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Survival and Effect of Exopolysaccharide-Producing *Lactobacillus plantarum* YW11 on the Physicochemical Properties of Ice Cream

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Ice cream was prepared with exopolysaccharide (EPS)-producing *Lactobacillus plantarum* YW11 by direct inoculation (DI), addition of pre-fermented skim milk (FSM), or addition of the lyophilized powder of the YW11 strain (LP) into the ice cream mix. After 4 weeks of storage, viable counts of the YW11 strain decreased in all groups by 0.8–1.61 log cfu/g. Furthermore, ice cream made using the LP method showed the highest survival rate. The ice cream processing and storage conditions also affected the YW11 strain's tolerance to acid and bile, with a decrease in survival rate of 38.8–63.2% and 10.8–51.8%, respectively. The degree of impact on the viability of strain YW11 was hardening>aging>freezing>storage ($p<0.05$). The YW11 strain produced a ropy EPS (up to 4.84 mg/g) in the ice cream mix made using the DI and FSM methods; it was present as a fine porous matrix as observed by Cryo-SEM. Formation of the EPS together with changes in the pH of the ice cream mix caused increased viscosity (up to 131.0 mPa·s), overrun and meltdown, decreased destabilization of fat, and firmness of ice cream. Hydrocarbons, ketones, and benzenes were found to be the major volatiles in the fermented ice cream samples, which also had decreased levels of dodecane, characterized by the smell of dirt.

INTRODUCTION

Traditional fermented dairy products are considered as main sources of functional microorganisms, *e.g.* lactic acid bacteria (LAB), and ingredients [Selhub *et al*., 2014]. Many LAB strains isolated from them have been shown with various promising bioactivities on human health, including antimicrobial activity, prevention and treatment of diarrhea, relief of symptoms caused by lactose intolerance, anti-mutagenic and anti-carcinogenic activities, and stimulation of the immune system [Shah, 2007]. However the uncertainties of influence from these LAB strains on the quality of functional foods and their bioactivity-keeping in the food matrix frequently hinder their application in modern food industry [Younesi & Ayseli, 2015].

Ice cream was suggested to be a good carrier for survival of LAB strains during storage in terms of its composition, which includes milk proteins, fat, and lactose, as well as other nutrients that might provide protection for the strains [Di Criscio *et al.*, 2010]. However, live cells in ice cream might be exposed to adverse conditions of cooling and freezing, osmotic stress, mechanical shearing, and oxygen stress during processing and storage [Mohammadi *et al.*, 2011]. The viability of several strains such as *Lactobacillus delbrueckii* [Dos Santos Leandro *et al.*, 2013], *L. rhamnosus* [Abghari *et al.*, 2011], *L. acidophilus* [Ferraz *et al.*, 2012; Akın & Dasnik, 2015] and *Bifidobacterium* [Da Silva *et al.*, 2015] were found to decrease to varying extents when they were used for ice cream processing.

Exopolysaccharides (EPSs) commonly produced by bacteria, fungi, and blue-green algae have been known to function as a shield to protect microbial cells against adverse environmental conditions [Tabibloghmany & Ehsandoost, 2014]. Formation of polysaccharide capsules on the surface of lactic acid bacteria was found to significantly enhance their survival in ice cream [Hong & Marshall, 2001]. EPSs produced by lactic acid bacteria also function as natural biothickeners for improving the rheology and texture of fermented food products [Ibarburu *et al.*, 2015; Zannini *et al.*, 2016]. The EPS produced by *Streptococcus thermophilus* was shown to significantly influence the sensory and rheological characteristics of ice cream [Dertli *et al.*, 2016].

In a previous study, *L. plantarum* YW11 isolated from Tibet Kefir was found to possess antimicrobial, antioxidant, antitumor, and immune regulatory activities [Wang, 2015]. This strain was also shown to produce a ropy acidic EPS composed of glucose and galactose (molar ratio of 2.71:1) with molecular mass of 1.1×10^5 Da, and it had a highly branched-porous microstructure [Wang *et al.*, 2015b]. These characteristics of strain YW11 make it an excellent candidate to be explored for application in functional products.

The aim of this study was to evaluate the suitability of *L. plantarum* YW11 for potential application in ice cream

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in terms of its viability through different steps of ice cream processing and storage. Its effect on the physicochemical properties of ice cream is also investigated. This EPS-producing strain was incorporated into ice cream in different ways to study the protective effect of the polymer on the viability, microstructure, and sensory characteristics of ice cream. To our knowledge, this is the first report on the viability of an EPS--producing *L. plantarum* strain affected by ice cream processing and storage. In addition, the effect of the EPS on the processing characteristics of ice cream is also reported for the first time.

MATERIALS AND METHODS

Chemicals, reagents and bacterial strains

L. plantarum YW11, originally isolated from Tibet Kefir, was stored in the Dairy Food Laboratory of Beijing Technology and Business University in lyophilized powder form (11.2 log cfu/g).

Bile salts, ether, glycerol, phenol, hydrochloric acid, sulfuric acid, and ethanol were purchased from Sigma (Shanghai, China), and de Man Rogosa Sharpe (MRS) agar was purchased from Difco (USA). Sugar, cream (Nestle, USA), non-fat milk powder (Fonterra, New Zealand), monoglyceride and gelatin were provided by Sangon Co., Ltd, Shanghai.

