

## Production of Functional High-protein Beverage Fermented with Lactic Acid Bacteria Isolated from Korean Traditional Fermented Food

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### Abstract

The aim of this study was to manufacture functional high protein fermented beverage, using whey protein concentrate (WPC) and *Lactobacillus plantarum* DK211 isolated from kimchi, and to evaluate the physicochemical, functional, and sensory properties of the resulting product. The fermented whey beverage (FWB) was formulated with whey protein concentrate 80 (WPC 80), skim milk powder, and sucrose; and fermented with *Lactobacillus plantarum* DK211 as single, or mixed with *Lactococcus lactis* R704, a commercial starter culture. The pH, titratable acidity, and viable cell counts during fermentation and storage were evaluated. It was found that the mixed culture showed faster acid development than the single culture. The resulting FWB had high protein (9%) and low fat content (0.2%). Increased viscosity, and antioxidant and antimicrobial activity were observed after fermentation. A viable cell count of  $10^9$  CFU/mL in FWB was achieved within 10 h fermentation, and it remained throughout storage at 15°C for 28 d. Sensory analysis was also conducted, and compared to that of a commercial protein drink. The sensory scores of FWB were similar to those of the commercial protein drink in most attributes, except sourness. The sourness was highly related with the high lactic acid content produced during fermentation. The results showed that WPC and vegetable origin lactic acid bacteria isolated from kimchi might be used for the development of a high protein fermented beverage, with improved functionality and organoleptic properties.

**Key words:** whey protein, fermented beverage, *Lactobacillus plantarum*, antioxidant activity, sensory analysis

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### Introduction

Probiotic bacteria has been shown to have effects in enhancement of the immune system, and in prevention of gut, vaginal, and urogenital infections, diarrhea, and gastritis, by inhibiting enteric and foodborne microbial pathogens (Walsh *et al.*, 2010). Consumers' interest in functional foods containing probiotic bacteria and prebiotics has created a huge market, and their market share is still expanding (Rathore *et al.*, 2012; Walsh *et al.*, 2010). The consumer request for food with nutritional benefits is also quickly improved, which has generated the manufacture of various value-added dairy products (Shiby *et al.*, 2013). Recently, the sales of ready-to-drink (RTD) protein drinks have been increased through mainstream supermarket distribution.

The value of proteins as an essential source of amino acids is well documented, but recently it has been recog-

nized that dietary proteins exert many other functionalities *in vivo*, by means of biologically active peptides. Such peptides are inactive within the sequence of the parent protein, and can be released by digestive enzymes during gastrointestinal transit, or by fermentation or ripening during food processing (Korhonen, 2009). In particular, milk proteins are regarded as a source of energy and of essential amino acids, which are needed for growth and maintenance of physiological functions (Unal and Akalm, 2012).

Whey protein, a by-product recognized as a valuable food ingredient with important nutritional and functional properties, is gaining acceptance as a functional food ingredient. Commercial whey proteins are considered a GRAS (Generally Recognized As Safe) substance for food product applications (Sinha *et al.*, 2007). A wide variety of whey ingredients are available for use in the manufacture of yogurt and fermented beverages, including sweet whey powder (SWP), whey protein concentrate (WPC), whey protein isolate (WPI) and specialized WPCs (Huginin, 2008). Whey proteins possess high biological value, and are superior to other proteins, such as those of egg, soy and caseins of milk, mainly due to their high content of branched essential amino acids (Pescuma *et al.*, 2010;

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Shiby *et al.*, 2013).

Whey-based lactic beverages represent an emerging segment of non-conventional dairy products that require sensory, physical, and chemical characterization for quality control and product development (Almeida *et al.*, 2009). However, consumer acceptance of these health drinks depends on the development of nutritional beverages that maintain their desirable appearance, texture, and flavor characteristics during storage and consumption (Shiby *et al.*, 2013). Numerous formulations of liquid products based on whey proteins have been developed to improve their characteristics (Almeida *et al.*, 2009; Athanasiadis *et al.*, 2004; Djurić *et al.*, 2004; Pescuma *et al.*, 2010; Shibby *et al.*, 2013). However, the protein contents of products in their studies are less than 5%. Whey proteins that are not modified to have more heat stability will not be stable as the sole ingredient at levels above 3% protein, that is, they will gel or precipitate under high heat treatment (Rittmanic, 2008). A typical yogurt and Greek yogurt provides on average 3 and 6.7 g protein/100 g serving, respectively. The protein contents in commercial protein drinks or shakes are mostly between 6 and 10%.

