Effects of oligosaccharides on the fermentation properties of *Lactobacillus plantarum*

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ABSTRACT

In the present work, we studied the effects of different oligosaccharides on *Lactobacillus plantarum* ATCC14917, focusing on growth and adhesion characteristics and fermented milk flavor. The results showed that mannan-oligosaccharide (MOS) had the greatest proliferative effect on *L. plantarum* ATCC14917 in vitro. In terms of adhesive properties, the autoaggregation rate of *L. plantarum* cultured in MOS was 23.76%, adhesion to mucin was 24.65%, and adhesion to Caco-2 cells was 14.71%. These results for *L. plantarum* cultured with MOS were higher than those for *L. plantarum* cultured in fructo-oligosaccharides (FOS) or galacto-oligosaccharides (GOS). Furthermore, the surface consistency and viscosity scores of fermented milk of the MOS group was higher than that of milks cultured with FOS or GOS, although MOS had the lowest scores for fermented milk flavor.

Key words: oligosaccharides, *Lactobacillus plantarum*, growth characteristics, adhesion characteristics, volatile organic component

INTRODUCTION

Lactic acid bacteria (LAB) are widely distributed in nature and have abundant species diversity. The LAB can produce large amounts of lactic acid from fermentable carbohydrates and play an important role in animals by maintaining normal function and establishing host resistance to pathogenic microorganisms in the intestinal tract (Hooper and Gordon, 2001; Bäckhed et al., 2005). Probiotics can also improve the physiological

balance of the gastrointestinal tract by adhering to intestinal epithelial cells and colonizing the intestine, and thus have beneficial effects on the host (Fuller, 1991). *Lactobacillus plantarum* has good probiotic properties and it can tolerate the simulated digestive tract environment. It can metabolize and synthesize bacteriocins, which have a strong inhibitory effect on the growth of gram-positive and gram-negative bacteria. The LAB are also used in the initial culture or as part of the microbiota for the fermentation of milk and other dairy products (Spano and Massa, 2006). Because *Lactobacillus plantarum* produces bacteriocins that render it resistant to acid and heat, it is often used for food fermentation (Gong et al., 2010).

Because foods with low or no sugar content have become an irreversible trend in the global food industry, oligosaccharides have attracted increasing attention because of their prebiotic potential, and they are widely used as a food ingredient in cereal foods and health foods, especially those for infants and young people (Vandenplas, 2002). Indigestible oligosaccharides act as substrates specifically for a host's intrinsic probiotic bacteria, and thus encourage their growth (Gibson and Roberfroid, 1995; Gibson et al., 2010). Oligosaccharides can improve blood lipid metabolism, regulate gastrointestinal function, prevent and treat constipation, increase vitamin synthesis, and improve human immunity. Oligosaccharides can also be used as a protective agent when bacteria encounter changes of temperature, pH, and other growth conditions (Chen et al., 2007). The synergistic benefits of prebiotics and probiotics could enhance and extend the therapeutic and nutritional benefits of fermented dairy products.

At present, studies on the utilization of oligosaccharides by LAB mainly focus on their use as all or part of a carbon source to analyze their growth-promoting effects or on the ability of LAB to cope with stress conditions. Rycroft et al. (2001) reported that xylo-oligosaccharide and fructo-oligosaccharides (FOS) are good sources of carbohydrates and can increase the

Received July 19, 2018.

Accepted December 10, 2018.

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number of lactobacilli. Some researchers have found that these oligosaccharides can reduce the damage of simulated gastrointestinal fluids and freeze-dried LAB, increasing their survival rate (Corcoran et al., 2004; Pan et al., 2009). Kim et al. (2001) found that the increased resistance to heat shock, oxidation, penetration, and acid stress in *Lactobacillus* was mainly attributed to structural and physiological changes, including expression levels of stress-related proteins, membrane composition, and cell wall structure (Kim et al., 2001; Prasad et al., 2003). From a consumer's point of view, flavor and health-promoting benefits are important features of fermented milk products. Flavor-related volatile organic compounds (VOC) determine the diverse flavor properties of fermented milk, and GC-MS allows detection of these components at very low quantities. However, few reports have described the functional properties of oligosaccharides, such as intestinal colonization of *Lactobacillus* or their influence on the flavor of fermented milk.