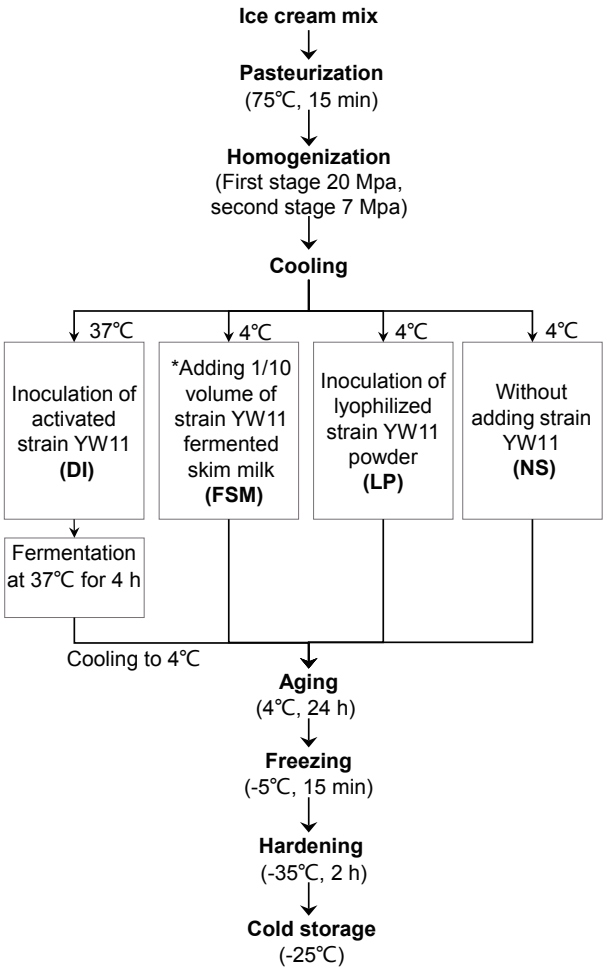

FIGURE 1. Ice cream processing method and scheme using incorporation of *L. plantarum* YW11. *Skim milk and 1/10 volume of ice cream mix were fermented with the YW11 strain for 4 h before incorporation into the ice cream mix.

Production of ice cream

Four experimental groups were defined according to the method of *L. plantarum* YW11 addition to the ice cream mix (Figure 1): DI, direct inoculation of strain YW11 precultured in MRS broth (1/10 volume of mix) at 37°C for 16 h; FSM, addition of 1/10 volume of strain YW11 fermented skim milk; LP, addition of the lyophilized powder of strain YW11 (1 g/L); NS, no addition of the strain. The ice cream mix formula consisted of 11% fat, 11% non-fat milk solid, 10% sucrose, 0.2% gelatin and 0.1% monoglyceride, the balance being made up with water. For the formula of FSM group, the non-fat milk solid and water were correspondingly reduced to achieve the same initial component of ice cream. The processing procedure for the ice cream is shown in Figure 1. Each batch (2 kg) of ice cream mix was frozen at -5°C and whipped for 15 min with a batch freezer BKY7118 (Donper, China).

Tolerance to acid and bile stress

Acid and bile tolerance tests were performed according to Anderson *et al.* [2010] with modifications. Half gram of ice cream was mixed with 4.5 mL of MRS broth of pH 2.0, 3.0, or 6.6. The mixtures were incubated at 37°C for 3 h before spreading on agar plates. For the bile tolerance assay, half gram of ice cream was mixed with 4.5 mL of MRS broth containing 0.3%, 0.5%, or 1.0% (w/v) ox gall (Sigma) and incubated at 37°C for 3 h, and the sample without ox gall was used as a control. The viable counts of *L. plantarum* YW11 were determined by plate counting using MRS agar. The plates were incubated at 37°C for 48 h under anaerobic conditions.

$$\text{Survival rate} = \frac{\text{Bacterial counts after incubation}}{\text{Initial bacterial counts}} \times 100\%$$

Chemical composition and pH of ice cream

All ice cream samples were analyzed for total solid content (% w/w) by drying at 100±5°C for 3.5 h. Total protein was measured using the Kjeldahl method. Fat content was measured using the Röse-Gottlieb method [Crocker *et al.*, 1955]. The pH values were obtained using a pH meter (PB10, Sartorius, Germany). Measurements were carried out in triplicate after 4 weeks of storage at -20±2°C.

Overrun

Overruns were determined according to the method proposed by Marshall *et al.* [2003]. A certain amount of frozen ice cream was weighed and the overrun was calculated using the following equation. The analysis was carried out in triplicate.

$$\text{Overrun (\%)} = \frac{V_1 - V_0}{V_0} \times 100\%$$

where: V_1=Volume of ice cream sample, and V_0=Volume of mix with same weight as ice cream sample.

Melting characteristics

Melting rates were measured according to the method described by Akin *et al.* [2007] with modifications, after 30 days of storage at -20±2°C. Approximately 40 g of samples, initially at -20°C, were placed on a 1.0 mm metal wire mesh screen over a graduated cylinder collector at 25°C. During 150 min of melting time, the melting volume of ice cream was recorded in minutes. Analysis was performed in triplicate.

Texture analysis

Measurement was conducted in a cold compartment at -20°C, using a Texture Analyzer XT2 (Stable Micro Systems, UK), fitted with a 6-mm diameter stainless steel probe. Ice cream samples were prepared by cutting into 2×2×2 cm^3 cube sections. Peak compression force (N) was recorded as firmness during the penetration depth of 0.5 cm at a speed of 1 mm/s.

Quantitation of destabilized fat

To evaluate the degree of destabilization of fat (DSF) in ice cream, samples were thawed and an aliquot of 10 mL were diluted 500 fold with distilled water. Absorbance was measured using a spectrophotometer (U-3010, HITACHI, Japan) at 540 nm with distilled water as the blank. Analysis was performed in triplicate.

Volatile analysis

Samples were prepared by mixing 10 g of ice cream with 1 g NaCl and filled in 20 mL glass vials. The headspace was balanced by stirring the mixture for 30–40 min at 50°C. Volatile compounds in the ice cream samples were analyzed as previously described [Wang *et al.*, 2015a]. Compounds were identified according to NIST 2.0 mass spectra libraries installed in the GC-MS equipment. GC-O was performed by three experienced panelists.

