

## Quality Characteristics of Stirred Yoghurt Added with Fermented Red Pepper

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### Abstract

Pungency of hot pepper has limited its usage even though it shows various health beneficial effects. This study was conducted to develop the novel yoghurt containing hot pepper with diminishing pungency and aimed to examine the quality characteristics of yoghurt prepared with fermented red pepper. Hot pepper was first fermented with *Bacillus licheniformis* SK1230 to reduce the pungency of capsaicin. We then examined the quality, sensory characteristics, and antioxidant activity of yoghurt containing the fermented red pepper. The titratable acidity of this yoghurt increased whereas the viscosity decreased with increasing amounts of added red pepper. The total polyphenol content increased in proportion to the amount of added red pepper. The antioxidant activity significantly increased with the addition of red pepper ( $p < 0.05$ ). Color evaluation showed that the *L* value decreased whereas the *a* and *b* values increased significantly with the amount of red pepper added ( $p < 0.05$ ). In the sensory evaluation, yoghurt prepared with higher amounts of fermented red pepper received lower scores. However, yoghurt containing fermented red pepper at a concentration of 0.05% received higher scores for taste, flavor, and overall acceptability than yoghurt prepared with non-fermented pepper. Therefore, it can be concluded that the application of red pepper fermented by *Bacillus licheniformis* SK1230 gives beneficial feature to the preparation of yoghurt.

**Keywords:** stirred yoghurt, quality characteristics, fermented red pepper, DPPH, antioxidant activity, *B. licheniformis*

### Introduction

Capsaicin is the principal pungent component of chili pepper. It is associated with enhanced lipolysis and the sparing of stored glycogen owing to an increase in circulating catecholamines (Lee *et al.*, 2010). Currently, there is an increasing interest in natural treatments to control dyslipidemia and to reduce the risk of cardiovascular disease. Adipose tissue has an important role in maintaining the energy balance and homeostasis (Lee *et al.*, 2011). Recently, research has been conducted on natural anti-obesity substances that prevent fat storage and accelerate fat disintegration, which could have applications in weight control and obesity management (Lee *et al.*, 2009). Capsaicin has numerous biological roles, such as the enhancement of thermogenesis and fat consumption as well

as analgesic, anti-inflammatory, and antioxidant activities (Hayman and Kam, 2008; Surh, 2002). However, despite its medicinal benefits, the strong pungency of red pepper restricts its widespread use. Therefore, this study aimed to reduce the pungency of hot pepper and produce healthy food made with red pepper with good flavor.

Yoghurt is a widely consumed functional food because of its high digestibility, bioavailability, and proteolytic activities (Shabboo and Ahmad, 2011). It strengthens the immune system, improves lactose ingestion and blood glucose management (Yadav *et al.*, 2007), helps relieve constipation and diarrhea, and has potential health benefits in colon cancer, inflammatory bowel disease, and allergies (Adolfsson *et al.*, 2004). Research on bioactive functional yoghurt include studies on the supplementation of dietary fiber (Damian, 2013; Min *et al.*, 2012), cultured ginseng as an anticancer agent (Lee *et al.*, 2003), micronutrients (Sazawal *et al.*, 2013), black garlic extract as an anti-inflammatory agent (Shin *et al.*, 2010), omega-3 polyunsaturated fatty acids (McCowen *et al.*, 2010), vitamin D (Shab-Bidar *et al.*, 2011), isoflavone (Cavallini *et al.*, 2009), and green tea powder (Jung *et al.*, 2005).

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The aim of this research was to manufacture functionally fortified yoghurt containing fermented red pepper with increased antioxidant activity. We measured the quality characteristics of stirred yoghurt added with red pepper that was fermented with *Bacillus licheniformis* SK1230 to reduce pungency.