In this study, we investigated the effects of galacto-oligosaccharides (GOS), FOS, and mannan-oligosaccharides (MOS) on the in vitro adhesion of *L. plantarum* ATCC14917. We analyzed differences in the flavor of fermented milks prepared using different oligosaccharides by use of an electronic nose and electronic tongue, GC-MS, and sensory evaluation. We aimed to determine the role of different oligosaccharides in adhesion and fermentation characteristics of *L. plantarum* to evaluate the potential role of oligosaccharides in probiotic fermented dairy products.

MATERIALS AND METHODS

Bacterial Strains and Culture Conditions

Lactobacillus plantarum ATCC 14917 (CGMCC 1.2437) was purchased from the China General Microbiological Culture Collection Center (Beijing, China) and grown statically at 37°C in modified de Man, Rogosa, and Sharpe (MRS) medium. The reagents used to prepare modified MRS medium were as follows (per liter of distilled water): carbohydrates, 20 g; proteose peptone (Oxoid, Basingstoke, UK), 10 g; beef extract, 8 g; yeast extract (Oxoid), 4 g; $K_2HPO_4 \cdot 3H_2O$, 2 g; $CH_3COONa \cdot 3H_2O$, 5 g; ammonium monohydrogen citrate, 2 g; $MgSO_4 \cdot 7H_2O$, 0.2 g; $MnSO_4 \cdot 4H_2O$, 0.04 g; and Tween 80, 1 mL (Fluka, Buchs, Switzerland).

Caco-2 cells were purchased from the Boster Biological Technology Co. Ltd. (Pleasanton, CA), cultured at 37°C in a gas mixture containing 5% CO_2 , and grown in Dulbecco's modified Eagle's medium (Sigma, Darmstadt, Germany) containing 20% (vol/vol) fetal bovine

serum and 1% (vol/vol) antibiotics (100 U·mL⁻¹ penicillin, 100 mg·mL⁻¹ streptomycin).

Growth Characteristics of *L. plantarum*

Three oligosaccharides were investigated for their possibility as a source of energy for lactobacilli: FOS and MOS were purchased from Solarbio Science & Technology Co. Ltd. (Beijing, China), and GOS was obtained from Source Leaf Biotech (Shanghai, China). *Lactobacillus plantarum* was cultured at 37°C in modified MRS medium in which glucose was replaced by the same amount of each of the oligosaccharides. Growth was measured at 600 nm and growth curves prepared; acid production was plotted by measuring pH every 3 h for 24 h and an acid production curve prepared.

Autoaggregation Assay

The autoaggregation assay was performed according to Del Re et al. (2000) with some modifications. *Lactobacillus plantarum* cells were grown for 18 h at 37°C in modified MRS medium with oligosaccharides, harvested by centrifugation at $6,000 \times g$ for 10 min, washed twice, and resuspended in PBS. Then, the suspension was adjusted to an optical density value of 1 ± 0.02 at 600 nm (A_0). Bacterial suspensions (4 mL) were mixed thoroughly and absorbance was measured at 600 nm after 4 h of incubation at room temperature (A_4). The autoaggregation rate was calculated as follows: Autoaggregation rate = $1 - A_4/A_0$, where A_4 and A_0 represent absorbance values at 4 and 0 h, respectively.

