007):

$$\% \text{ Inhibition} = (A_{\text{control}} - A_{\text{extract}}) / A_{\text{control}} \times 100$$

Where A was absorbance at 517 nm.

Sensory analysis

Sensory analyses were carried out by 10 trained assessors aged 20 and 40 years. Samples were coded with three-digit numbers and randomly served at 7 to 10°C in plastic cups (10 mL). Assessors completed a test assessment form to compare five sensory attributes (general taste, flavor, appearance, hot taste, and overall acceptability) using a five-point hedonic scale (1, extremely poor; 2, poor; 3, fair; 4, good; 5, excellent). Pungency was scored as follows: 5, strong; 3, medium; and 1, mild.

Statistical analysis

Three separate experiments with triplicate assays were performed. Data are expressed as means+standard error of the mean (SEM). Statistical analysis was performed using one-way analysis of variance (ANOVA; SPSS 20) followed by Duncan's *post hoc* test for mean comparison. Statistical significance was set at $p<0.05$.

Results and Discussion

Nutritional compositions in yogurt added with fermented red or green pepper juice

Results of fundamental nutritional components of yogurt added with fermented pepper juice at 1–5% are shown in Table 1. For the preparation of yogurt, we used red or green pepper juice containing 300 or 250 ppm of capsaicin, respectively. The yogurt without adding any pepper juice served as control group. Its moisture, crude protein, fat, and lactose contents were 82.78, 3.52, 3.96, and 6.44%, respectively. For yogurt added with fermented red pepper juice, moisture, crude protein, fat, and lactose contents were 82.59–82.70%, 3.44–3.73%, 3.98–4.25%, and 6.63–6.74%, respectively. For yogurt added with fermented green pepper juice, moisture, crude protein, fat, and lactose contents were 82.77–83.08%, 3.48–3.70%, 3.91–

Table 1. Proximate analysis of yogurt with pepper juice

Component	Addition levels of fermented pepper juice (%)	Total amount (%)	
		Red pepper	Green pepper
Moisture	0	82.78±0.20	82.78±0.20^{ab}
	1	82.67±0.20	82.77±0.12^{b}
	3	82.70±0.22	82.86±0.20^{ab}
	5	82.59±0.10^{B}	83.08±0.11^{aA}
Ash	0	0.20±0.00	0.20±0.00
	1	0.20±0.00	0.20±0.00
	3	0.20±0.00	0.20±0.00
	5	0.20±0.00	0.20±0.00
Protein	0	3.52±0.05^{bc}	3.52±0.05^{b}
	1	3.44±0.04^{c}	3.50±0.14^{b}
	3	3.58±0.06^{b}	3.48±0.04^{b}
	5	3.73±0.01^{a}	3.70±0.11^{a}
Fat	0	3.96±0.08^{b}	3.96±0.08^{b}
	1	3.98±0.05^{b}	3.91±0.19^{b}
	3	4.06±0.09^{a}	4.04±0.05^{b}
	5	4.25±0.01^{a}	4.26±0.08^{a}
Lactose	0	6.44±0.19^{b}	6.44±0.19
	1	6.74±0.12^{a}	6.61±0.02
	3	6.63±0.02^{ab}	6.66±0.01
	5	6.71±0.00^{a}	6.66±0.06
Total solids	0	14.36±0.26^{b}	14.36±0.26^{b}
	1	14.51±0.08^{b}	14.44±0.32^{b}
	3	14.68±0.15^{b}	14.59±0.08^{b}
	5	15.08±0.01^{a}	15.03±0.19^{a}

Values are mean±SEM (n=3).
Different small letters in the same column and capitalized letters in the same row indicated significant difference ($p<0.05$).

4.26%, and 6.61–6.66%, respectively. The moisture content in yogurt added with 5% of green pepper juice was significantly higher than that in yogurt added with 5% of red pepper juice. Contents of total solids, protein, fat, and lactose were all significantly higher in yogurt added with 5% of green or red pepper juice compared to those in the control or yogurt added with 1–3% of pepper juice.

Changes in pH, titratable acidity, growth of probiotics, and viscosity

Results of pH and TA of yogurt added with 1, 3, 5% of fermented red or green pepper juice are shown in Fig. 1A and 1B, respectively. The pH of prepared control yogurt ranged from 4.48 to 4.61. The pH value of yogurt added with red pepper juice was lower than that of yogurt added with green pepper juice. The pH of pepper juice decreased significantly with the added amount of pepper juice. The pH of yogurt added with red pepper juice at 3% was lower than that added with red pepper juice at 5%. TA value of yogurt added with red or green pepper juice was increased significantly compared to that of the control (Fig. 1B). TA value of yogurt added with fermented red pepper juice was increased 0.80 to 0.90% and that of yogurt added with fermented green pepper juice was increased 0.81 to 0.87%. This might be due to the presence of organic acid promoted by the addition of fermented pepper juice. In our previous report on stirred yogurt added with fermented red pepper powder (Yu et al., 2014), the pH was decreased to around 4.3 while TA was increased 0.90 to 0.95%. This suggests that the addition of red pepper juice results in slightly less decrease in pH and acidity compared to that of pepper powder, depending on the additional level and the type of hot pepper.