## Materials and Methods

### Preparation of red pepper powder

Red pepper (*Capsicum annuum* cv. Chungyang), which is cultivated in Cheong-Yang, Chungchung Nam-do province, during September, was purchased fresh from a local market, washed with clean water, and ground (without adding water) using a mixer (Dasung Health Mix, DA 5000). The ground pepper was sterilized in an autoclave at 120°C for 20 min. The red pepper powder was then freeze-dried and stored in a refrigerator.

### Preparation of fermented red pepper

*Bacillus licheniformis* SK1230 was grown in LB broth or solid medium (Difco™, BD, Franklin Lakes, NJ, USA). The overnight culture was inoculated at 1% in bacillus minimal medium (BMM). The BMM was composed of Na<sub>2</sub>HPO<sub>4</sub>·7H<sub>2</sub>O, 33.5 mM; KH<sub>2</sub>PO<sub>4</sub>, 22 mM; NaCl, 8.6 mM; NH<sub>4</sub>Cl, 18.7 mM; MgSO<sub>4</sub>, 1 mM; CaCl<sub>2</sub>, 0.1 mM; glucose, 10 mM. Red pepper powder (3%) was added to the BMM, and the mixture was sterilized and fermented at 37°C for 48 h. The fermented red pepper was pasteurized at 90°C for 10 min and used for the preparation of yoghurt.

### Preparation of stirred yoghurt

Fermented or non-fermented red peppers was added to milk at concentrations of 0-0.1% and homogenized (Homogenizer T 25, Janke and Kunkel type, Ika, Staufen, 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 detailed in Fig. 1 and fermented for 5 h. The yoghurt was stored at 4°C for 24 h.

### Analysis of yoghurt component, pH and titratable acid

The yoghurt component was analysed by Milkoscan (Milkosan Minor 78110, Foss Co., Denmark). The pH of the homogenized yoghurt was determined using a digital pH meter (ISTEC 735P, Korea). Titratable acid (TTA) was

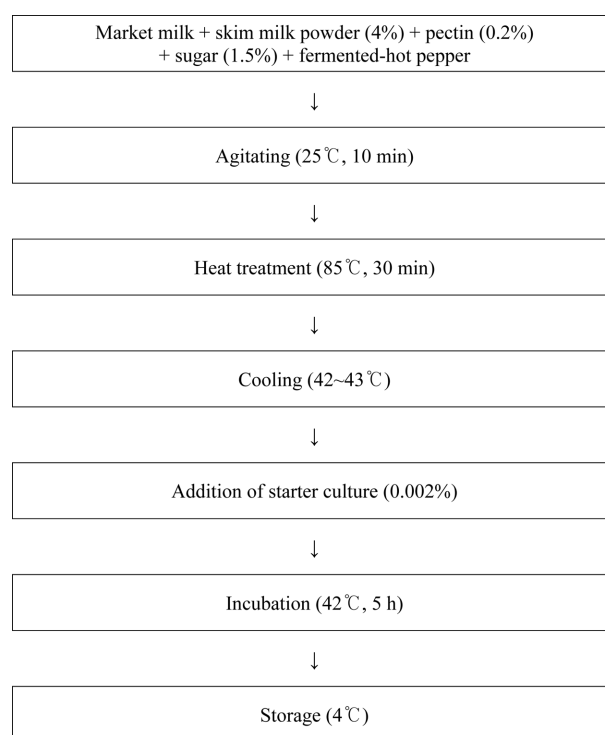


Fig. 1. Procedure for the manufacture of stirred yoghurt with added fermented red pepper.

determined by titration with 0.1 N NaOH. Yoghurt sample (3 g) was transferred into an Erlenmeyer flask containing 27 mL of dH<sub>2</sub>O. Three to five drops of 0.1% phenolphthalein as pH indicator were added. The yoghurt mixture was then titrated with 0.1 N NaOH with continuous stirring until a stable pink color was achieved. The amount of acid produced during fermentation was calculated as follows:

$$\text{TTA (\% Lactic acid)} = \text{Dilution factor} \times V_{\text{NaOH}} \times 0.1 \text{ N NaOH Factor} \times 0.009 / \text{weight of sample (g)} \times 100$$

Where  $V_{\text{NaOH}}$  is the volume (mL) of 0.1 N NaOH required to neutralize the acid; a dilution factor of 10 was used.