The aim of this study was to formulate a novel functional fermented whey beverage containing high protein content, by using whey protein, and lactic acid bacteria isolated from Korean traditional fermented foods. However, a potential issue with culture is the use of vegetable-origin probiotic in a milk-based product containing high protein content; consequently, this probiotic would be potentially unsuited to growth in milk-based product. Moreover, the incubation time is highly related with the production capacity in plant. The reduction in fermentation time could increase the production capacity of plant, and significantly reduce production costs (Huginin, 2008). A commercial strain, *Lactococcus lactis* R704 was selected for mixture use with vegetable-origin lactic acid bacteria isolated from kimchi in previous study, as it is extensively used for the industrial production of fermented milk, cheese, and yogurt (Khalid *et al.*, 2011).

Hence, this study was conducted (1) to optimize the conditions to develop a fermented whey beverage with acceptable organoleptic properties, (2) to determine functional properties, such as antioxidant activity and antimicrobial activity, and (3) to assess the shelf life of the product.

## Materials and Methods

### Strains and materials

The strain, *Lactobacillus plantarum* DKL 121 was iso-

**Table 1. The composition of whey protein concentrate (WPC) and skim milk powder (SMP)**

	Concentration (g/100 g powder)	
	WPC	SMP
Protein	80	35
Fat	0.1	52
Lactose	6.5	1.0

lated from kimchi samples, and maintained in glycerol stocks at -20°C. Four commercial strains: *Lactobacillus helveticus* (LH 166, Culture System Inc, USA), *Lactobacillus plantarum* (LP-5, Culture System Inc, USA), *Streptococcus thermophilus* (ST-Body 1, Christian Hansen, Denmark), and *Lactococcus lactis* (*L. lactis* R704, Christian Hansen, Denmark) were used in this study depending on the purpose of use.

Whey protein concentrate (WPC 80) and skim milk were purchased from Sung Poon Co. (Korea) and Seoul milk (Korea), respectively. Table 1 shows the compositions of these dairy ingredients. Sucrose was obtained from CJ Co. (Korea). All other chemicals and reagents used were of analytical grade, and were purchased from Sigma-Aldrich (USA).

### Fermentation condition

Samples were withdrawn every 2 h during 24 h incubation, for the measurements of pH, titratable acidity, and viable cell counts. Bacterial growth was observed with enumerated viable colony at the MRS agar plates incubated at 37°C for 48 h.

### Manufacture of fermented whey beverage

Fermented whey beverage was prepared, using 11% (w/v) WPC 80, 2% (w/v) skim milk powder, and 10.3% sugar. Each strain was subcultured triplicate in MRS broth at 37°C. All dry ingredients were dissolved in sterile water, and homogenized with a homomixer (IKA, Japan) at 10,000 rpm. This mixture was then pasteurized at 70°C for 30 min, cooled to about 40°C, inoculated culture at a rate of 20 mL/L ( $10^8$  CFU/mL), and fermented at 37°C for 10 h. The resulting FWB was distributed in sterile glass bottles, and stored at 4, 10, and 15°C for 28 d. Viable cell count, pH, and titratable acidity were measured after 0, 7, 14, 21, and 28 d of storage. The FWBs containing commercial strains (LH 166 or ST-Body 1) were also prepared as above, except for the fermentation time. The fermentation time of FWB with *Lactobacillus helveticus* LH 166 and that with ST-body were 13 h and 14.5 h, respectively.