Scanning electron microscopy

The microstructure of ice cream was imaged using a low temperature FEI Dual Beam Helios NanoLab 600i focused ion beam scanning electron microscope (LT-SEM/FIB). Ice cream specimens (approximately 100 mm^3) were taken from the inner bulk of hardened samples at -25°C with a surgical blade, immediately suited on the specimen holder, and then immersed into liquid nitrogen (-196°C). The holder and specimen under liquid nitrogen were transferred into the cryo-preparation unit (FEI/ Quorum PP3000T, UK). At -150°C inside the unit, the specimen was fractured to expose a fresh surface of the ice cream for scanning. The specimen was sublimated at -150°C for 15 min and then coated with 30 nm layer of gold. The holder was transferred under vacuum into the cold stage (-150°C) of FEI LT-SEM/FIB, where samples were viewed and photographed at 2.00 kV accelerating voltage at different magnifications.

Quantitation of EPS

The EPS was separated as previously described [Wang *et al.*, 2015b] with some modification. Fat and proteins in the ice cream mix were precipitated by adding 80% (w/v) trichloroacetic acid solution in 100 mL of mix to a final concentration of 4% (w/v). The mixture was thoroughly mixed by stirring at room temperature for 30 min, then centrifuging at 3000×*g* for 30min, using a Gerber centrifuge to separate fat, and at 10,000×*g* for 20 min with refrigerated centrifuge to separate protein. Two volumes of cold ethanol (4°C) were added to the supernatant and stored at 4°C overnight. The precipitate was collected by centrifugation at 10,000×*g* and dialyzed with deionized water after being totally dissolved in ddH_2O. The dialyzed water was changed 3 times every 8 h. The amount of EPS was quantified using the phenol-sulfuric acid method with glucose as a standard.

Statistical analysis

Statistical analysis was performed using SPSS 16.0 and Sigmaplot 12.0. Significant differences between treatments were tested by ANOVA. All data were presented as means ± standard deviation of means. Principal component analysis was performed using SPSS 16.0 software with a non-linear iterative partial least-squares algorithm [Scott, 2015]. Relative abundance for volatiles was input as data matrix. Zero was filled in the matrix for undetected volatiles.

RESULTS AND DISCUSSION

Viability of *L. plantarum* YW11 during ice cream processing and storage

Maintaining the viability of strains with bioactivities in food matrix till the end of shelf life is an important criterion for exerting their health-beneficial effect. To our knowledge, the survivability of *L. plantarum* strains during ice cream processing has been rarely studied. Figure 2 compares the effect of ageing, freezing, hardening, and storage during ice cream processing on the survivability of *L. plantarum* YW11 when added in different ways. The viable counts of *L. plantarum* YW11 generally decreased with ice cream processing and 4 weeks of storage ($p<0.05$). The impact of each processing step on the viability of *L. plantarum* YW11 was in the or-

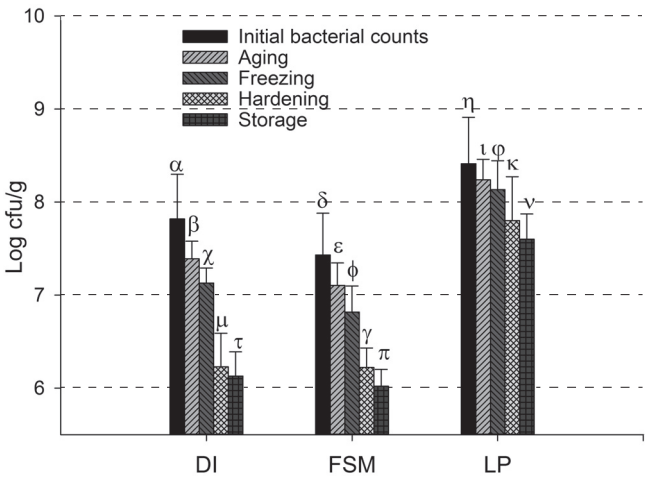

FIGURE 2. Viability of *L. plantarum* YW11 during ice cream processing (ageing, freezing, and hardening) and storage. DI, direct inoculation of strain YW11 pre-cultured in MRS broth (1/10 volume of mix) at 37°C for 16 h; FSM, addition of 1/10 volume of strain YW11 fermented skim milk; LP, addition of the lyophilized powder of strain YW11 (1 g/L); α, β, γ represent significant differences ($p<0.05$).

TABLE 1. Effect of acidity and bile salt on the survival rate of *L. plantarum* YW11 in ice cream (%).

Groups		Culture duration in acid environments		Bile salt concentration		
		1h	3h	0.3% (w/v)	0.5% (w/v)	1.0% (w/v)
Fresh strain	pH2.0	14.23±1.78[a]	0	87.10±12.74[a]	77.62±9.81[b]	54.95±6.35[c]
	pH3.0	47.19±2.47[e]	220.47±18.68[h]			
DI	pH2.0	0	0	11.28±3.06[g]	9.23±2.86[h]	3.21±1.14[i]
	pH3.0	18.29±2.64[b]	87.34±5.28[f]			
FSM	pH2.0	0	0	13.43±2.77[j]	8.41±1.75[k]	2.28±0.38[l]
	pH3.0	22.74±2.41[c]	89.57±3.92[f]			
LP	pH2.0	0	0	43.28±8.48[d]	40.19±4.72[e]	32.15±5.02[f]
	pH3.0	29.82±3.47[d]	153.71±21.91[g]			

DI, direct inoculation of strain YW11 pre-cultured in MRS broth (1/10 volume of mix) at 37°C for 16 h; FSM, addition of 1/10 volume of strain YW11 fermented skim milk; LP, addition of the lyophilized powder of strain YW11 (1 g/L); Values are expressed as mean ± standard deviation, n = 3. [a, b, c] Values with different symbols are significantly different ($p < 0.05$).