Adhesion Activity In Vitro

Experiments to measure the adhesion of *L. plantarum* ATCC 14917 to mucin were based on Izquierdo et al. (2009) with some modifications. Freshly grown bacteria were labeled with 100 μM carboxyfluorescein diacetate (CFDA) in PBS (37°C, 30 min). Adhesion was expressed as the percentage of fluorescence recovered after binding to mucin relative to the total fluorescence added to each well, corresponding to 200 μL of labeled bacteria. Adhesion to Caco-2 cells was evaluated according to Celebioglu et al. (2017) with some modifications, as previously described. Cultured cells (2 mL) were seeded in antibiotic-free 6-well tissue culture plates and incubated at 37°C overnight. They were then incubated with CFDA-labeled bacteria (2 mL) for 2 h at 37°C. Unbound bacteria were discarded and washed 3 times with PBS, and the bound cells were lysed by using trypsin. Fluorescence was measured and the percentage of adherent bacteria was calculated. Three

independent experiments were conducted in each test, each in quadruplicate, and data were analyzed using SPSS software (version 14.0; SPSS Inc./IBM Corp. Chicago, IL).

Expression of Adhesion Factors

Total RNA was extracted from *L. plantarum* (stationary phase, 18 h) using a Bacterial RNA kit (Omega Bio-Tek Inc., Norcross, GA) according to the kit instructions. Reverse transcription of RNA was performed using the TransScript All-in-One First-Strand cDNA Synthesis Super Mix kit (One-Step gDNA Removal, TransGen Biotech, Beijing, China), according to the manufacturer's instructions. Gene expression levels were determined by quantitative (q)PCR and performed according to the instructions in the TransStart Tip Green qPCR SuperMix Kit (TransGen Biotech) and quantified by using a LightCycler 96 (Roche, Basel, Switzerland). The qPCR cycle threshold (CT) results were modified using the comparative CT method ($2^{-\Delta\Delta CT}$ method (Wagner, 2013)). The genes encoding adhesion-associated proteins of *L. plantarum* ATCC 14917 were mannose-specific adhesion (*msa*), mucin-binding proteins (*mub1*, *mub2*, and *mub3*), lipoprotein signal peptidase (*lspA*), and elongation factor thermal instability EF-Tu (*tuf*).

Fermentation Process

The mother culture was prepared using *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus thermophilus* in a 1:1:1 combination with each *L. plantarum* strain anaerobically at 37°C for 10 h, and maintained at 4°C until the milk fermented (within 48 h). Before flavor analysis and sensory evaluation, the mother culture was added to fresh pasteurized milk (6% inoculum); fermentation was performed at 42°C for 6 h and post-ripening at 4°C for 18 h. To achieve the different fermentations, 5% GOS, 5% FOS, or 5% MOS was added to each milk sample during milk fermentation.

Electronic Nose and Tongue Analysis

The smell and taste of the fermented milks were analyzed by using an electronic nose and electronic tongue, respectively. For smell, samples were taken from fermented milk containing the 3 oligosaccharides (FOS, GOS, and MOS), glucose, and fresh commercial milk. Five milliliters of each sample was placed in a 25-mL glass vial with a silicone cap and then introduced into an electronic nose sampling device (Yu and Wang, 2007; Mamat et al., 2011). Linear discriminant analysis

(LDA) and principal component analysis (PCA) of the major VOC of various fermented milk were performed using the Airsense PEN 3 electronic nose (Airsense Analytics GmbH, Schwerin, Germany).

For taste, the prepared fermented milks were homogenized and poured into an electronic tongue special cup. Using the Astree II electronic tongue system (Isenso, New York, NY) with an Ag/AgCl reference electrode, data acquisition was performed at room temperature. Before data acquisition, the electronic tongue system was passively activated, calibrated, and diagnosed, and other steps were performed to ensure that the collected data were reliable and stable. After homogenizing the fermented milk, 25 mL was poured directly into an electronic tongue special cup for testing. Ultra-pure water was used as the cleaning solvent. Five replicates were selected and 3 stable data points were selected for analysis. The instrument includes software for PCA and discriminant factor analysis (Fung et al., 2009). Ten trained panelists were invited and asked to rate samples for firmness, sweetness, sourness, astringent, and butter flavor using a 10-point intensity scale, scoring attributes as low (1–3), medium (5), or high (8–10) intensity for each fermented milk product.