Results of lactic acid bacteria (LAB) counts of yogurt added with 1, 3, 5% of red or green pepper juice are shown in Fig. 1C. The LAB count of yogurt showed an increasing tendency following the addition of pepper juice at increasing amount.

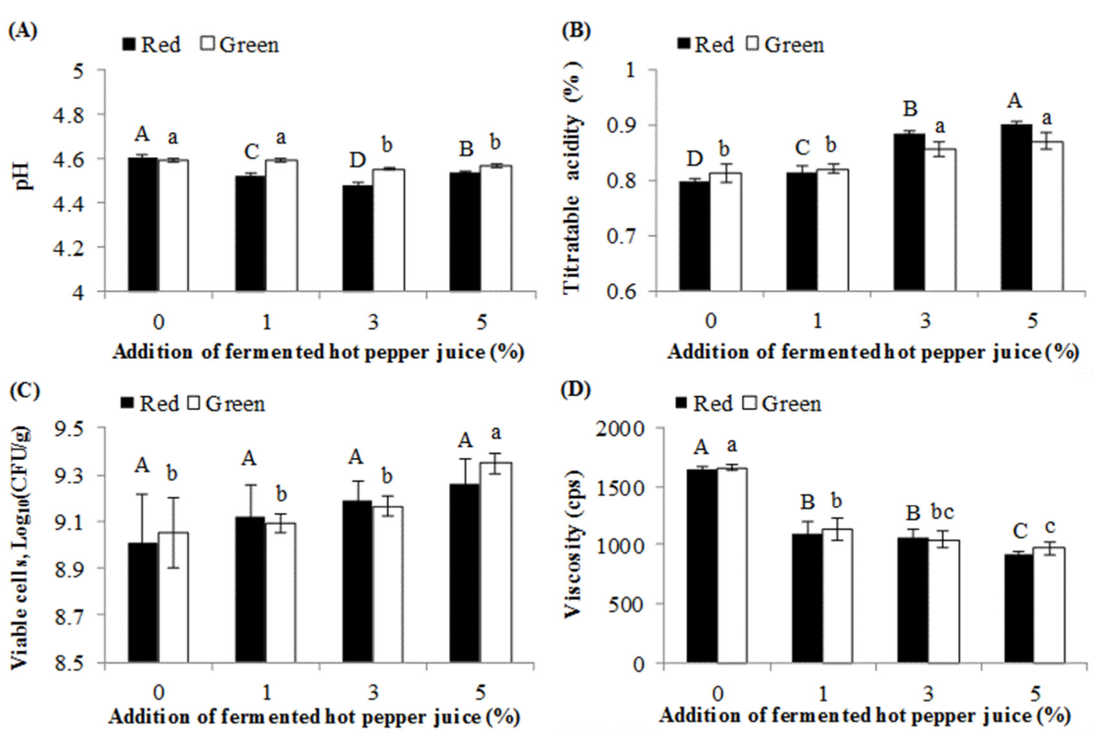

Fig. 1. Changes in pH, titratable acidity (TA), viable cell count, and viscosity of yogurt added with fermented red or green pepper juice. (A) pH, (B) TA, (C) Viable cell count, (D) Viscosity. Different capitalized letters and small letters indicate significant difference in yogurt added with red or green pepper juice (p<0.05).

LAB counts of yogurt added with 5% of green pepper juice were significantly higher than those of yogurt added with 0–3% of green pepper juice. LAB counts of yogurt prepared with 1, 3, and 5% of fermented red pepper juice were 9.12, 9.19, and 9.26 Log CFU/mL, respectively, while those of yogurt prepared with green pepper juice were 9.09, 9.16, and 9.35 Log CFU/mL, respectively. These were higher than those of yogurt added with red pepper powder (6.5–7.5 Log CFU/mL, Yu et al., 2014). These results indicate that fermented pepper juice can promote the growth of probiotics better than fermented pepper powder. Hot pepper contains complex carbohydrates and oligosaccharides in fruits and vegetables. They are sources of prebiotics (Jovanovic-Malinovska et al., 2014). Shin et al. (2010) have reported that LAB count of yogurt added with black garlic is increased significantly with increasing amount of addition. This might be due to increased production of reducing sugar during the proceeding of fermentation which might have promoted the growth of LAB.

Results of viscosity of plain yogurt added with fermented red or green pepper juice are shown in Fig. 1D. The viscosity of yogurt was decreased with increasing addition of fermented red or green pepper juice. This suggests that the addition of fermented pepper juice can promote the growth of LAB as prebiotics and decrease the gel strength by promoting degradation of milk solid components or pH-induced changes in casein micelles, resulting in increased acid production (Gastaldi and Lagaude, 1996). It has been reported that yogurt added with mulberry pekmez or black garlic extract has lower viscosity compared to control yogurt (Celik and Bakirci, 2003; Shin et al., 2010).