### Enumeration of lactic acid bacteria in yoghurt

The agar plate was incubated at 37°C for 48 h. Total microorganism counts were determined on MRS agar and expressed as CFU (colony-forming units)/mL.

### Measurement of viscosity

The viscosity of the yoghurt, which was stored at 4°C, was measured using a Brookfield-Viscometer (Model LV DV 1+, Version 3.0, Strongton, M. CoA, USA) with spindle No. 63 at 100 rpm for 5 to 8 min.

### Measurement of color

The color of the yoghurt was measured using the Hunter Lab color meter (Minolta chromameter CR-210) and expressed as  $L$ ,  $a$ , and  $b$  values.  $L$  value represents lightness,  $a$  value represents redness, and  $b$  value represents yellowness. The  $L$ ,  $a$ , and  $b$  values on a standard color plate were 97.83, -0.45, and 2.00, respectively.

### Total polyphenol content assay

Water extract from yoghurt containing red pepper (1.0 mL) was mixed with methanol [9.0 mL, 95% (v/v)] and 9 mL of dH<sub>2</sub>O. Folin-Ciocalteu reagent (1 mL, 1N) was added to each supernatant, and after being thoroughly mixed, the solutions were allowed to stand for 3 min at room temperature. Na<sub>2</sub>CO<sub>3</sub> (300 μL, 1 N) was then added, and absorbance at 725 nm (UV-1601, Shimadzu, Japan) was read after incubation for a 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 the water extracts of yoghurt, and the regression of gallic acid standards was used to convert unknown samples to total phenolic content [μg gallic acid equivalent (μg GAE)/mL].

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

DPPH inhibition was determined as described by Shetty *et al.* (1995). Briefly, 1 mL of 0.15 mM DPPH (Sigma-Aldrich, Germany) was mixed with 4 mL of yoghurt 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. The constant absorbance readings at 517 nm were recorded using a spectrophotometer (UV-1601, Shimadzu, Japan) at 5 min, and 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  $A$  is absorbance at 517 nm.

### Sensory analysis

Sensory analyses were carried out by 10 trained assessors between 20 and 50 years of age. The samples were coded with three digit numbers and randomly served at 7 to 10°C in plastic cups (10 mL). The assessors completed a test assessment form to compare the five sensory attributes (taste, flavor, appearance, hot taste, and overall ac-

ceptability) by 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; 1, mild.

### Statistical analysis

Three separate experiments with triplicate assays were performed. Data were expressed as means + SEM (standard error of the mean). 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 established as  $p < 0.05$ .