GC-MS Analysis and Sensory Evaluation

Volatile organic compounds in fermented milk were identified by solid-phase microextraction (SPME)-GC-MS. Divinylbenzene/carboxen on polydimethylsiloxane on a StableFlex fiber (DVB/CAR/PDMS) SPME (Supelco, Bellefonte, PA) was performed according to Roberts et al. (2000) with some modifications. A Vocol capillary column was used for the GC-MS analysis (60 m × 0.32 mm × 1.8 µm, Supelco).

The constant flow rate of the carrier gas (helium) was 3 mL/min, with an inlet temperature of 210°C and splitless injection mode. The initial temperature of the column oven was 40°C, which was maintained for 3 min, increased to 140°C at 4°C/min, held for 1 min, and then increased to 200°C at 10°C/min for 20 min. The signal acquisition of the mass spectra was in full scan mode, ionization method EI, electron bombardment energy of 70 eV, interface temperature of 220°C, ion source temperature of 230°C, quadrupole rod temperature of 150°C, scan mass range of m/z 40 to 600, and scan frequency of 3.6 scans/s.

For the sensory evaluation, 10 trained panelists were recruited and asked to taste the samples in the order given and to evaluate the appearance, taste, and aroma of the fermented milk samples. For each test, replicates were conducted to improve the power of analysis and detect true discriminators. Sensory attributes were

evaluated using a scale of 1 to 9, scoring attributes as low (1–3), medium (5), and high (8–9) intensity for each fermented milk product.

Data Analysis

All statistical analyses were performed using SPSS (SPSS Inc./IBM Corp, Chicago, IL) and Origin 8.5 (OriginLab, Northampton, MA) software. The differences between the mean values of the groups were analyzed using a one-way ANOVA with Duncan's multiple range test; P -values < 0.05 were considered significant.

RESULTS

Growth Characteristics of *L. plantarum* Influenced by Different Oligosaccharides

The growth characteristics of *L. plantarum* in the presence of 3 oligosaccharides were observed for 24 h, and viable counts were taken every 3 h to prepare a bacterial growth curve. As shown in Figure 1, when *L. plantarum* was cultured with different oligosaccharides, cell density increased with culture time and the pH of the culture supernatant decreased. Thus, we demonstrated that all 3 oligosaccharides could be used as a carbon source for *L. plantarum*. The growth rate of *L. plantarum* was lower when cultured in oligosaccharides than when grown in glucose. This may be because glucose is a monosaccharide and most suitable for the growth of LAB.

Adhesion Ability of *L. plantarum*

As shown in Figure 2A, *L. plantarum* cultured in MOS exhibited the highest rate of autoaggregation (23.76%), followed by *L. plantarum* cultured in FOS (21.10%) and GOS (20.64%), but the autoaggregation rate did not differ significantly among the groups ($P > 0.05$). Figure 2B and Figure 2C show that *L. plantarum* cultured with MOS showed the highest ($P < 0.05$) adhesion to mucin (24.65%) and to Caco-2 cells (14.71%), compared with the other oligosaccharide groups, followed by *L. plantarum* cultured in FOS (14.64 and 11.21% for adhesion to mucin and Caco-2 cells, respectively). *Lactobacillus plantarum* cultured in GOS showed weaker adhesion ability (8.60% to mucin and 6.23% to Caco-2 cells).

Expression of Adhesion Factors

The expression of adhesion factors in *L. plantarum* cultured with different oligosaccharides is shown in Fig-

ure 3. The expression of all adhesion-associated genes and the elongation factor thermo-unstable *EF-Tu* was significantly higher in *L. plantarum* cultured in MOS compared with that grown in the FOS and GOS treatments. The expression of adhesion factors *mub2*, *msa*, and *lspa* was higher in the GOS group than in FOS, with the expression of *msa* being significantly different. The expression of *mub1*, *mub3*, and *tuf* was higher in FOS than in GOS, with the expression of *tuf* being significantly different.