der of hardening>aging>freezing>storage ($p<0.05$) for all three experimental groups. The decrease in the viable counts of *L. plantarum* in ice cream of the DI, FSM, and LP groups after processing and storage was 1.61 log cfu/g, 1.28 log cfu/g, and 0.80 log cfu/g, respectively. Direct inoculation of the YW11 strain into the ice cream mix (DI group) achieved the least viable counts, but addition of the strain in freeze-dried form (LP group) retained the highest viability. This correlates with previous studies [Arslan *et al.,* 2016; Shao *et al.,* 2014; Wang *et al.,* 2014] showing that the bacterial strain after experiencing cold stress, *i.e.* freeze-drying treatment in this study, exhibited enhanced resistance to such freezing conditions as those during ice cream processing and storage. The effect of low temperature pretreatment on bacterial cells was mainly due to change of the intracellular enzymatic activity that reinforced the freezing tolerance of the bacterial strains [Kandil & El Soda, 2015]. Overall, the viable counts in the ice cream of all experimental groups in this study were higher than 6 log cfu/g after 4 weeks of storage. Similarly, a *L. rhamnosus* strain maintained higher than 6 log cfu/g of viable count in ice cream although viability of the strain decreased significantly during production and storage [Champagne *et al.*, 2015]. The *L. delbrueckii* [Dos Santos Leandro *et al.*, 2013] and *L. casei* [Homayouni & Norouzi, 2016] strains survived well during ice cream processing and storage, but poor survival of *Bifidobacteria* [Lu *et al.*, 2009; Champagne *et al.*, 2015] in ice cream was also reported. Therefore, necessary protection for added strain is generally required to achieve maximal viability in ice cream.

Since resistance to acid and bile is a prerequisite for a particular microorganism to survive the stomach and intestinal environment when consumed [Dianawati *et al.*, 2016], it was necessary to evaluate the ability of tolerance to acid and bile of *L. plantarum* YW11 as affected by ice cream processing and storage conditions. Table 1 shows the results of the acid and bile tolerance tests for the fresh strain and the strain incorporated into ice cream in three different ways. After 1-h incubation at pH 2.0, the bacterial count was significantly decreased ($p<0.01$) by 85.77% for the fresh culture and 100% (undetectable) for the strain in the DI, FSM, and LP experimental groups. After 3 h of incubation at pH 2.0, no viable strain could be detected in all groups. This behavior of strain YW11 in an acid environment is similar to that previously reported for *L. rhamnosus* GG [Alamprese *et al.*, 2005] and *L. johnsonii* La1 [Alamprese *et al.*, 2002]. However, at pH 3.0, the YW11 strain demonstrated a different extent of tolerance with the three addition methods tested. Incubation for 1 h resulted in survival rates of 18.29% for the DI group and higher than 20% for the FSM and LP groups. After 3 h of incubation, the survival rates of all groups significantly increased with the highest for the LP group (153.71%). These results suggest that after adaption to the pH 3.0 condition, the YW11 strain recovered its growing and reproducing activity. To determine the bile tolerance of strain YW11 after ice cream processing, the fresh strain and the strain added to ice cream in three different ways were examined by addition to MRS broth containing 0.3%, 0.5%, and 1.0% (w/v) of ox gall bile salt. As the bile salt concentration increased, the survival rates of strain YW11 in all groups decreased by 54.95% (fresh strain), 3.21% (DI), 2.28% (FSM), and 32.15% (LP) when the bile salt concentration was 1.0% (w/v). The results indicate that after experiencing the ice cream processing and storage conditions, the YW11 strain significantly decreased its tolerance to bile salt. Furthermore, the strain added *via* the LP method preserved activity better. A similar decrease in bile tolerance has also been observed for other *L. plantarum* strains [Zhang *et al.*, 2014].

Texture analysis of ice cream

The significance of texture characteristics, including viscosity, overrun, and melting properties on consumer acceptance has been affirmed by several researchers [Méndez-Velasco & Goff, 2012; McGhee *et al.*, 2015]. We therefore examined the effect of addition and fermentation of the YW11 strain on the composition, pH, and firmness of ice cream (Table 2). The pH values of ice cream in the NS, DI, FSM, and LP groups were 6.60, 6.06, 6.40, and 6.60, respectively, indicating the degree of fermentation of the ice

TABLE 2. Composition, pH and firmness of ice cream of all experimental groups.

Ice cream	Fat (%)	Protein (%)	Total solids (%)	pH	Firmness (g)
NS	11.26±0.12[a]	5.29±0.24[b]	35.15±0.83[d]	6.60±0.05[a]	530.0±8.0[a]
DI	11.29±0.24[a]	5.35±0.26[b]	34.34±2.04[d]	6.06±0.07[c]	483.0±12.0[c]
FSM	11.23±0.43[a]	5.19±0.10[b]	34.65±1.47[d]	6.40±0.01[b]	505.0±9.0b
LP	11.20±0.36[a]	5.28±0.43[b]	35.00±1.00[d]	6.60±0.08[a]	525.0±5.0[a]

DI, direct inoculation of strain YW11 pre-cultured in MRS broth (1/10 volume of mix) at 37°C for 16 h; FSM, addition of 1/10 volume of strain YW11 fermented skim milk; LP, addition of the lyophilized powder of strain YW11 (1 g/L); NS, no addition of the strain.
[a, b, c] different symbols means significant difference between rows.

cream mix was in the order of DI>FSM>LP=NS. The mean values (g force) of the firmness of ice cream between NS (530 g) and LP (525 g) groups were not significantly different ($p<0.05$), and they were greater than those of the FSM (505 g) and DI (483 g) groups ($p<0.05$).