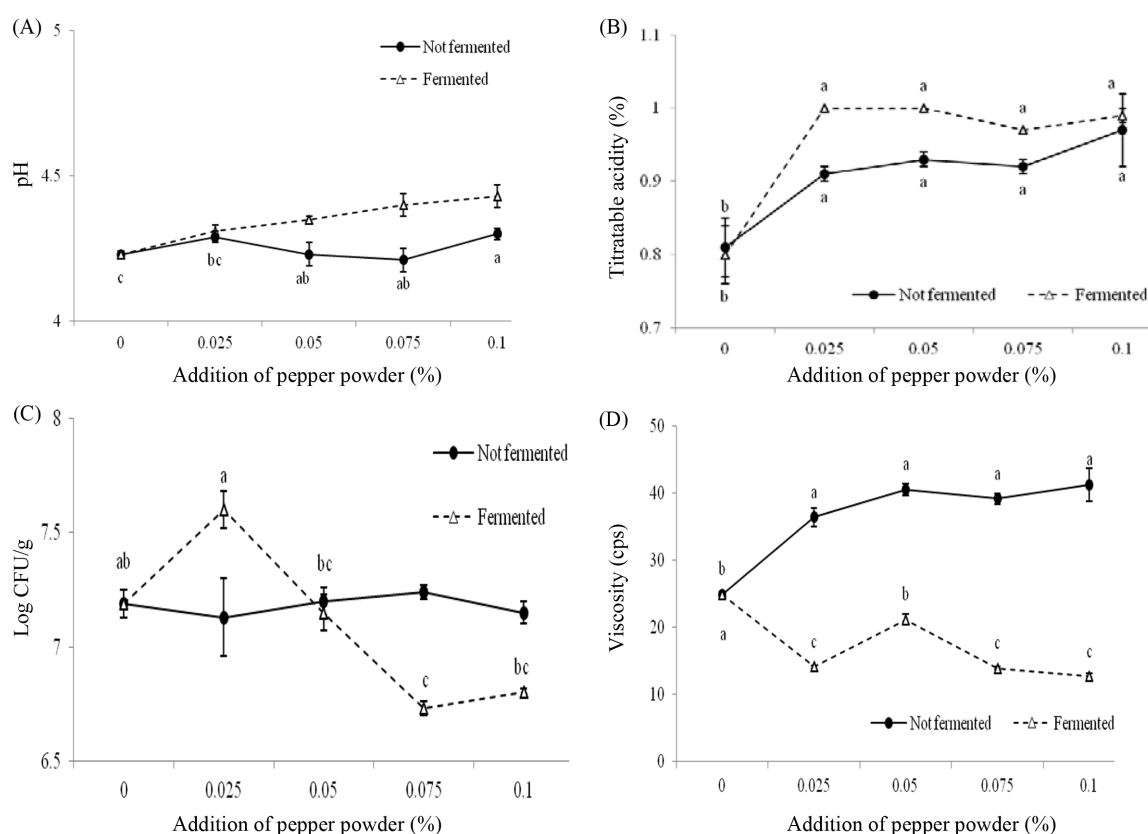
## Results and Discussion

### pH, TTA, growth of probiotics, and viscosity

The control yoghurt without red pepper contained total solids 14.3%, moisture 82.84%, protein 3.53%, fat 3.92%, lactose 6.38%, and ash 0.2%. The pH and TTA of yoghurt prepared with 0 to 0.1% fermented or non-fermented red pepper are shown in Fig. 2A and B, respectively. The pH was decreased to around 4.3 after the fermentation of yoghurt mixture. Yoghurt with 0.05-0.1% fermented red pepper showed a slightly higher pH than yoghurt with the same concentration of non-fermented pepper (Fig. 2A). Lee *et al.* (2010) reported that the pH of pepper fermented with *Bacillus subtilis* P3-3 increased with incubation time.

The constantly higher TTA in yoghurt added with fermented red pepper compared to that in non-fermented yoghurt (Fig. 2B) could be attributed to higher acid production due to the addition of fermented red pepper during the preparation of yoghurt. Yoghurt added with red pepper showed TTA contents in the range of 0.91 to 1.0%, similar to the TTA content of domestic commercial yoghurt (0.92-1.40%). TTA is generally high depending on the decrease of pH. However, the addition of fermented red pepper showed a different tendency with some unknown reason.

The lactic acid bacteria (LAB) count of yoghurt with 0 to 0.1% red pepper is shown in Fig. 2C. The LAB count of yoghurt prepared with 0.025% fermented red pepper increased significantly compared with that of yoghurt with non-fermented red pepper. However, with the addition of fermented red pepper at concentrations of 0.075 to 0.1%, the LAB count of yoghurt decreased significantly, whereas that of non-fermented pepper-containing yoghurt was not changed. These results showed that the addition of 0.05% or higher concentrations of red pepper was not good for the LAB growth.