### Assay for proteolytic activity

Proteolytic activity was assessed by an agar diffusion method. Milk agar plates containing 1.6%(w/v) skim milk and 1.5% (w/v) agar were prepared. Three hundred  $\mu\text{L}$  of each strain at a final concentration of  $10^8$  CFU/mL was inoculated onto plates. After incubation at  $37^\circ\text{C}$  for 48 h, absence of an inhibitory zone was observed. Each plate was examined for clear zones.

### Measurement of physical properties

The measurement of pH was conducted at room temperature, using a digital pH meter (Orion 3 star, Thermo Scientific, Korea). Titratable acidity expressed as a percentage of lactic acid was measured by titrating 9 mL of the sample with 0.1 N NaOH, until the substance reached to the end point of the phenolphthalein. The viscosity was measured by a dynamic viscometer (RVDI, Brookfield, USA).

### Measurement of chemical analysis

The organic acid contents were determined using high-performance liquid chromatography (HPLC), consisting of UV/visible detector (Waters 410, Waters, USA). Five mL of samples were mixed with 5 mL of 0.0085 N  $\text{H}_2\text{SO}_4$ , and stood at room temperature for 2 h. This mixture was centrifuged at 4,000 g for 10 min, and then filtered with a syringe filter (pore size 0.22  $\mu\text{m}$ ). The chromatographic analysis was conducted using hydrosphere C 18 column (4.6 mm  $\times$  150 mm, particle size 5  $\mu\text{m}$ , YMC, Japan). The mobile phase used was 0.0085 N  $\text{H}_2\text{SO}_4$ . The chromatographic separation of samples (20  $\mu\text{L}$ ) was performed at a constant flow rate of 0.6 mL/min. The concentration was calculated by using the peak area obtained with standard organic acids (acetic acid and lactic acid).

### Measurement of DPPH radical scavenging activity

This method is based on reduction of the free radical DPPH (1,1-diphenyl-2-picrylhydrazyl). A 0.2 mM/L DPPH radical solution in 99% ethanol was prepared. A 100  $\mu\text{L}$  aliquot of each sample was added to 1 mL of 0.2 mM DPPH solution containing 50  $\mu\text{L}$  of ethanol. After gentle mixing, the solution was incubated at  $37^\circ\text{C}$  for 30 min. The absorbance was measured at 515 nm, using an UV spectrophotometer (X-ma 1200, Human Co., Korea). Trolox was used as a reference antioxidant, at a concentration of 0.1 mg/mL. Free radical scavenging activities were calculated as

$$[(1 - (\text{sample O.D.} / \text{blank O.D.})) \times 100]$$

### Measurement of ferric reducing antioxidant power (FRAP)

This method was used to measure the reductive power of samples. The FRAP reagent was prepared by mixing 300 mM sodium acetate buffer (pH 3.6), 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ), and 20 mM  $\text{FeCl}_3$  at a ratio of 10:1:1 (v/v/v). One mL of each sample was mixed with 100  $\mu\text{L}$  of trichloroacetic acid with vortexing, then centrifuged for 10 min at 9,300 g. The supernatant (40  $\mu\text{L}$ ) was mixed with 1.2 mL of FRAP reagent and 20  $\mu\text{L}$  of distilled water; and then this mixture was incubated at  $37^\circ\text{C}$  for 5 min. The absorbance was measured at 593 nm using a spectrophotometer, using deionized water as the blank. Trolox was used as the positive control. The results were expressed as micromoles of Fe(II), using a linear calibration curve obtained with different concentrations of  $\text{FeSO}_4$ .

### Antimicrobial activity assay

*Staphylococcus aureus* and *Salmonella enterica* were used as test strains, to evaluate the antimicrobial effects of FWB samples. These strains were incubated in LB broth for 18 h at  $37^\circ\text{C}$ , then 0.15 mL of cell culture was spread onto LB agar. The paper disc (diameter, 10 mm) was soaked with 100  $\mu\text{L}$  of each FWB sample. The soaked paper discs were placed on the surface of each plate. After incubation at  $37^\circ\text{C}$  for 18 h under aerobic condition, each plate was examined for the clear inhibition zones around the paper disc.