Figure 1. Growth kinetics of *Lactobacillus plantarum* fermented with different oligosaccharides, as measured by optical density (OD) values (A) and pH changes (B) of *L. plantarum* ATCC 14917 cultured with glucose (Glu; control), galacto-oligosaccharide (GOS), fructo-oligosaccharide (FOS), or mannan-oligosaccharide (MOS) for different culture times.

Figure 2. Adhesion properties of *Lactobacillus plantarum* under treatment with different oligosaccharides: galacto-oligosaccharide (GOS), fructo-oligosaccharide (FOS), or mannan-oligosaccharide (MOS). Autoaggregation (A) of *L. plantarum* after culture with different oligosaccharides, and adhesion of *L. plantarum* to (B) mucin and (C) Caco-2 cells. Bars with different letters (a, b, c) differ significantly ($P < 0.05$). Error bars represent SD.

Sensory Evaluation and Correlation Analysis of Fermented Milk

The smell and taste of fermented milk were detected by electronic nose and electronic tongue, respectively, and the effects of different oligosaccharides on the flavor of fermented milk were analyzed by PCA and LDA. Figure 4A shows that the contribution to variance of principal component (PC)1 was 77.82%, and that of PC2 was 21.56%; thus, PC1 and PC2 together accounted for 99.38% of the total data difference. The differences between oligosaccharide groups can be seen in Figure 4A and Figure 4B. The results of the electronic tongue analysis are shown in Figure 5; PC1 contributed 56.54% of the total variance and PC2 contributed 29.38%, for a cumulative contribution of 85.92%, which reflects most of the information in the whole sample. The discriminant index for the PCA was 94.56, indicating that discrimination between different fermented milk samples was good. The discriminant index value for the LDA was 96.92, again reflecting that the different fermented milk products were well differentiated. From the results of the sensory evaluation (Figure 6), we can see that the sweetness, acidity, and creamy feeling scores of the fermented milk in the MOS group were the lowest, and the creamy feeling score in the GOS group was higher than that in the FOS group. The sweetness and acidity scores of the GOS group were close to those of the Glu and FOS groups. The fermented milk of the MOS group had the highest scores for surface consistency and viscosity.

GC-MS Analysis

Fermented milk contains a complex mixture of VOC; however, only a few have important effects on flavor. In the present study, we used SPME-GC-MS to identify the VOC present in fermented milk and to evaluate their distribution in different groups of fermented milk samples. Table 1 summarizes the main VOC and their contents. The flavor substances detected in the samples were divided into 9 categories: 3 acids, 7 alkanes, 4 ketones, 3 esters, 4 benzenes, 2 aldehydes, 2 olefins, 1 ether, and 1 hetero compound. As shown in Table 1, 22 flavor compounds were detected in the GOS and glucose groups, 23 in the FOS group, and 19 in the MOS group. The main flavor components in each group were 2-heptanone, 2-nonanone, and hexanoic acid. 2,3-Butanedione, an important flavor component, was detected in the GOS group. The contents of these major flavor compounds were significantly higher in the GOS and glucose groups than in the FOS and MOS groups.

DISCUSSION

Studies have shown that oligosaccharides can promote the proliferation of LAB in vitro to varying degrees (Gopal et al., 2001; Mital and Steinkraus, 2010). However, because LAB may lack an enzyme system that metabolizes oligosaccharides, not all oligosaccharides can be utilized by LAB (Ward et al., 2006; Lee et al., 2008). We found that *L. plantarum* could grow in medium containing oligosaccharides (GOS, FOS, MOS), indicating that the *L. plantarum* used in this study has a protease system that can hydrolyze and transport oligosaccharides. The 3 oligosaccharides tested had different effects on the growth of LAB at different growth stages. In the logarithmic phase, the growth rate of *L. plantarum* was greatest in the FOS group. Carbohydrates are transported and concomitantly phosphorylated by the phosphotransferase system (PTS). *Lactobacillus plantarum* can metabolize a diversity range of carbon sources, including all major categories of oligosaccharides, but FOS are highly preferred substrates (Gänzle and Rainer, 2012). Some research has suggested that the association of a sucrose PTS and a β -fructofuranosidase could be used for short-chain FOS degradation in *L. plantarum* (Saulnier et al., 2011). However, fewer in-depth studies have examined the mechanism by which *L. plantarum* utilizes GOS and MOS.