Color change in yogurt added with pepper juice

Results of color changes in yogurt added with 1, 3, and 5% of fermented pepper juice measured as Hunter L*, a*, and b* values are shown in Table 2. The addition of fermented pepper juice decreased the brightness of yogurt, with significant differences among samples added with various amounts of pepper juice (p<0.05). The L* value (lightness) of yogurt added with 5% fermented red pepper juice ranged from 85.55 to 77.61 while that of yogurt added with 5% green pepper juice ranged from 87.90 to 84.95. They were lower compared to that of the control (L* value=90.33). These results indicate that increasing amount of pepper juice will lead to decreased brightness. The a*-value (redness) was increased by the addition of pepper with significant difference, especially with the addition of red pepper. By the addition of fermented pepper juice at 5%, the a*-value of yogurt was increased significantly (p<0.05) from –3.46 in the control to 8.08 and –2.68 for red and green pepper yogurts, respectively. The b*-value (yellowness) of yogurt was also increased by increasing amount of fermented pepper juice added, giving yogurt a more yellowish color. This might be due to the natural color from red or green pepper. The color of red or green of peppers depends on their maturities. Fully matured red pepper showed the highest b*-value

Table 2. Color change in yogurt added with fermented pepper juice

Color value	Treatment	Addition of fermented pepper juice (%)			
		0	1	3	5
L*	Red pepper	90.33±0.89^{A}	85.55±0.01^{bB}	81.12±0.06^{bC}	77.61±0.38^{bD}
	Green pepper	90.33±0.89^{A}	87.90±0.34^{aB}	87.09±0.47^{aB}	84.95±0.67^{aC}
a*	Red pepper	–3.46±0.37^{D}	3.08±0.03^{aC}	6.67±0.16^{aB}	8.08±0.27^{aA}
	Green pepper	–3.46±0.37^{B}	–2.91±0.10^{bA}	–2.89±0.09^{bA}	–2.68±0.10^{bA}
b*	Red pepper	7.42±1.19^{D}	21.62±0.06^{aC}	28.26±0.10^{aB}	32.19±0.72^{aA}
	Green pepper	7.42±1.19^{B}	6.78±0.35^{bB}	9.08±0.52^{bA}	9.59±0.16^{bA}

Values are mean±SEM (n=3).
Different small letters in the same column and capitalized letters in the same row indicated significant difference (p<0.05).

(32.19) of yogurt. However, for yogurt added with green pepper juice at 5%, b*-value was only 9.59. L* values of yogurt added with green pepper juice were all significantly higher than those of yogurt added with red pepper juice ($p<0.05$). The a*- and b*-values of yogurt added with red pepper juice were all significantly higher than those of yogurt added with green pepper juice ($p<0.05$).

Antimicrobial activity

Antimicrobial activities of yogurt added with fermented red or green pepper juice were tested. The yogurt with no pepper juice showed antimicrobial activities on the pathogens such as *Escherichia coli, Pseudomonas aeruginosa*, and *Salmonella* sp. (Table 3). The yogurts added with fermented red or green pepper juice showed almost the same antimicrobial activities, implying that hot pepper juice did not inhibit the activity produced by LAB.

The most antimicrobial activity was observed in *E. coli* VTII/O104. In *P. aeruginosa*, the activity of yogurt added with green pepper juice was significantly higher than that of control whereas the yogurt added with red pepper juice showed lower activity. In *Salmonella london* E, the yogurt with red or green pepper juice showed significantly higher antimicrobial activity than the control, indicating that hot pepper juice may promote the activity.

In this study, squeezed juice of red or green pepper showed no antimicrobial activity in the tested pathogens shown in Table 3. Wahba et al. (2010) reported that the ethanol extract of green pepper showed antimicrobial activity against *Staphylococcus aureus*. Keskin and Toroglu (2011) reported that the extracts of red pepper by organic solvents showed weak antimicrobial activity against *P. aeruginosa* and no antimicrobial activity of hot pepper by other researchers was also mentioned therein, suggesting that different environments of plants grown, and extraction methods may affect the antimicrobial activity.

Total polyphenol content and antioxidant activity

The results of total polyphenol contents (TPC) in water and methanol extracts of red or green pepper yogurt are shown in Fig. 2A and 2B, respectively. TPC in methanol extract was higher than that in water extract. TPC of yogurt was significantly

Table 3. Antimicrobial activities of yogurt with pepper juice

Pathogen	Antimicrobial activity (mm)		
	No addition	Red pepper (5%)	Green pepper (5%)
Escherichia coli	19.00±0.25[a]	18.78±0.44[ab]	18.39±0.60[b]
Escherichia coli VTI/O55	18.67±0.71	20.00±1.80	18.94±2.27
Escherichia coli VTI/O91[1)]	17.94±1.16	17.72±1.03	17.78±0.62
Escherichia coli VTII/O104	24.78±2.28[ab]	25.61±0.86[a]	24.11±0.60[b]
Pseudomonas aeruginosa	16.22±0.36[b]	15.11±1.29[c]	17.39±1.11[a]
Salmonella auatum E1	17.22±1.09[b]	18.28±0.51[a]	17.78±0.62[ab]
Salmonella london E	14.61±0.70[b]	16.83±0.43[a]	16.89±0.74[a]
Salmonella oyonnox C1	14.06±1.84	13.78±1.56	13.33±1.41
Salmonella teddington	13.61±1.41	14.06±0.95	14.67±1.41

Values are mean±SEM (n=3).
Different small letters in the same row indicated significant difference ($p<0.05$).
[1)] Clear zone showing a ring type.

increased following the addition of red or green pepper juice. Yogurt added with fermented red pepper juice showed significantly higher TPC than that added with green pepper juice. TPC of control yogurt without pepper juice was the lowest at 37 μg/mL in water extract. It was 158 μg/mL in methanol extract. TPC of yogurt added with fermented red pepper juice was 52–123 μg /mL in water extract and 220–334 μg/mL in methanol extract. TPC in yogurt added with fermented green pepper juice was 45–79 μg/mL in water extract and 229–240 μg/mL in methanol extract. The yogurt added with red pepper juice had higher TPC than that added with green pepper juice at 3–5%.