### Sensory evaluation

The samples were subjected to sensory evaluation. The control was a commercial protein drink, a banana-flavored processed milk fortified with milk protein concentrate (MPC). Thirty-five panelists were employed, and the members of this panel were students selected for their familiarity with yogurt products, but who received no training at all, in order to represent the average consumer. The FWB and commercial product were presented to panels in identical plastic cups labeled with random numbers, and in random order. Each panelist was asked to rate the samples for the four attributes (overall taste, flavor, off-flavor, freshness, and aftertaste) using a 7-point hedonic scale (1="I dislike extremely", 7="I like extremely"). The intensities of sweetness, sourness, and off-flavor were evaluated using a 7-point "just-about-right" scale, ranging from 1="much too mild", to 7="much too strong". Statistical analysis was conducted using ANOVA, where the significant effect was found. Significance of differences

between samples was determined on the basis of Turkey's test, at a significance level of  $p < 0.05$ . The statistical analysis was performed using Statistica 8.0 software (StatSoft Inc., USA).

## Results and Discussion

### Selection of culture

In previous study, a total of 106 colonies were isolated from Kimchi samples. Of them, 60 colonies were identified as *Lactobacillus* species, through observation of phenotypic characters, and identification using API kit and 16S rRNA gene sequencing. Further studies on acid tolerance, bile tolerance, and proteolytic activity were conducted, to select strains for commercial use as probiotics and starter culture in dairy products. It was found that *L. plantarum* DK 211 had tolerance under acid and bile conditions, as its viable cell count remained at pH 2.0 and under 1.0 % bile salt condition, after 2 h incubation time (data not shown). Other studies (Lee *et al.*, 2011; Lim and Im, 2009) conducted on *L. plantarum* strain isolated from kimchi, also indicated that *L. plantarum* strain had acid and bile tolerance, compared with other species.

The proteolytic system of lactic acid bacteria is essential for their growth in milk and ripening of fermented milk products, because it leads to the liberation of peptides and amino acids, which consequently has considerable effects on the physical structure, taste, and flavor development (Beganović *et al.*, 2013; Cho *et al.*, 2013; Khalid and Marth, 1990). Skimmed milk agar plate assays allow principally for qualitative determinations of protease activity, as the hydrolysis zone produced on the skim milk agar could be related to the amount of protease produced by the bacteria (Vijayaraghavan and Vincent, 2013). For the determination of proteolytic activity, a zone of proteolysis was detected on the skimmed milk agar plates. As shown in Fig. 1, this strain possesses strong proteolytic activity, as it produced larger clear zones on milk agar than commercial *Lactobacillus plantarum* strain, LP-5. It showed similar proteolytic activity to 911 LC. The high proteolytic activity of 911LC was proven by the study of Ko *et al.* (2005). The proteolytic activities of all cultures studied in this study followed a time-dependent pattern.

### Growth patterns of culture

Whey proteins will gel or precipitate under high heat treatment, when they are used at levels above 3% protein as the sole ingredient. Therefore, whey protein beverages

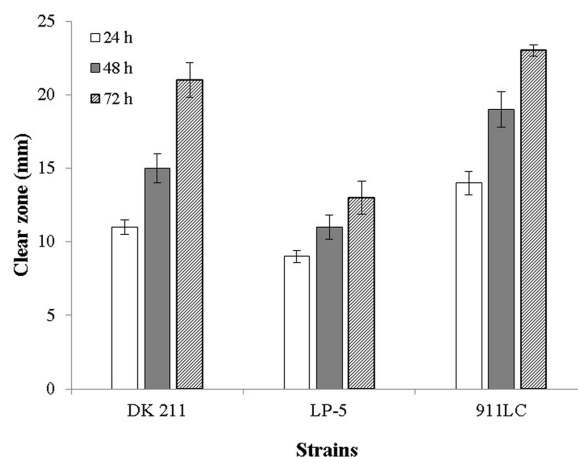


Fig. 1. Comparison of proteolytic activity of *L. plantarum* DK 211 and commercial strains; *L. plantarum* LP-5 and *L. casei* 911 LC using skim milk agar plate. The plates were incubated at 37°C for 24, 48 and 72 h.

are generally formulated to a pH of less than 4.5, because they are subjected to a mild thermal pasteurization process (Rittmanic, 2008). The current minimum standard for yogurt manufacture is about 0.9% acidity, at a final pH between 4.2 and 4.5 (Campagne *et al.*, 2010).