Figure 3 shows the overrun of the ice cream mix and viscosity of ice cream of all experimental groups. The overrun of the ice cream mixes from the DI and FSM groups were higher than those of the NS and LP groups. The viscosity between each group was similar, implying that fermentation of *L. plantarum* YW11 significantly affected the textural properties of ice cream. Sofjan & Hartel [2004] reported that overrun of ice cream tended to provide a light texture by affecting melting and firmness characteristics. Fermentation of ice cream with *L. rhamnosus* GG changed the viscosity of the ice cream mix [Alamprese *et al.*, 2005]. These findings were contradictory to previous conclusions that addition of non-starter strains had little effect on the texture of ice cream since addition and fermentation behavior of these strains did not change the main composition of ice cream [Cruz *et al.*, 2010]. Although fermentation of the YW11 strain did not change the primary composition of ice cream in this study (Table 3), the presence of ropy EPS in ice cream (up to 4.84 mg/g) was detected, and this might be responsible for the change in texture of the ice cream as described above. *L. plantarum* YW11 was reported earlier to produce a viscous EPS [Wang *et al.*, 2015b]. An increase in the viscosity of freezing yogurt was also observed when EPS was present in the yogurt mix [Dertli *et al.*, 2016]. Application of EPS-producing *L. plantarum* in other food was found to effectively change the food matrix and improve the texture [Bindhumol & Nampoothiri, 2014]. Considering the EPS yield of 90 mg/L of *L. plantarum* YW11 reported earlier [Wang *et al.*, 2015b], different EPS yields of strain YW11 in ice cream mix observed in this study (Figure 3) might be due to differences in the fermentation media, temperature, pH and fermentation time, *etc.* Variation of capacity of EPS production by lactic acid bacteria with fermentation conditions has been widely reported [Li *et al.*, 2013; Hermann *et al.*, 2015; Meng *et al.*, 2015].

Destabilized fat and melting characteristics

DSF is a parameter used to measure the amount of the coalesced fat globules, which affects the stability and sensory effect of the product [Goff, 1997]. Figure 4 shows the effect of *L. plantarum* YW11 incorporated into ice cream by three different methods on DSF. The occurrence of DSF in ice cream between NS (18.23%) and LP (17.27%) groups was not significantly different, and they had less formation of DSF than observed in the DI (21.13%) and FSM (19.87%) groups, being in the order of DI>FSM>LP=NS. This was consistent

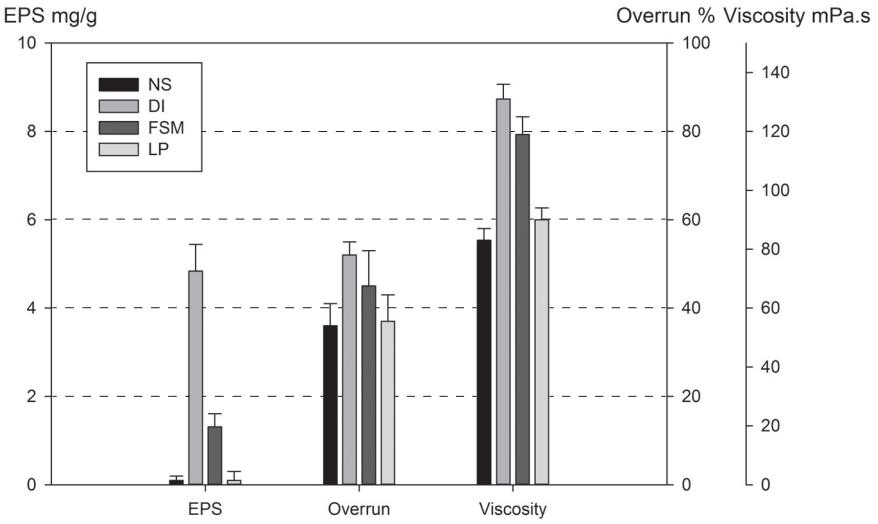

FIGURE 3. Exopolysaccharide content, viscosity, and overrun of ice cream incorporated with *L. plantarum* YW11 by DI, direct inoculation of strain YW11 pre-cultured in MRS broth (1/10 volume of mix) at 37°C for 16 h; FSM, addition of 1/10 volume of strain YW11 fermented skim milk; LP, addition of the lyophilized powder of strain YW11 (1 g/L); NS, no addition of the strain. Values in each group of bars are significantly different ($p < 0.05$).

TABLE 3. Main volatile compounds in ice cream incorporated with *L. plantarum* YW11 by different methods.

No.	Volatiles	RT[1]	RI[2]	Aroma[3]	Relative content (%)			
					NS	DI	FSM	LP
1	Ethyl acetate	5.30	905	Pineapple	2.00±0.21^{c}	4.60±0.32^{b}	4.94±0.44^{ab}	5.31±0.29^{a}
2	Decane	6.65	968	Alkane	–	3.24±1.02^{a}	–	–
3	Dodecane	11.93	1200	Dirt	16.55±0.47^{a}	7.99±0.25^{d}	11.36±0.98^{c}	14.00±0.77^{b}
4	Hexadecane	14.36	1247	Alkane	10.26±0.97^{a}	8.00±0.82^{b}	8.54±0.69^{b}	7.01±0.11^{c}
5	Naphthalene	27.12	1751	Faint scent	2.10±0.32^{a}	0.92±0.41^{b}	1.04±0.29^{b}	1.29±0.35^{b}
6	2-Heptanone	12.69	1189	Fruity	14.77±0.52^{b}	16.11±0.28^{a}	13.90±0.38^{c}	13.86±0.45^{c}
7	2-Pentanone	7.16	992	Orange peel	2.31±0.22^{b}	3.19±0.17^{a}	3.06±0.38^{a}	2.15±0.12^{b}
8	3-Hydroxy-2-butanone	15.65	1286	Sour milk	–	3.96±0.36^{a}	1.28±0.21^{b}	–
9	2-Nonanone	18.35	1389	Soap	9.70±0.33^{b}	9.14±0.18^{c}	11.53±0.24^{a}	8.10±0.31^{d}
10	2-Undecanone	23.50	1589	Orange	1.21±0.61^{a}	2.40±0.95^{a}	2.06±1.08^{a}	1.96±0.81^{a}
11	Butyric acid	24.26	1561	Cheese	–	8.25±0.67^{a}	1.89±0.72^{b}	–
12	Acetic acid	19.97	1435	Vinegar	1.63±0.43^{a}	1.51±0.25^{a}	1.33±0.41^{a}	1.39±0.15^{a}
13	Benzaldehyde	21.84	1515	Almond	–	1.92±0.86^{a}	–	–
14	9-Octadecenal	18.46	1100	No	–	–	–	4.15±1.23^{a}
15	2-Ethyl-1-hexanol	21.01	1494	Fruity	10.54±0.96^{b}	8.86±1.08^{b}	14.23±0.27^{a}	9.42±0.91^{b}
16	Benzene	5.97	924	Fragrance	11.35±0.48^{a}	9.10±0.12^{c}	9.51±0.21^{c}	10.25±0.38^{b}
17	Toluene	8.37	1028	Nutty, bitter	10.64±0.52^{a}	6.83±0.04^{d}	7.59±0.48^{c}	9.79±0.37^{b}
18	Ethylbenzene	10.48	1122	Aromatic odor	6.93±0.08^{a}	2.83±0.17^{d}	6.64±0.22c	5.81±0.31^{b}
19	o-Xylene	11.17	1183	Geranium	–	–	–	5.64±0.09^{a}
20	Dimethyl sulfone	30.02	1273	Sulfur, burnt	–	1.13±1.02^{a}	–	–