Yu et al. (2014) have also shown that yogurt added with fermented red pepper powder has significantly higher TPC than that added with non-fermented one. The increase of TPC by the addition of pepper juice is because pepper fruits contain high contents of polyphenols (Oboh and Rocha, 2007; Materska and Perucka, 2005). Yogurt fortified with polyphenols can be used as bioactive product. Vauzour (2012) has reported that polyphenols could exert their neuroprotective actions against injury due to neurotoxins and neuroinflammation.

Antioxidant activities of water and methanol extracts of yogurt added with fermented red pepper juice were increased significantly ($p<0.05$) compared to those of yogurt added with fermented green pepper juice (Fig. 2C and 2D). These results are consistent with previous studies showing that total polyphenol content and antioxidant activity of fermented red pepper juice are higher than fermented green pepper juice (Cho et al., 2015). However, antioxidant activities of water and methanol extracts of yogurt added with fermented green pepper juice were not significantly different. Antioxidant activity of yogurt was significantly ($p<0.05$) increased when the amount of red or green pepper juice added to yogurt was increased. Higher antioxidant activity could be due to polyphenol compounds contained in hot peppers. Interestingly, antioxidant activities of

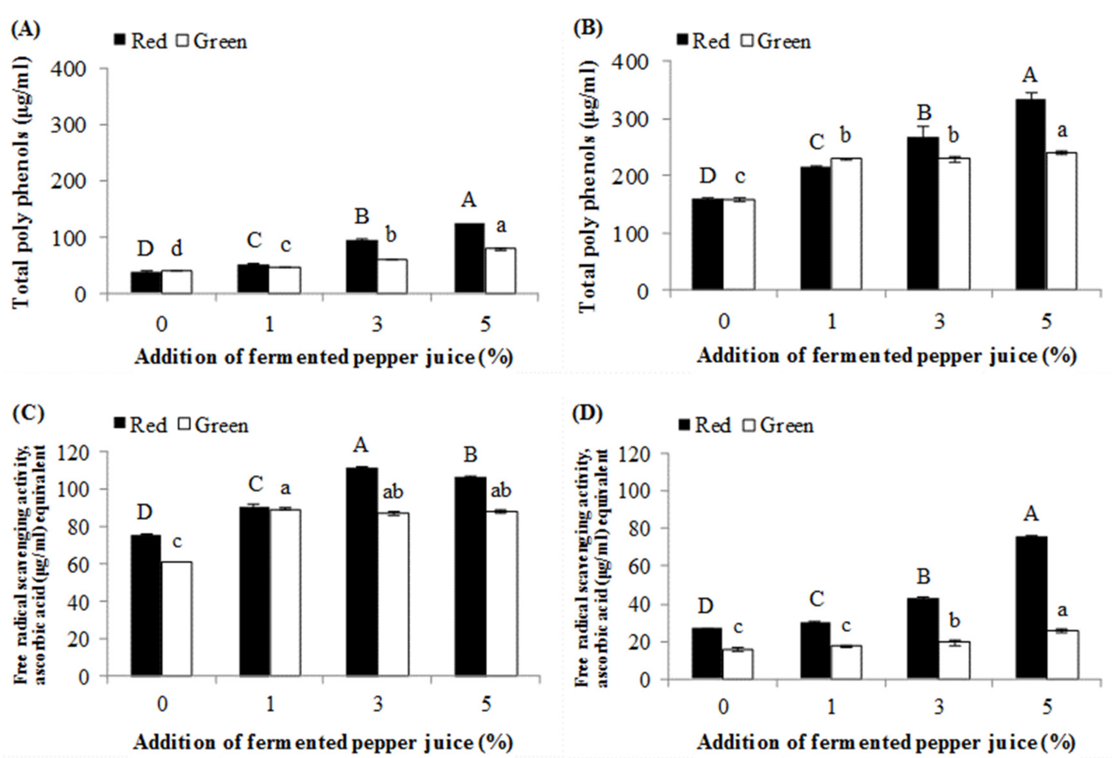

Fig. 2. Total polyphenol contents and antioxidant activity in extracts prepared using different solvents from pepper yogurt with different addition levels of fermented red or green pepper juice. (A) and (B), Total polyphenol contents, (C) and (D) Antioxidant activity. (A) and (C), Water extracts. (B) and (D) Methanol extracts. Different capitalized letters and small letters mean significant difference in yogurt added with red or green pepper juice ($p<0.05$).

water and methanol extracts were not proportion to amounts of polyphenols. For example, water extracts had higher antioxidant activities although they had lower contents of polyphenols compared to methanol extracts. It may suggest that other compounds with some polyphenols in hot pepper may mainly act on the antioxidant activity.