**Fig. 2.** Changes in pH, titratable acidity, viable cell count, and viscosity of yogurt with fermented red pepper. (A) pH; (B) Titratable acidity; (C) Viable cell count; (D) Viscosity. Means with different superscript letters are significantly different ( $p < 0.05$ ).

The viscosity of plain yoghurt with red pepper was determined with a viscometer as shown in Fig. 2D. The viscosity of yoghurt with added fermented red pepper decreased significantly compared with that of yoghurt with non-fermented red pepper, whereas the viscosity of non-fermented red pepper-containing yoghurt was significantly higher than that of plain yoghurt without red pepper. The decrease in viscosity could be attributed to the fermentation activity of LAB. There was no correlation between viscosity and pH in either fermented or non-fermented red pepper-containing yoghurts. Chung *et al.* (2006) reported that the viscosity of yoghurt with added enzymatic oyster hydrolysates increased proportionally with the amount of LAB. Shin *et al.* (2010) reported that the viscosity of yoghurt decreased because of increased acid production.

### Color changes

The color changes in yoghurt added with 0 to 0.1% fermented red pepper, measured using Hunter *L*, *a*, and *b* values, are shown in Table 1. The addition of fermented pepper led to a decrease in brightness, with significant differences observed upon varying the amounts of red pep-

per compared with no addition. The *L* value of fermented pepper-containing yoghurt ranged from 84.4 to 88.38, indicating a relatively bright color. On the other hand, the *L* value of non-fermented pepper-containing yoghurt gradually decreased from 88.38 to 85.58 with the addition of up to 0.1% of pepper. These data showed that increasing the amount of red pepper led to a decrease in brightness. The *a* value (redness) significantly decreased after the addition of red pepper, whereas the *b* value (yellowness) increased. The *a* value (redness) decreased from -3.12 in plain yoghurt to -4.02 with the addition of up to 0.1% fermented red pepper, whereas the *b* value (yellowness) increased significantly. Increasing the amount of red pepper in yoghurt gave it a more yellowish color. Yoghurt prepared with Maca (*Lepidium meyenii*) hot water extract also showed a decrease in *L* value, whereas the Hunter *a* and *b* values increased (Chung *et al.*, 2010).

### Total polyphenol content

Phenolic compounds are widely found in various plants. They are generally water-soluble and have excellent antioxidative effects. The total polyphenol contents (TPC) from water and ethanol extracts of red pepper in yoghurt