Values (relative content, %) presented are means ± standard deviation on duplicate trials. Compounds not detected are indicated by "-". [1] Retention time; [2] Retention index; [3] Odor description at the GC-sniffing port. [abcd] Means in the same row followed by different letter are significantly different ($p < 0.05$). DI, direct inoculation of strain YW11 pre-cultured in MRS broth (1/10 volume of mix) at 37°C for 16 h; FSM, addition of 1/10 volume of strain YW11 fermented skim milk; LP, addition of the lyophilized powder of strain YW11 (1 g/L); NS, no addition of the strain.

with the results of the effect on overrun and viscosity of ice cream, which varied depending on the method of adding strain YW11 (Figure 3). The increment of overrun was found to increase air bubbles that destabilized fat globules in the ice cream mix [Bolliger *et al.*, 2000]. During ice cream processing, the freezer was kept at a stationary shearing speed, and the increased viscosity of the ice cream mix and the shearing force destabilized fat globules to induce partial coalescence [Goff, 1997]. A positive correlation between the viscosity increment of the ice cream mix and the formed shearing force was also confirmed by a rheology test [Rossa *et al.*, 2012].

The meltdown behavior of ice cream is an empirical characteristic that reflects the melting resistance of ice cream when exposed to warm temperatures, and it is closely related to thermal conductivity, heat capacity, and microstructure of ice cream [Sun-Waterhouse *et al.*, 2013]. Melting of the ice cream when *L. plantarum* YW11 was added by different methods was similar during the 150 min of the melting test, with almost the same melting rate at about 0.27 mL/min as calculated by the polynomial, linear fitting mode using the Sigmaplot software (Figure 5). However, in the first 20 min, the melting rate of each experimental group was different, leading to a remaining amount of 4.0 g, 8.0 g, 5.0 g, and 4.0 g of ice cream for the NS, DI, FSM, and LP groups, respectively. This result coincides with the overrun difference between the groups as described above (Figure 3); the lower the overrun, the faster the melting rate [Sofjan, 2002]. Contrary to the observations of Muse & Hartel [2004], overrun was not a determining factor to the melting rate by statistical results. The effect of overrun on the melting characteristics of ice cream needs to be further investigated.

Microstructure of ice cream

Figure 6 shows the Cryo-SEM micrographs of ice cream when *L. plantarum* YW11 was added by different methods. Samples from the NS, DI, FSM, and LP groups exhibited different microscopic morphologies. For the ice cream samples from DI and FSM groups that contain EPS produced by strain YW11, the ice cream matrix demonstrated a web and porous structure due to the EPS network formed within proteins present in the matrix. Especially in DI samples, the structure of ice cream mainly consisted of big air bubbles and fine porous fabric matrices, which is similar to

the microstructure observed in ice cream made with EPS--producing *Streptococcus thermophilus* [Dertli *et al.*, 2016] and in other dairy products containing EPS [Hassan *et al.*, 2003]. The micrographs of ice cream samples from the NS and LP groups differ slightly, and they both contain a similar number and size of air bubble, ice crystal in the ice cream matrix, which is in accordance with the previous report on the microstructure of ice cream [Goff *et al.*, 1999]. These results were also in agreement with the overrun and firmness experiments described above (Table 2, Figure 3), confirming that the higher EPS concentration in ice cream leads to higher overrun and lower firmness of the matrix. However, the process and mechanism of EPS interacting with ice cream components to form characteristic microstructures of the ice cream matrix requires further investigation.

Volatile compounds in ice cream

Flavor is often the first indicator when consumers choose a food; their interest is not aroused in consuming a functional food if the bioactive ingredients result in disagreeable flavors [Cruz *et al.*, 2010]. Volatile analysis is widely applied in objective sensory evaluation for dairy foods in order to determine consumer acceptance of novel functional products [Arancibia *et al.*, 2015].

Analysis of volatile compounds in ice cream by solid-phase micro-extraction and GC-O-MS methods revealed a total of 20 volatiles detected in four batches of ice cream samples when *L. plantarum* YW11 was added by different methods. There were 13, 18, 15, and 15 compounds identified in the ice cream samples from NS, DI, FSM, and LP groups, respectively. These volatile compounds belong to different chemical families including 5 ketones, 4 hydrocarbons, 4 benzenes, 2 free fatty acids (FFAs), 2 alcohols, 1 aldehyde, 1 ester, and 1sulfur compound (Table 3).