Sensory evaluation

Sensory properties of yogurts prepared with red or green pepper juice at concentrations of 0–5% are shown in Table 4. General taste scores of yogurt added with fermented pepper juice were decreased from 4.07 to 1.43 with increasing amount of pepper juice added to yogurt. General taste scores of yogurt added with 5% of red or green pepper juice were significantly lower than those of yogurt added with pepper juice at 0–3% (p<0.05). Scores of appearance, texture, flavor, and overall acceptance of yogurt added with 3% of red pepper juice were relatively higher than those of yogurt added at other concentrations. However, yogurt added with green pepper juice showed relatively higher scores at 1% addition. The overall acceptability score was decreased with increasing amounts of added pepper juice due to increasing hot taste.

In this study, more amounts (10–50 times) of hot pepper could be added for the preparation of stirred yogurt by using fermented pepper juice compared to the previous yogurt prepared by Yu et al. (2014). In sensory evaluation, the addition of fermented red pepper juice at concentration of 3% or green pepper juice at concentration of 1% resulted in moderate scores for general acceptance. As a result, the optimal concentration of pepper juice was determined.

Changes of pH, titratable acidity, growth of probiotics, and viscosity during storage of yogurt

Physicochemical characteristics of yogurt added with fermented red pepper juice (final 3%) and fermented green pepper juice (final 1%) after storage at 4℃ were investigated for 16 days at 4-day intervals. The pH of control yogurt during the storage period ranged from 4.41 to 4.31. It was 4.39–4.26 for yogurt added with fermented red pepper juice at 3% and 4.42–4.27 for that added with fermented green pepper juice at 1% (Fig. 3A). The pH of yogurt was decreased very slowly during the storage period. It maintained at >4.2, indicating the production of acids. The addition of spirulina can also decrease pH to

Table 4. Sensory characteristics of yogurt with pepper juice

Attributes	Pepper juice	Addition of fermented pepper juice (%)			
		0	1	3	5
General taste	Red pepper	4.07±0.16^{A}	3.71±0.49^{A}	2.86±0.69^{B}	1.43±0.53^{C}
	Green pepper	4.07±0.16^{A}	3.14±0.69^{B}	3.29±0.76^{B}	1.86±0.38^{C}
Hot taste	Red pepper	1.21±0.58^{C}	2.00±0.58^{B}	2.71±0.76^{AB}	3.29±1.11^{A}
	Green pepper	1.21±0.58^{C}	2.29±0.76^{B}	2.57±0.79^{B}	3.71±0.95^{A}
Appearance	Red pepper	4.57±0.51^{A}	3.43±0.79^{B}	4.57±0.53^{aA}	2.14±0.38^{C}
	Green pepper	4.57±0.51^{A}	3.86±0.69^{B}	3.29±0.49^{bC}	2.29±0.49^{D}
Texture	Red pepper	3.57±0.94	3.43±1.13	3.71±1.11	3.14±1.21
	Green pepper	3.57±0.94	3.00±0.58	2.86±0.69	3.29±0.95
Flavor	Red pepper	3.79±0.58^{AB}	3.14±0.69^{B}	3.86±0.90^{aA}	1.71±0.49^{C}
	Green pepper	3.79±0.58^{A}	3.86±0.90^{A}	2.71±0.76^{bB}	2.14±0.69^{B}
Overall acceptability	Red pepper	4.07±0.92^{A}	3.71±0.76^{A}	3.86±0.69^{aA}	2.00±0.00^{B}
	Green pepper	4.07±0.92^{A}	3.71±0.49^{A}	2.71±0.95^{bB}	2.29±0.76^{B}

Values are mean±SEM (n=3).
Different small letters in the same column and capitalized letters in the same row indicated significant difference (p<0.05).

around 4.2 compared to plain yogurt at pH 4.65 (Son et al., 2008). However, mulberry pekmez yogurt shows increased pH to 4.65–5.57 compared to pH 4.47 for plain yogurt Celik and Bakirci, 2003). This indicates that the amount and kind of added substrate in yogurt manufacture can affect pH change during storage. A shown in Fig. 3B, TA value was increased from 0.87% to 0.91% for control yogurt without adding any pepper juice. It was increased from 0.93% to 0.96% for yogurt added with fermented red pepper juice at 3% and from 0.90% to 0.93% for yogurt added with fermented green pepper juice at 1%. The increased acidity might be due to slow growth of LAB during storage, resulting in decreased pH.

The number of LAB of all yogurts was increased significantly at 8 days after storage and decreased at 12 days after storage (Fig. 3C). According to the Korean Food Standards Codex (2011), viable cell count of yogurt should be 10^8 CFU/mL or more. All yogurt samples prepared in this study had >10^9 CFU/mL. Therefore, they should have beneficial effects on human intestinal microflora. The viscosity of fermented yogurt added with red pepper juice was increased until 8 days after storage. It was then decreased (Fig. 3D). The viscosity of fermented pepper juice yogurt showed a decreasing tendency compared to that of the control, consistent with a previous report (Son et al., 2008) showing that the viscosity of spirulina added yogurt was increased until 6 days after storage followed by a gradual decrease after that. The addition of spirulina increased viscosity compared to control whereas red or green pepper juice decreased viscosity, suggesting that different kinds of functional substrates added to yogurt could have various effects on viscosity of yogurt.