**Table 1. Color changes in yoghurt with added fermented red pepper**

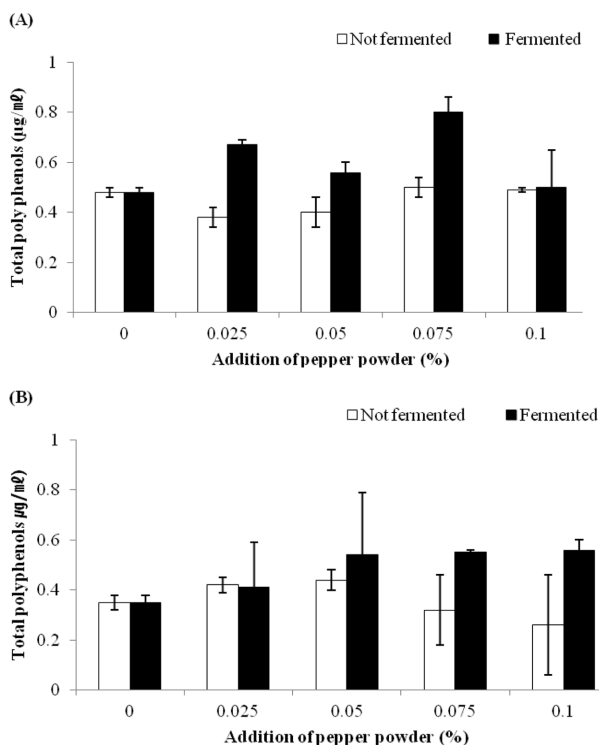
	Pepper powder	Addition of pepper powder (%)					p value
		0	0.025	0.05	0.075	0.1	
L (Lightness)	Non-fermented	88.38±1.13 <sup>a</sup>	84.87±1.14 <sup>c</sup>	<sup>A</sup> 87.88±0.64 <sup>a</sup>	<sup>A</sup> 86.77±0.92 <sup>ab</sup>	85.58±0.94 <sup>bc</sup>	0.0058
	Fermented	88.38±1.13 <sup>a</sup>	84.83±0.54 <sup>c</sup>	<sup>B</sup> 85.80±0.86 <sup>bc</sup>	<sup>B</sup> 84.40±0.60 <sup>c</sup>	86.27±0.33 <sup>b</sup>	0.0005
	p-value	1.0000	0.9554	0.0283	0.0202	0.2993	-
a (Redness)	Non-fermented	-3.12±0.23 <sup>b</sup>	-2.59±0.42 <sup>a</sup>	-3.48±0.18 <sup>bc</sup>	<sup>B</sup> -3.57±0.25 <sup>bc</sup>	-3.79±0.23 <sup>c</sup>	0.0026
	Fermented	-3.12±0.23 <sup>b</sup>	-2.46±0.16 <sup>a</sup>	-3.16±0.26 <sup>b</sup>	<sup>A</sup> -3.06±0.19 <sup>b</sup>	-4.02±0.06 <sup>c</sup>	<0.0001
	p-value	1.0000	0.6503	0.1574	0.0456	0.1658	-
b (Yellowness)	Non-fermented	7.64±1.47 <sup>ab</sup>	6.22±0.91 <sup>b</sup>	8.26±0.75 <sup>a</sup>	8.63±0.16 <sup>a</sup>	<sup>B</sup> 8.86±0.81 <sup>a</sup>	0.0675
	Fermented	7.64±1.47 <sup>b</sup>	5.23±0.31 <sup>c</sup>	7.19±0.81 <sup>b</sup>	7.39±0.49 <sup>b</sup>	<sup>A</sup> 10.28±0.33 <sup>a</sup>	0.0003
	p-value	1.0000	0.1509	0.1676	0.1526	0.0476	-

Data are means±SD.

Means with different superscripts are significantly different ( $p < 0.05$ ).

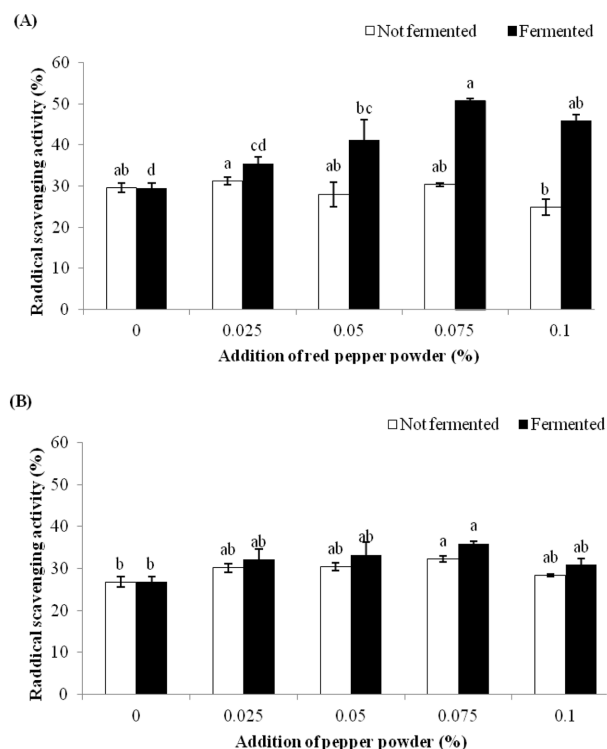
<sup>A-B</sup> Within a column, different letters indicate significant differences ( $p < 0.05$ ).