The growth kinetic of *L. plantarum* DK 211 was estimated by measuring the pH, titratable acidity, and viable cell count during fermentation. The growth curve obtained by measuring the pH and titratable acidity was used to estimate at what point in time the bacteria reached to pH 4.5. As shown in Fig. 2, the pH of culture media sharply decreased, until it reached to pH 4.5 at 18 h of incubation. When mixture culture of *L. plantarum* DK 211 and *Lactococcus lactis* was used, there was almost 30% reduction

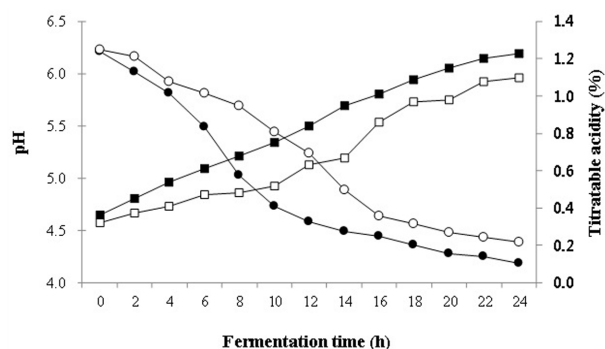


Fig. 2. Changes in pH and titratable acidity of fermented whey beverage with *Lactobacillus plantarum* DK 211 and mixed culture (*Lactobacillus plantarum* DK 211+ *Lactococcus lactis* R704) during fermentation. ●: pH in mixed culture, ○: pH in *Lactobacillus plantarum* DK 211, ■: titratable acidity in mixed culture, □: titratable acidity in *Lactobacillus plantarum* DK 211.

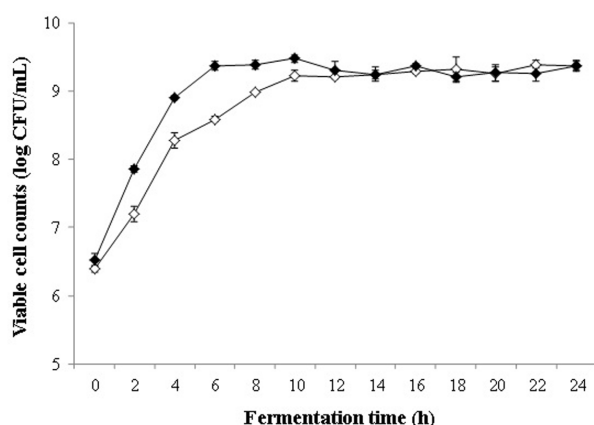


Fig. 3. Changes in viable cell counts of whey fermented beverage with *Lactobacillus plantarum* DK 211 (◇) and mixed culture (*Lactobacillus plantarum* DK 211+ *Lactococcus lactis* R704: ◆) during fermentation.

in the incubation time required to reach pH 4.5. Almeida *et al.* (2009) also observed that the bases fermented by co-culture were generally faster in reaching pH 4.5.

High viable counts are necessary to get the desired acid production and reduction in pH, which affect organoleptic properties of the final product and its shelf-life, and prevent product contamination (Rathore *et al.*, 2012). During fermentation, the population of *L. plantarum* DK 211 rapidly increased at the beginning, and the growth reached a stationary phase after 10 h (Fig. 3). The viable cell counts increased from an initial value of 6.5 Log CFU/mL, to above 9.0 Log CFU/mL at 10 h of incubation time. On the other hand, the population of mixed culture of *L. plantarum* DK 211 and *L. lactis* R704 reached above 9 Log CFU/mL at 6 h of incubation time. An ideal starter culture should be quick and steady in acid production (Cho *et al.*, 2013). Campagne *et al.* (2010) also stated that mixing strains could affect the growth and acidification rates, which are important benefits for sanitary and economic standpoints. According to these results, mixed culture of *L. plantarum* DK 211 and *L. lactis* R704 with 10 h of fermentation time was selected for the preparation of FWB in the further study.