Color change during storage of yogurt

Results of color changes of yogurt prepared with the addition of pepper juice are shown in Table 5. L* values (lightness) of

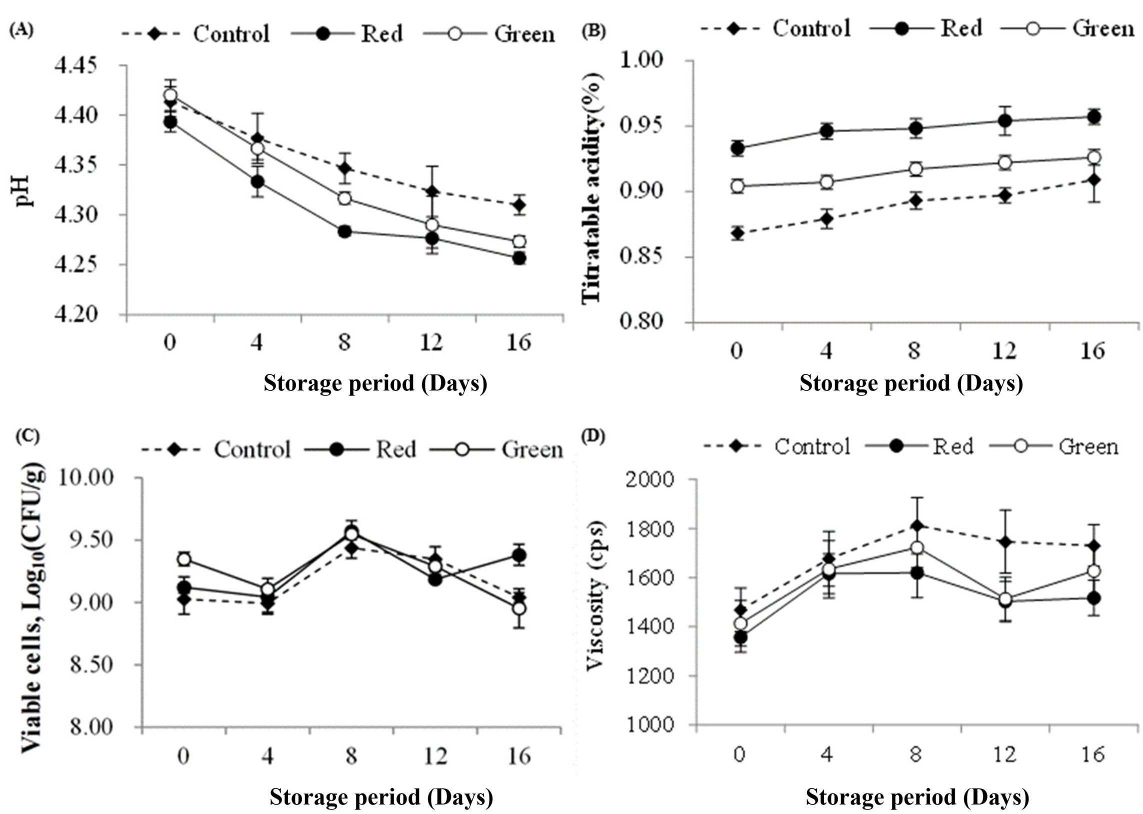

Fig. 3. Changes in pH, titratable acidity (TA), viable cell count, and viscosity during storage period of yogurt added with fermented red or green pepper juice. (A) pH, (B) TA, (C) Viable cell count, (D) Viscosity. Values are mean±SEM (n=3).

Table 5. Color change in yogurt with pepper juice during the storage at 4℃

Color value	Storage period (days)	Treatment		
		Control	Red	Green
L*	0	85.58±0.05^{cA}	80.46±0.22^{aC}	84.96±0.24^{dB}
	4	87.84±0.57^{bA}	79.81±0.41^{bC}	86.42±0.46^{cB}
	8	88.66±0.19^{aA}	80.15±0.34^{abC}	81.82±0.45^{eB}
	12	88.69±0.61^{aA}	80.61±0.31^{aC}	87.60±0.32^{bB}
	16	87.12±0.29^{bB}	80.70±0.09^{aC}	89.23±0.15^{aA}
a*	0	–2.63±0.07^{aB}	7.55±0.47^{aA}	–2.51±0.07^{aB}
	4	–3.28±0.18^{bB}	4.95±0.49^{dA}	–2.89±0.19^{bB}
	8	–3.57±0.07^{cB}	6.01±0.58^{cA}	–3.06±0.27^{bcB}
	12	–3.65±0.21^{cB}	7.00±0.50^{abA}	–3.33±0.08^{cB}
	16	–3.08±0.11^{dB}	6.65±0.26^{bcA}	–3.62±0.02^{dC}
b*	0	5.26±0.27^{cB}	25.58±0.40^{aA}	5.70±0.18^{cB}
	4	7.10±0.50^{aB}	23.88±0.34^{bA}	5.98±0.71^{cC}
	8	7.79±0.19^{aB}	24.36±0.66^{bA}	6.24±0.27^{bcC}
	12	7.83±0.63^{aB}	25.31±0.60^{aA}	7.20±0.20^{aB}
	16	6.25±0.24^{bC}	24.75±0.11^{abA}	6.84±0.42^{abB}

Values are mean±SEM (n=3).
Different small letters in the same column and capitalized letters in the same row indicated significant difference ($p<0.05$).