<sup>a-c</sup> Within a row, different letters indicate significant differences ( $p < 0.05$ ).



**Fig. 3. Total polyphenol contents of yoghurt with fermented red pepper.** (A) Water extract; (B) Methanol extract.

are shown in Fig. 3. For both water and methanol extracts, yoghurt with added fermented red pepper showed significantly higher TPC than yoghurt with non-fermented red pepper, and TPC increased proportionally with the amount of fermented red pepper added. We suggest that the elevated TPC in yoghurt is associated with an increase in the antioxidant activities of fermented red pepper. TPC in the methanol extracts from fermented red pepper yoghurt was also higher than non-fermented one. Jeon



**Fig. 4. DPPH radical-scavenging activity of fermented red pepper-containing yoghurt.** (A) Water extract; (B) Methanol extract.

(2011) reported that yoghurt showed increased TPC when *Dioscorea batatas* fermented by *Monascus* sp. and *Lactobacillus* sp. was added to it. The higher TPC in red pepper-containing yoghurt may be due to the herb's indigenous phytochemical compounds.

**Antioxidant activity**

The antioxidant activity of water extract from fermen-

ted red pepper-containing yoghurt was increased significantly ( $p<0.05$ ) compared to that of non-fermented red pepper-containing yoghurt (Fig. 4A). Non-fermented red pepper-containing yoghurt showed little change in antioxidant activity. Similar to the result for TPC, the antioxidant activity of the water extract was higher than that of the methanol extract, as shown in Fig. 4B. The addition of red pepper water extract resulted in an increase in antioxidant activity (Fig. 4A), similar to the pattern observed for TPC (Fig. 3A). This finding indicates that the polyphenols of red pepper mainly contribute to the antioxidant properties of yoghurt. The higher antioxidant activities in red pepper-containing yoghurt were likely due to the herb's antioxidant properties (Thompson *et al.*, 2007). The reduction in antioxidant activities during refrigerated storage of yoghurt is attributed to increasing degradation of phenolic compounds with antioxidant activities (Yildiz and Eydurán, 2009) or increasing milk protein-polyphenol interaction (Yuksel *et al.*, 2010).

### Sensory evaluation

The sensory properties of the yoghurt were evaluated by 10 trained panels of ages 20 to 50 years, and the results are summarized in Table 2. The yoghurt was prepared with red pepper at concentrations of 0 to 0.1%. The taste scores for yoghurt with fermented red pepper ranged from 3.45 to 2.43, which were higher than those for yoghurt with non-fermented peppers (2.91 to 2.26). The taste, flavor, color, and overall acceptability decreased in propor-

tion to the added amount of red pepper. The flavor score of non-fermented red pepper-containing yoghurt ranged from 3.39 to 2.52, whereas that of fermented red pepper-containing yoghurt ranged from 3.64 to 2.52. The color value of non-fermented red pepper-containing yoghurt ranged from 3.57 to 2.78, which was comparable to that of fermented red pepper-containing yoghurt, 3.91 to 2.71. The hot taste increased with the amount of red pepper added. Fermented red pepper-containing yoghurt had a lower score than non-fermented red pepper-containing yoghurt, indicating a reduced pungency of red pepper after fermentation. In the addition of 0.025% fermented red pepper, its hot taste decreased significantly compared with non-fermented red pepper-containing yoghurt ( $p<0.05$ ). The fermented red pepper contained reduced capsaicinoids (data not shown). The overall acceptability decreased with increasing amounts of added red pepper. High scores were received by non-fermented red pepper-containing yoghurt with 0.025% red pepper and fermented red pepper-containing yoghurt with 0.05% red pepper. Therefore, the addition of fermented red pepper in yoghurt improved the functional properties such as antioxidant activity as well as the sensory characteristics of yoghurt. These results indicate that the addition of fermented red pepper at a concentration of 0.05% would be good for the production of pungency-reduced and functionally enriched yoghurt fortified with antioxidant, anti-obesity, and anti-inflammatory components.