Results of the principal component analyses (PCA) of the volatiles in ice creams are shown in Figure 7. PCA of the hydrocarbons, ketones, and benzenes demonstrated that they were scattered near the F1 axis, and accounted for 73.45% of the total variability in the data, with 87.85% and 8.55% of the variance explained by F1 and F2, respectively. Among the 4 ketones detected, 2-heptanone (fruity aroma) and 2-nonanone were present in all the 4 batches of ice cream as the most abundant ketones (>13%). These two ketone compounds were found to comprise the typical flavor of heat-treated milk, and they were synthesized generally during the pasteurization of ice cream mix by decarboxylation of β-oxidized saturated fatty acids or decarboxylation of β-keto acids [Vazquez-Landaverde *et al.*, 2005]. For hydrocarbons, dodecane had a higher concentration in the ice cream sample from the NS (~ 16%) group than in samples from the DI (~ 8%), FSM (~ 11%), and LP (~ 14%) groups. This suggests that fermentation with *L. plantarum* YW11 in ice cream played a role in reducing the formation of dodecane that is usually found in cheese with a smell of dirt [Buchin *et al.*, 1998]. The 4 benzenes, which are common flavor compounds of skim milk powder, together with the ketones might be responsible for the primary pleasant smell of the ice cream in this study. Some volatiles such as decane, benzaldehyde, and dimethyl sulfone were only detected in the DI sample. Other volatiles such as butyric acid and 3-hydroxy-2-butanone were found only in DI and FSM samples. The content of butyric acid in DI samples was relatively high as reported by Michaud *et al.* [2008] that contribute to a cheese-like flavor. The volatile compound 3-hydroxy-2-butanone, a typical fermented milk flavor component, was also found in fermented soymilk produced by EPS-producing lactic acid bacteria [Li *et al.*, 2014]. O-xylene and 9-octadecenal detected in the LP sample, might be derived from the freeze-dried powder of strain YW11.

PCA of the correlation between the factors (F1, F2) with the test groups is also shown in Figure 7. The lines of the four groups were scattered along the F1 axis, and they had a simi-

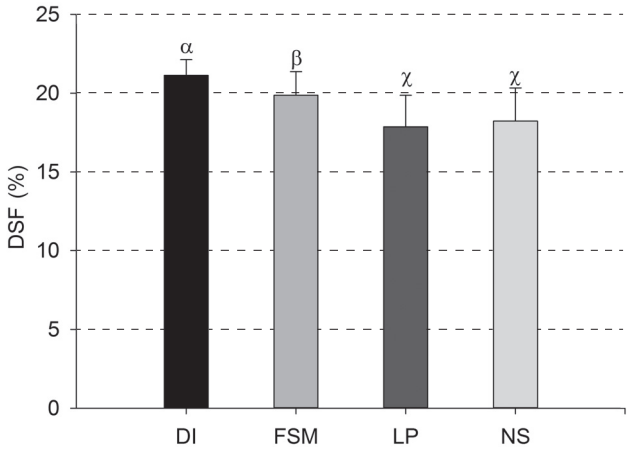

FIGURE 4. Coalescence of dairy fat globules in ice cream as affected by *L. plantarum* YW11 added by DI, direct inoculation of strain YW11 pre-cultured in MRS broth (1/10 volume of mix) at 37°C for 16 h; FSM, addition of 1/10 volume of strain YW11 fermented skim milk; LP, addition of the lyophilized powder of strain YW11 (1 g/L); NS, no addition of the strain.α, β, χ represent significant differences ($p<0.05$).

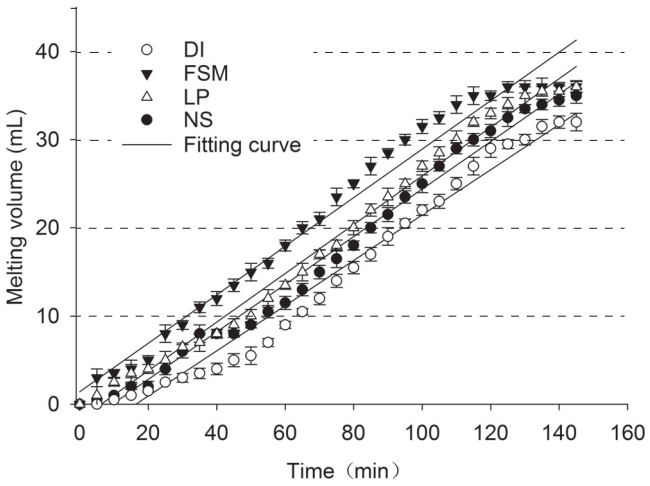

FIGURE 5. Melting behavior of ice cream incorporated with *L. plantarum* YW11 by DI, direct inoculation of strain YW11 pre-cultured in MRS broth (1/10 volume of mix) at 37°C for 16 h; FSM, addition of 1/10 volume of strain YW11 fermented skim milk; LP, addition of the lyophilized powder of strain YW11 (1 g/L); NS, no addition of the strain. Values of the same time point in different test groups are significantly different.