control yogurt and yogurt added with green pepper juice were significantly increased with storage period except for yogurt added with red pepper juice. The L value of yogurt added with red pepper juice was significantly lower compared to that of other yogurt. The a*-value (redness) was significantly decreased during storage for all yogurt samples. The highest a*-value was observed for yogurt added with red pepper juice during storage. From 4 days after storage, the b*-value (yellowness) was significantly increased in control yogurt without adding any fermented pepper juice. However, the b*-value of yogurt added with 3% fermented red pepper juice was significantly decreased during storage whereas that of yogurt added with 1% fermented green pepper juice was significantly increased from 8 days after storage. Yogurt added with red pepper juice also had higher b*-value than that in other yogurt samples during storage. These results indicate that carotenoids of red pepper juice might have contributed to the increase of redness and yellowness and the decrease of lightness in the development of yogurt color.

Total polyphenol content and antioxidant activity during the storage of yogurt

Total polyphenol content (TPC) and antioxidant activity of control yogurt (prepared without any pepper juice), yogurt added with 3% fermented red pepper juice, and yogurt added with 1% of fermented green pepper juice were measured during storage at 4°C. Results are shown in Fig. 4. TPCs in water extracts of red and green pepper yogurts were higher than those in the control (Fig. 4A). For yogurt added with 3% of fermented red pepper juice, TPCs were 90–100 μg/mL for water extracts and 200–220 μg/mL for methanol extracts during the storage period (Fig. 4 A and B). For yogurt added with 1% of fermented green pepper juice, TPCs were 60–90 μg/mL for water extracts and 180–200 μg/mL for methanol extracts. For methanol extracts of yogurt, TPCs of yogurt added with 3% of fermented red pepper juice were slightly higher than those of other yogurts.

Antioxidant activities of water and methanol extracts from yogurt added with pepper juice showed no significant changes according to storage time (Fig. 4C and 4D). In both water and methanol extracts, yogurt added with fermented red or green

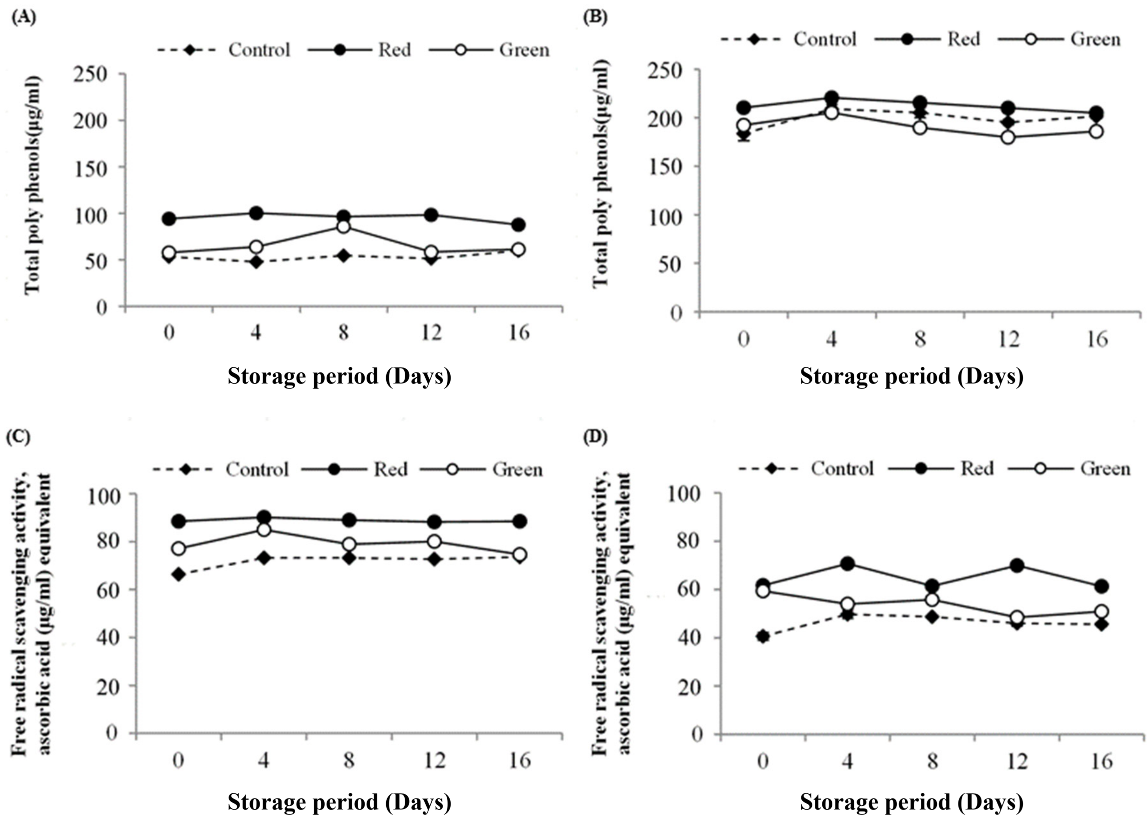

Fig. 4. Changes of total polyphenol contents and antioxidant activity during storage period of yogurt added with fermented red or green pepper juice. (A) and (B), Total polyphenol contents, (C) and (D), Antioxidant activity. (A), (C) and (B), (D) are water and methanol extracts, respectively.

pepper juice showed higher antioxidant activity compared to plain yogurt. These results indicated that TPC and antioxidant activities were correlated with the amount of pepper juice added to yogurt. They were constantly kept during storage at 4°C.