Bacterial adhesion to intestinal epithelial cells or mucus through biochemical action is a key process for the probiotic action of LAB in the intestinal tract, which can provide organisms a competitive advantage in this ecosystem (Xu et al., 2009; Kotzamanidis et al., 2010). The adhesion of LAB to intestinal cells is directly or indirectly related to bacterial surface components such as lipoteichoic acid, surface layer proteins, polysaccharide, and whole peptidoglycan (Servin and Coconnier, 2003). Surface proteins are important factors influencing the adhesion of LAB. Mucin-binding protein (MUB) was first discovered in *Lactobacillus reuteri* 1063 and *Lactobacillus acidophilus* NCFM (Roos and Jonsson, 2002; Buck et al., 2005), and has a sortase-dependent LPXTG anchor motif. In this study, expression of the genes encoding MUB in the MOS group was significantly higher than that in GOS and FOS groups. Many environmental factors, such as pH, temperature, and oxygen availability, have potential roles in the adhesion characteristics of *Lactobacillus* strains by affecting the expression of surface proteins in the cell membrane (Granato et al., 1999; Garrote et al., 2004). Some research has found that the carbon sources cellobiose and polydextrose could increase probiotic adhesion to intestinal cells and mucin (Celebioglu et al., 2017). This might explain why adhesion proteins were differentially expressed in an environment of complex carbon sources

Figure 3. Expression of adhesion factors (*mub1*, *mub2*, *mub3*, *msa*, *lspa*, and *tuf*) in different oligosaccharide groups: galacto-oligosaccharide (GOS), fructo-oligosaccharide (FOS), or mannan-oligosaccharide (MOS). Bars with different letters (a, b, c) differ significantly ($P < 0.05$). Error bars represent SD.

Figure 4. Principal components analysis (A) and linear discriminant analysis (B) of the major volatile organic compounds, measured using an electronic nose, of milk fermented with glucose (Glu; control), galacto-oligosaccharide (GOS), fructo-oligosaccharide (FOS), or mannan-oligosaccharide (MOS). The points within the ellipses represent the same set of data points, and the 2 ellipses indicate intra-group differences. Data points in the same ellipse had small differences and good repeatability.

in the current study. Human diet has some effect on microbial colonization and relative abundance in the gastrointestinal tract, which is why food intake can maintain bacterial storage in the mucus layer. The intake of different oligosaccharides by humans could influence the homeostasis of microorganisms in the epithelial tract surface (Donaldson et al., 2016). Our research showed that when *L. plantarum* was cultured with different oligosaccharides, its adhesion characteristics changed, and *L. plantarum* cultured in MOS had significantly greater adhesion ability than the other groups. We also showed that MOS affected adhesion characteristics through high expression of adhesion factor genes and a variety of LAB surface proteins.

To further explore the use of oligosaccharides in fermented food products, we evaluated the effects of different oligosaccharides on the flavor of fermented milk. The main aroma compounds in fermented milk with different oligosaccharides were 2-heptanone, 2-nonanone, and caproic acid. More than 90 flavor compounds have been identified so far in fermented milk, including alcohols, aldehydes, ketones, acids, esters, sulfur compounds, and furan derivatives (Ziadi et al., 2008; Settachaimongkon et al., 2014). Acids and ketones are

Figure 5. Principal components analysis (A) and linear discriminant analysis (B) of the major taste compounds, measured using an electronic tongue system, of milk fermented with glucose (Glu; control), galacto-oligosaccharide (GOS), fructo-oligosaccharide (FOS), or mannan-oligosaccharide (MOS). DI = discriminant index.