Table 2. Antimicrobial activity of fermented whey beverage

Test microorganisms	Clear zone (mm)			
	NFWB	FWB	FWB-LH 166	FWB-ST Body1
<i>Stapylococcus aureus</i>	0	11 ± 0.5	11 ± 0.1	12 ± 0.3
<i>Salmonella enterica</i>	0	10.5 ± 0.5	10 ± 0.5	9.5 ± 0.2

NFWB, non-fermented whey beverage; FWB, whey fermented beverage with mix culture (*Lactobacillus plantarum* DK 211+ *Lactococcus lactis* R704); FWB-LH 166, whey fermented beverage with *Lactobacillus helveticus* LH 166; FWB-ST Body1, whey fermented beverage with *Streptococcus thermophilus* ST-Body1.

All values are mean±SD of triplicates.

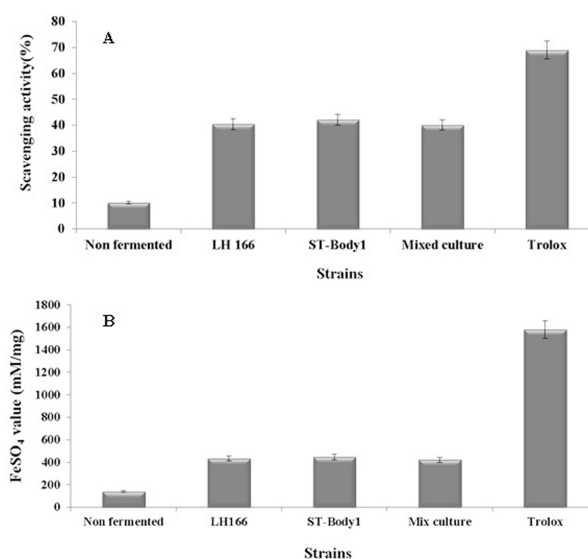


Fig. 4. Comparison in DPPH radical scavenging activity (A) and FRAP values (B) for non-fermented whey beverage, whey fermented beverage with *Lactobacillus helveticus* LH166, whey fermented beverage with *Streptococcus thermophilus* ST-Body1 and whey fermented beverage with mixed culture (*Lactobacillus plantarum* DK 211+ *Lactococcus lactis* R704), positive control: 0.01% Trolox.

#### Characteristics of FWB

As shown in Table 2, the protein, carbohydrate, and fat of FWB were 9.0, 11.5, and 0.2%, respectively. The protein content of FWB showed almost twice higher than that of commercial yogurt products, and was similar with that in commercial protein drinks or shakes. Regarding viscosity, fermentation by lactic acid bacteria caused increasing viscosity of the FWB produced. According to Hassan *et al.* (2012), the viscosity behavior is related to the pH, so the increase in viscosity takes place at acidic value.

One of the characteristic features of fermentation by lactic acid bacteria is the production of different organic acids, due to the degradation of some components in the raw material, and the resultant decrease in pH (Wu *et al.*, 2011). Therefore, the organic acid profile in fermented

dairy foods is an indicator of the metabolic activity of added bacterial cultures. These acids act as natural preservatives, and contribute to the characteristic sensory properties (Adhikari *et al.*, 2002). Lactic acid contents greatly increased as much as 3.5 times during fermentation, while there was less increase in the content of citric acid. These results were similar with those of Adhikari *et al.* (2002), in which there was a 3-fold increase in the content of lactic acid during fermentation. However, in their study, the unfermented mix had a higher amount of citric acid than the fermented product. They explicated that it was because of the utilization of citric acid by the yogurt starter during fermentation.