Sensory evaluation during storage of yogurt

Sensory properties of yogurt added with 3% of fermented red pepper juice or 1% of fermented green pepper juice are shown in Table 6. Yogurt supplemented with fermented red pepper juice showed lower scores in general sensory taste regardless of storage period ($p<0.05$). Yogurt added with fermented green pepper juice also showed slightly lower scores compared to control yogurt. Regardless of storage period, hot taste was significantly higher ($p<0.05$) for yogurt added with fermented red pepper juice. During storage, hot taste was not changed. This indicates that there is no further degradation of capsaicin, resulting in a constant hotness. This is due to the addition of fermented pepper juice for the preparation of yogurt.

The appearance of yogurt added with fermented red pepper juice was similar to that of the control. On the other hand, the appearance score of yogurt added with fermented green pepper juice was significantly lower than that of the control ($p<0.05$). There was no difference in the texture of yogurt among treatment groups during the storage period. Sensory evaluation of flavor was significantly ($p<0.05$) lower for yogurt added with fermented red pepper juice regardless of storage period. Regarding overall acceptability, yogurt added with fermented pepper juice showed no significant ($p>0.05$) difference compared to control yogurt. Taken together, these results suggest that fermented pepper juice does not cause any dramatic sensory problem while keeping various physicochemical and biological characteristics for the manufacturing of functional yogurt.

Table 6. Sensory characteristics in yogurt with pepper juice during the storage at 4℃

Attributes	Storage period (days)	Treatment		
		Control	Red	Green
General taste	0	4.86±0.38^{A}	2.86±0.38^{aC}	4.00±0.58^{aB}
	4	4.71±0.49^{A}	2.71±0.49^{abB}	4.14±0.90^{aA}
	8	4.86±0.38^{A}	2.71±0.49^{abC}	4.00±0.82^{aB}
	12	4.43±0.79^{A}	2.43±0.79^{abC}	3.43±0.53^{abB}
	16	4.29±0.76^{A}	2.14±0.69^{bC}	3.00±0.58^{bB}
Hot taste	0	1.14±0.38^{C}	3.43±0.53^{A}	2.43±0.53^{B}
	4	1.29±0.49^{C}	3.29±0.49^{A}	2.29±0.49^{B}
	8	1.29±0.49^{C}	3.29±0.49^{A}	2.29±0.49^{B}
	12	1.29±0.49^{C}	3.29±0.49^{A}	2.29±0.49^{B}
	16	1.29±0.49^{C}	3.29±0.49^{A}	2.29±0.49^{B}
Appearance	0	4.29±0.76^{A}	4.14±0.69^{A}	2.57±0.79^{B}
	4	4.14±0.90^{A}	4.00±0.58^{A}	2.71±0.49^{B}
	8	4.00±0.82^{A}	4.00±0.58^{A}	2.57±0.79^{B}
	12	3.86±0.69^{A}	3.71±0.49^{A}	2.43±0.79^{B}
	16	3.71±0.76^{A}	3.57±0.53^{A}	2.14±0.90^{B}
Texture	0	4.29±0.95	4.00±1.00	4.00±1.00
	4	4.00±1.15	3.86±0.90	3.86±0.90
	8	4.00±0.82	3.71±0.76	3.71±0.76
	12	3.86±1.07	3.71±0.76	3.71±0.76
	16	3.86±1.46	3.57±0.98	3.57±0.98
Flavor	0	4.14±0.90^{A}	2.43±0.79^{B}	3.29±0.95^{AB}
	4	4.00±0.82^{A}	2.29±0.76^{B}	3.14±0.90^{AB}
	8	4.14±0.90^{A}	2.43±0.79^{B}	3.29±0.95^{AB}
	12	4.00±0.82^{A}	1.86±0.69^{B}	3.14±0.90^{A}
	16	3.43±0.53^{A}	1.86±0.69^{B}	2.86±0.90^{A}
Overall acceptability	0	3.43±0.98	3.29±1.25	3.29±1.38
	4	3.57±0.98	3.43±1.13	3.57±1.51
	8	3.57±0.98	3.57±0.98	3.57±1.13
	12	3.29±0.95	3.14±1.46	3.29±1.70
	16	3.57±0.53	3.29±1.50	3.29±1.50

Values are mean±SEM (n=3).
Different small letters in the same column and capitalized letters in the same row indicated significant difference (p<0.05).

Conclusion

Polyphenols containing capsicum are cardioprotectants, inhibitors of neurodegenerator, and anti-obesity effectors. The polyphenol content of yogurt was significantly increased with the addition of fermented pepper juice. DPPH scavenging activity of yogurt added with fermented red pepper juice was higher than that of yogurt added with fermented green pepper juice. In sensory evaluation, yogurt added red pepper juice at 3% or green pepper juice at 1% had higher scores in flavor, appearance, texture, and overall acceptance compared to yogurt in other groups. The yogurt containing pepper juice might become a popular dairy product for consumers who prefer a unique taste of hotness. In conclusion, the results suggest that the fermented pepper juice can be used for manufacturing functionally fortified yogurt with reduced pungency.

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