Table 1. Volatile organic compound analysis of fermented milk samples fermented with different oligosaccharides¹

Volatile organic compound	Retention time (s)	Content (mg/kg; mean $\pm$ SD)			
		Glu	GOS	FOS	MOS
(Acridin-9-ylamino)-acetic acid, ethyl ester	3,275			0.36 $\pm$ 0.04	0.85 $\pm$ 0.20
Octanoic acid	2,102	17.53 $\pm$ 2.77 ^{ab}	21.35 $\pm$ 8.29 ^b	11.01 $\pm$ 0.63 ^a	12.65 $\pm$ 2.23 ^a
2-Nonanone	2,006	66.64 $\pm$ 4.95 ^b	95.75 $\pm$ 5.59 ^c	56.11 $\pm$ 1.06 ^a	52.52 $\pm$ 5.04 ^a
Benzene, (octyloxy)-	1,998	1.09 $\pm$ 0.45	—	0.77 $\pm$ 0.10	0.37 $\pm$ 0.22
3,3-Diethylheptadecane	1,922	1.70 $\pm$ 0.39	—	1.53 $\pm$ 0.68	1.72 $\pm$ 0.14
Bicyclo[2.2.1]heptane, 7,7-dimethyl-2-methylene	1,874	1.03 $\pm$ 0.33 ^{ab}	2.51 $\pm$ 0.21 ^c	1.57 $\pm$ 0.36 ^b	0.90 $\pm$ 0.23 ^a
Benzaldehyde	1,847	3.41 $\pm$ 0.54 ^b	3.64 $\pm$ 0.36 ^b	2.56 $\pm$ 0.14 ^a	2.16 $\pm$ 0.18 ^a
Ether, hexyl <i>t</i> -butyl	1,793	1.56 $\pm$ 0.27 ^c	1.02 $\pm$ 0.19 ^b	1.10 $\pm$ 0.17 ^b	0.37 $\pm$ 0.02 ^a
Hexanoic acid	1,690	38.41 $\pm$ 3.01 ^b	56.44 $\pm$ 10.74 ^c	23.61 $\pm$ 5.22 ^a	27.29 $\pm$ 1.67 ^{ab}
Decane	1,677	1.58 $\pm$ 0.21 ^b	1.33 $\pm$ 0.28 ^b	1.44 $\pm$ 0.06 ^b	0.88 $\pm$ 0.08 ^a
2-Heptanone	1,516	122.70 $\pm$ 3.06 ^b	157.4 $\pm$ 10.88 ^c	95.63 $\pm$ 1.52 ^a	97.73 $\pm$ 2.23 ^a
<i>p</i> -Xylene	1,467	5.99 $\pm$ 0.49 ^c	4.28 $\pm$ 0.62 ^b	5.10 $\pm$ 0.68 ^{bc}	1.50 $\pm$ 0.07 ^a
Ethylbenzene	1,452	2.37 $\pm$ 0.26	1.71 $\pm$ 0.32	2.07 $\pm$ 0.34	—
Oxime-, methoxy-phenyl-	1,432	1.72 $\pm$ 0.29 ^{bc}	2.45 $\pm$ 0.49 ^c	0.91 $\pm$ 0.06 ^a	1.18 $\pm$ 0.56 ^{ab}
2,3,3-Trimethyl-1-hexene	1,267	2.35 $\pm$ 0.15 ^b	3.04 $\pm$ 0.30 ^c	2.46 $\pm$ 0.28 ^b	1.74 $\pm$ 0.33 ^a
Toluene	1,162	7.03 $\pm$ 0.46 ^c	4.57 $\pm$ 0.09 ^b	5.06 $\pm$ 0.05 ^b	3.31 $\pm$ 0.25 ^a
1,2,4-Benzenetricarboxylic acid, 1,2-dimethyl ester	923	—	9.88 $\pm$ 1.05	6.52 $\pm$ 0.43	—
Butanal	665	1.16 $\pm$ 0.23	—	0.17 $\pm$ 0.02	0.39 $\pm$ 0.10
<i>n</i> -Hexane	566	41.30 $\pm$ 2.91 ^b	54.20 $\pm$ 5.48 ^c	6.79 $\pm$ 0.59 ^a	8.07 $\pm$ 0.91 ^a
2-Ethyl-oxetane	553	—	—	19.32 $\pm$ 3.71	—
Acetone	441	2.43 $\pm$ 0.15 ^a	7.69 $\pm$ 1.62 ^c	3.99 $\pm$ 0.50 ^b	1.88 $\pm$ 0.64 ^a
Heptane, 2,2,4,6,6-pentamethyl-	1,655	—	—	—	1.15 $\pm$ 0.29
Acetic acid	611	23.32 $\pm$ 1.99	19.65 $\pm$ 1.70	0.26 $\pm$ 0.01	—
Ammonium acetate	589	11.78 $\pm$ 3.00	3.42 $\pm$ 0.29	3.23 $\pm$ 0.39	—
2,3,8-Trimethyl-1H-pyrrolo[3,2-H]quinoline-7-carboxylic acid ethyl ester	2,244	—	0.74 $\pm$ 0.23	—	—
Eicosane, 9-octyl-	2,093	0.83 $\pm$ 0.20	1.47 $\pm$ 0.45	—	—
2-Ethyl-5-tridecylpyrrolidine	2,029	1.11 $\pm$ 0.52	1.05 $\pm$ 0.13	—	—
2,3-Butanedione	649	—	1.64 $\pm$ 0.43	—	—