FWB sample stored at 4°C had slight acid development during the storage period, while the samples stored at 10 and 15°C showed post-fermentation acidification, as the pH decreased from 4.5, to around 3.7 at 28 d of storage (Fig. 4). A similar behavior was suggested by Almeida *et al.* (2009), who observed that the post-acidification behavior of most co-cultures was faster in a milk-whey base.

To exert beneficial health effects, the amount of probiotic bacteria in the food product should be adequately high, i.e.,  $10^6$ - $10^8$  CFU/mL throughout the entire shelf life (Najgebauer-Lejk, 2014). Table 3 shows that the level of probiotics in this study maintained  $10^9$  CFU/mL during 28 d of the storage period, regardless of the storage temperature, which was beyond this criterion.

### Functional properties of FWB

Antioxidant activity has been reported for milk proteins, sodium caseinate and whey protein concentrate, yoghurt, and lactic acid bacteria (Unal and Akalm, 2012). Two antioxidant assays were employed to measure the antioxidant activity of FWB, i.e., DPPH and FRAP assay. DPPH assay is used to evaluate the ability of antioxidant to scavenge free radicals, which is known to give reliable information concerning the antioxidant ability of the tested compounds (Zha *et al.*, 2009). Transition metal ions are also involved in many oxidation reactions *in vivo*, as the reduced form of iron causes oxygen toxicity, by converting the less reactive hydrogen peroxide to the more

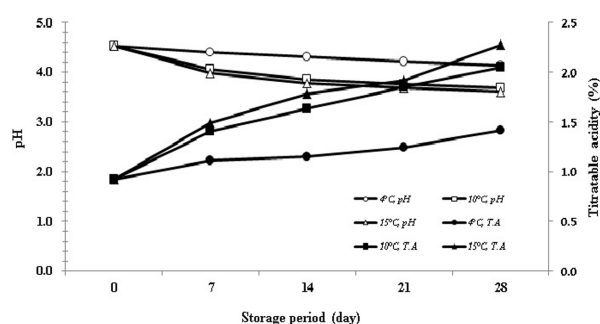


Fig. 5. Changes in pH and titratable acidity of whey fermented beverage during storage.

reactive oxygen species, such as hydroxyl radical, via the Fenton reaction (Unal and Akalm, 2012; Zha *et al.*, 2009). The FRAP assay measures the reduction of ferric to ferrous ion at low pH in the presence of antioxidants, which causes a colored ferrous-tripyridyltriazine complex to form (Gliszczynska-Swiglo, 2006).

To ensure that the measured antioxidant activity was produced during fermentation, the antioxidant activity of non-fermented whey beverage was measured. The DPPH scavenging activity of samples ranged from 10.2% (non-fermented sample) to 42.07%, when Trolox at a concentration of 0.1 mg/mL showed a DPPH scavenging activity of 68.93% (Fig. 5A). Regardless of the starter culture, all fermented whey beverage samples had a higher DPPH scavenging activity than the non-fermented one, indicating that fermentation by lactic acid bacteria increased antioxidant levels. FRAP values in all fermented products were also 3 times higher than in the non-fermented one (Fig. 5B). Increased scavenging effect of FWB might be due to antioxidant peptides released during the fermentation of milk by lactic acid bacteria.

Unal and Akalm (2012) who studied the antioxidant activity of yoghurt fortified with different types of milk protein, revealed that whey protein concentrate showed higher scavenging activity than sodium calcium caseinate. They stated that high scavenging activity of the whey proteins could be attributed to lactoferrin,  $\alpha$ -lactalbumin, and  $\beta$ -lactoglobulin. Species of *Lactobacillus* being widely

Table 3. Changes in viable cell counts of fermented whey beverage during storage

Storage temp. (°C)	Viable cell counts (Log CFU/mL)			
	0 d	7 d	14 d	28 d
4	9.62±0.05	9.60±0.03	9.57±0.10	9.59±0.09
10	9.67±0.06	9.41±0.11	9.48±0.04	9.49±0.05
15	9.60±0.08	9.46±0.10	9.60±0.05	9.45±0.10