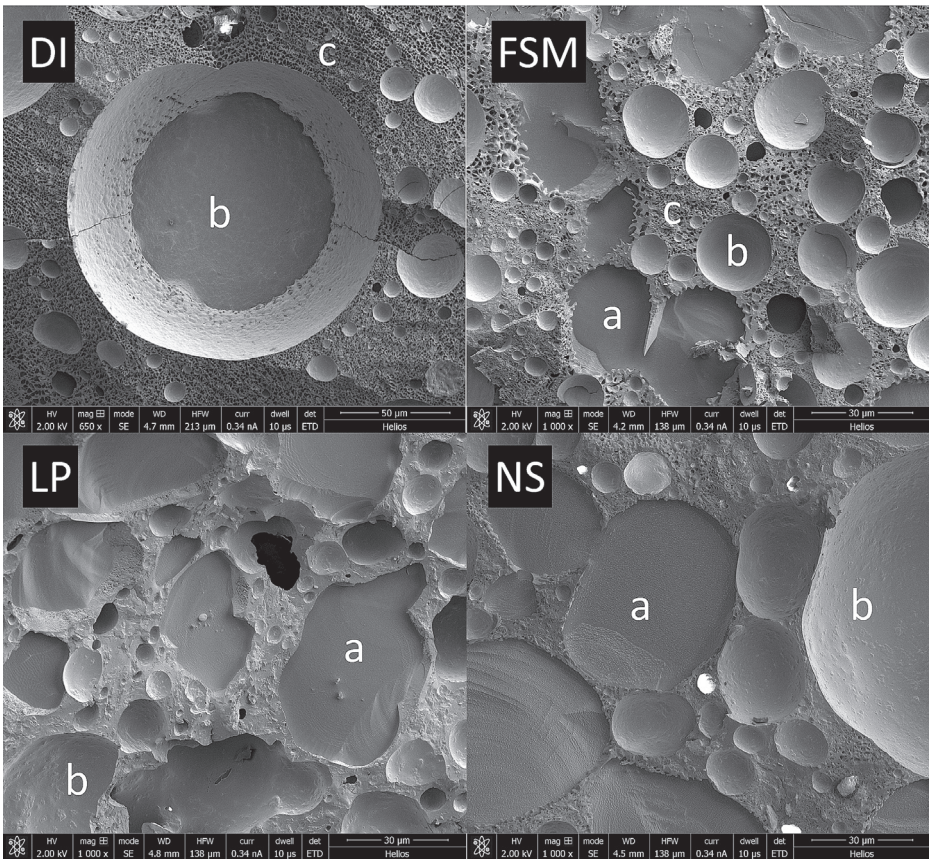

FIGURE 6. Cryo-SEM micrographs of ice cream incorporated with *L. plantarum* YW11 by different methods: DI, direct inoculation of strain YW11 pre-cultured in MRS broth (1/10 volume of mix) at 37°C for 16 h; FSM, addition of 1/10 volume of strain YW11 fermented skim milk; LP, addition of the lyophilized powder of strain YW11 (1 g/L); NS, no addition of the strain. a, ice crystals; b, air bubbles; c, EPS structure.

lar projection length on the horizontal axis, indicating a strong correlation between F1 and the test groups. The result suggested that the addition and fermentation of strain YW11 did not change the basic flavor of the ice cream. Mohammadi *et al.* [2011] also reported that supplementing ice cream with lactic acid bacteria had little effect on its flavor. However, the scatter direction and projection length from DI and FSM samples were significantly different from those of the LP and NS samples on the F2 axis, with the DI sample having the longest positive projection length on the F2 axis. These results suggest that although the basic flavors of the ice cream samples of all groups were similar, the difference in the degree of ice cream fermentation brought about by the different methods of adding the YW11 strain still has an effect on ice cream flavor.

CONCLUSION

To examine the effect of addition of EPS-producing *L. plantarum* YW11 on ice cream and its viability and functionality during ice cream processing and storage, the YW11 strain was incorporated into ice cream *via* the DI, FSM, and LP methods. The viability of strain YW11 after ice cream processing and storage was found to decrease in the order of hardening>aging>freezing>storage. However, the ice cream of all groups had viable counts higher than 6 log cfu/g. Production of a ropy EPS by strain YW11 seemed to play a favorable role in modifying the physicochemical properties of ice cream, including its viscosity, firmness, overrun, destabilized fat, and meltdown behavior of ice cream. The presence of EPS in the ice

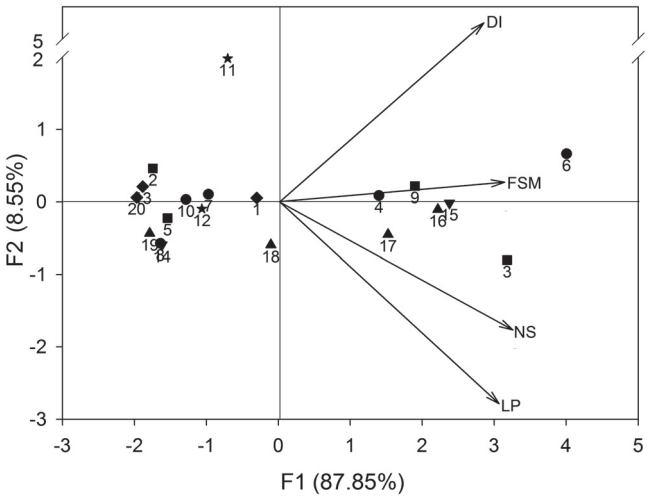

FIGURE 7. Plots by principal component analyses of mean values for ketones (●), hydrocarbons (■), benzenes (▲), alcohol(▼), free fatty acids (★), aldehyde, ester and sulfur compound(♦). DI, direct inoculation of *L. plantarum* YW11 pre-cultured in MRS broth (1/10 volume of mix) at 37°C for 16 h; FSM, addition of 1/10 volume of strain YW11 fermented skim milk; LP, addition of the lyophilized powder of strain YW11 (1 g/L); NS, no addition of the strain.

cream mix improved the microstructure of ice cream through formation of a fine porous fabric matrix as observed by Cryo-SEM. GC-MS and PCA analysis showed that the main volatiles in the ice cream samples were hydrocarbons, ketones, and benzenes. Although incorporation of the YW11 strain did not change the basic flavor of the ice cream, fermentation by the strain played a role in reducing the formation of dodecane with a smell of dirt, thus improving the sensory characteristics of ice cream. The results of this study indicate that strain YW11, when incorporated properly, *e.g.* by the DI method, could survive well during ice cream processing and storage. However, strain protection requires further investigation in order to maintain its tolerance to acid and bile, and to survive passing through the intestine when consumed.

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