In conclusion, the addition of fermented pepper to yo-

**Table 2. Sensory characteristics of yoghurt with fermented red pepper**

Attributes	Pepper powder	Addition of pepper powder (%)					p value
		0	0.025	0.05	0.075	0.1	
Taste	Non- fermented	2.91±1.24	2.70±1.06	2.91±1.31	2.26±1.21	2.26±1.14	0.1448
	Fermented	3.45±1.13 <sup>a</sup>	3.14±0.85 <sup>ab</sup>	3.24±1.14 <sup>ab</sup>	2.71±1.06 <sup>bc</sup>	2.43±0.75 <sup>c</sup>	0.0174
	p-value	0.2296	0.1338	0.3866	0.1953	0.5701	-
Flavor	Non- fermented	3.39±1.08 <sup>a</sup>	2.87±0.97 <sup>abc</sup>	3.26±1.21 <sup>ab</sup>	2.61±0.94 <sup>bc</sup>	2.52±1.24 <sup>c</sup>	0.0267
	Fermented	3.64±1.12 <sup>a</sup>	3.29±1.06 <sup>ab</sup>	3.14±1.11 <sup>abc</sup>	2.67±1.00 <sup>bc</sup>	2.52±0.75 <sup>c</sup>	0.0114
	p-value	0.5440	0.1797	0.7388	0.8412	0.9947	-
Color	Non- fermented	3.57±1.20 <sup>a</sup>	3.22±1.00 <sup>ab</sup>	3.30±0.97 <sup>ab</sup>	2.78±0.90 <sup>b</sup>	2.83±0.98 <sup>b</sup>	0.0504
	Fermented	3.91±1.30 <sup>a</sup>	3.52±1.03 <sup>ab</sup>	3.10±0.62 <sup>bc</sup>	2.86±0.85 <sup>c</sup>	2.71±0.85 <sup>c</sup>	0.0022
	p-value	0.4520	0.3223	0.4065	0.7802	0.6894	-
Hot taste	Non- fermented	1.22±0.52 <sup>d</sup>	<sup>A</sup> 2.39±1.08 <sup>c</sup>	2.96±1.02 <sup>b</sup>	3.22±1.04 <sup>b</sup>	3.83±1.03 <sup>a</sup>	<0.0001
	Fermented	1.27±0.65 <sup>c</sup>	<sup>B</sup> 1.71±0.78 <sup>c</sup>	2.38±1.07 <sup>b</sup>	3.10±1.00 <sup>a</sup>	3.38±1.12 <sup>a</sup>	<0.0001
	p-value	0.7898	0.0227	0.0753	0.6936	0.1761	-
Overall acceptability	Non- fermented	3.13±1.14 <sup>a</sup>	2.91±1.08 <sup>a</sup>	2.83±1.15 <sup>a</sup>	2.13±1.06 <sup>b</sup>	2.09±1.00 <sup>b</sup>	0.0023
	Fermented	3.55±1.21 <sup>a</sup>	3.05±1.07 <sup>ab</sup>	3.33±0.80 <sup>a</sup>	2.57±0.98 <sup>b</sup>	2.43±1.03 <sup>b</sup>	0.0054
	p-value	0.3379	0.6812	0.1001	0.1597	0.2695	-

Data are means±SD.

Means with different superscripts are significantly different ( $p<0.05$ ).

<sup>A-B</sup>Within a column, different letters indicate significant differences ( $p<0.05$ ).

<sup>a-c</sup>Within a row, different letters indicate significant differences ( $p<0.05$ ).

ghurt resulted in increased titratable acidity and decreased viscosity. The polyphenol content and antioxidant activity of yoghurt significantly increased with the amount of fermented pepper added. In sensory evaluation, yoghurt containing fermented pepper received higher scores for taste, flavor, and overall acceptability.

### Acknowledgements

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