All values are mean±SD of triplicates.

used as starter cultures in the dairy industry have been reported to play a significant role in the production of bioactive peptides in fermented dairy products (Ramesh *et al.*, 2012). Farvin *et al.* (2010) also indicated that the milk peptides act as electron donors, and could react with free radicals, to convert them to more stable products. The strong proteolytic activity of *L. plantarum* DK 211 was already observed in Fig. 1. Therefore, the antioxidant activity of FWB fermented with the mixture culture of *L. plantarum* DK 211 and *Lactococcus lactis* could be due to the peptides produced by the action of *L. plantarum* DK 211.

The antibacterial activity of FWB prepared with selected strains was estimated by the agar diffusion method. A strong inhibitory effect of FWB on the growth of *Staphylococcus aureus* and *Salmonella enteritica* was detected (Table 4). It has recently been reported that *Lactobacillus* sp. strains inhibit the growth of Gram-negative pathogenic bacteria, which has generally been attributed to the production of lactic acid during the *Lactobacillus* growth (Nikolova *et al.*, 2009).

### Sensory evaluation

By the addition of a high amount of whey protein, FWB had higher protein content as much as twice, and significantly lower fat content, than the commercial product. However, FWB had a low acceptability score, because of the unappealing taste and excessive acidity of whey protein, which consequently affects the overall taste of the product in preliminary study (data not shown). Djurić *et al.* (2004) also stated that numerous procedures have been developed for characteristics of whey protein. This defect can be overcome by adding flavor components, such as vanilla or fruit flavor. Vanilla flavor was selected to add to the final product, because this flavor was found through the preliminary sensory test to be better matched with FWB than fruit flavors, such as banana and strawberry (data not shown).

Table 5 shows the mean panelists rating of the sensory attributes of the FWB and control. There were no significant differences in the ratings of all attributes, except sourness. The FWB was found to contain sour characteristics, which seemed highly correlated with the lactic acid content. The stinky smell of whey protein in FWB seems to have been improved by the addition of vanilla flavor, because there were no significant differences in the ratings of aftertaste and off-flavor with the commercial product. Sensory evaluation of FWB showed that it was a favorable product, as the panelist ratings for overall taste

**Table 4. The chemical composition and physical property of non-fermented whey beverage (NFWB), fermented whey beverage (FWB) and commercial product (control)**

	NFWB	FWB	Control
Protein (%)	ND	9.0	4.5
Carbohydrate (%)	ND	11.5	9.0
Fat (%)	ND	0.2	3.0
Lactic acid (mg/mL)	1.44	5.04	1.70
Citric acid (mg/mL)	0.48	1.44	1.41
Viscosity (cp)	7.20±0.1	33.6±0.1	9.6±0.2

ND, not detected.

The values for organic acid contents and viscosity are mean±SD of triplicates.

**Table 5. Mean panelist ratings for FWB and commercial product (control)**

Parameters	Samples	
	FWB	Control
Overall taste	4.92 <sup>a</sup>	5.03 <sup>a</sup>
Flavor	5.50 <sup>a</sup>	5.16 <sup>a</sup>
Freshness	4.66 <sup>a</sup>	4.80 <sup>a</sup>
Aftertaste	4.83 <sup>a</sup>	4.93 <sup>a</sup>
Off-flavor	4.33 <sup>a</sup>	4.30 <sup>a</sup>
Sourness	4.14 <sup>a</sup>	2.83 <sup>b</sup>
Sweetness	3.80 <sup>a</sup>	3.77 <sup>a</sup>

FWB, Fermented whey beverage; control, a commercial brand, a banana flavored processed milk fortified with milk protein concentrate.

<sup>a,b</sup>means with different letters are significantly different ( $p < 0.05$ ).

and aftertaste were above the mid-point.

The results obtained in this study provide evidence that a novel high-protein beverage having functional properties and acceptable organoleptic character could be produced by fermentation of whey protein using *L. plantarum* DK 211, a vegetable-origin lactic acid bacterium.

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