^{a-c}Means with different letters indicate significant differences ($P < 0.05$).

¹Glu = glucose; GOS = galacto-oligosaccharide; FOS = fructo-oligosaccharide; MOS = mannan-oligosaccharide.

important flavor compounds in fermented milk and contribute to its unique flavor. Other flavor substances such as acetic acid and diacetyl were at barely detect-

able levels in the FOS and MOS groups. This may be due to the influence of oligosaccharides on carbohydrate metabolism in the starter cultures, which leads to lower contents of flavor compounds in these products (Goh and Klaenhammer, 2015). Although MOS had beneficial effects on growth and adhesion characteristics of *L. plantarum* and on the texture of fermented milk, it did not have a favorable effect on fermented milk in terms of flavor and taste compared with the GOS group. The effects of MOS on fermented milk texture may be due to the use of MOS by *L. plantarum* to produce extracellular polysaccharides, including capsular polysaccharides and mucopolysaccharides, which adhere to the cell surface to promote stickiness and increase the viscosity of fermented milk (Yang et al., 2010; Jiaojiao et al., 2015). The negative effects of MOS on flavor and taste of fermented milk may be related to its low sweetness, and the lesser contribution to the flavor formation (Shi et al., 1999).

Figure 6. Sensory evaluation of fermented milk products fermented with different oligosaccharides. Scores of 1 to 3 indicate low, scores of 5 to 7 medium, and scores of 8 to 10 indicate high intensity for attributes of milk fermented with glucose (Glu; control), galacto-oligosaccharide (GOS), fructo-oligosaccharide (FOS), or mannan-oligosaccharide (MOS).

CONCLUSIONS

A carbon source is critical for probiotic bacteria to grow and release energy for adhesion to the gastroin-

testinal tract. Oligosaccharides serve as prebiotics for intestinal microbes and can selectively stimulate the growth and activity of beneficial microbial strains residing in the host gastrointestinal tract. The effects of different oligosaccharides on growth and adhesive properties of *L. plantarum* are varied. *Lactobacillus plantarum* cultured with MOS may have potential because of the greater expression of adhesion factors and abundance of surface proteins. Furthermore, the surface consistency and viscosity score of the fermented milk in the MOS group was superior, although it had the lowest flavor scores. It is necessary to further study the flavor of fermented milk and adhesion characteristics of *L. plantarum* under different sugar combinations based on prebiotic MOS.

ACKNOWLEDGMENTS

This work was supported by the Natural Science Funding of China (31671869, 31471598, and 31601487), the Natural Science Funding of Zhejiang (LY19C200005), the Science and Technology Bureau of Ningbo (2016C10022), and the K. C. Wong Magna Fund in Ningbo University.

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