The fermentation characteristics of soy yogurt with different content of D-allulose and sucrose fermented by lactic acid bacteria from *Kimchi*

Hee Jin Kim¹ · Myung Joo Han¹

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Abstract In this study, to understand whether D-allulose, an ultra-low calorie sweetener, was available in soy yogurt fermentation, we isolated *Leuconostoc mesenteroides* and *Lactobacillus plantarum* from kimchi and fermented in soymilk at various contents of D-allulose and sucrose. The lactic acid bacteria counts in soy yogurt had the highest range of 9.23–9.49 log CFU/g at 24 h fermentation and then decreased. At 48 h fermentation, the pH showed 4.31 and 4.52 in the samples containing 75% and 100% D-allulose as sweetener. DPPH radical scavenging activity showed a decreasing tendency as the amount of D-allulose increased. Soy yogurt samples containing D-allulose had higher scores in sweet taste, sour taste and overall preference in sensory evaluation. These findings suggest that D-allulose is beneficial for the development of a low calorie soy yogurt.

Keywords Soy yogurt · D-Allulose · *Leuconostoc mesenteroides* · *Lactobacillus plantarum* · Fermentation

Introduction

Soymilk is widely recognized as an alternative beverage of milk because it does not provoke lactose intolerance and allergic reaction by lactose (Lee et al., 2013). However, some people may not prefer soymilk because of its

disagreeable beany flavor (Wang et al., 2006). Therefore, many studies recommended to ferment soymilk using lactic acid bacteria (LAB) to improve its sensory properties (Cha et al., 1990; Jang and Yoon, 1997). Because soybean contains soy oligosaccharide, a low calorie sugar, such as raffinose and stachyose, LAB can ferment soybean products by breaking down soy oligosaccharide and produce lactic acid (Choi et al., 1995). By fermenting soymilk with LAB, it is possible to improve absorption of isoflavones and free amino acids (Lee et al., 2013), and to increase antioxidant activity (Morazza et al., 2012; Zhao and Shah, 2014). They also enhance the flavor and nutritional values of food by breaking down some ingredients such as sugar, protein, and fat (Jung et al., 2016). Kimchi, one of the fermented food in Korea, contains high levels of LAB (about 10^7 – 10^9 CFU/g) (Park et al., 2014). *Leuconostoc mesenteroides* and *Lactobacillus plantarum* are the major LAB contributing to kimchi fermentation (Kim et al., 2017). These kimchi LAB are considered to be bioactive probiotics (Son et al., 2017).

D-Allulose, a C-3 epimer of D-fructose (Hossain et al., 2015), is a non-fermentable component of commercial carbohydrates (Park et al., 2016). Current consumption of sugar and high-fat diets is one of the biggest problems causing metabolic syndromes including obesity and diabetes. However, D-allulose is an ultra-low calorie sweetener and ideal substitute for sucrose with 70% of sweetness, because D-allulose is absorbed in the small intestine and excreted from the body via the urine (Young et al., 2016; Zhang et al., 2016). Accordingly, D-allulose is expected to be used as a medicinal food source for the prevention or treatment of obesity and related lipid disorders (Han et al., 2016).

In the present study, we isolated *Leuconostoc mesenteroides* and *Lactobacillus plantarum* strains from kimchi,

✉ Myung Joo Han
mjhan@khu.ac.kr

Hee Jin Kim
mdcl9393@hanmail.net

¹ Department of Food and Nutrition, Kyung Hee University,
26 Kyungheedaero, Dongdaemoongu, Seoul 02447, Korea

fermented soymilk at various contents of D-allulose and sucrose with *Leuconostoc mesenteroides* and *Lactobacillus plantarum*, and investigated the quality characteristics of soy yogurt and the sensory evaluation results.

Materials and methods

Materials

Soybeans harvested in 2016 were purchased from Suanbo Nonghyub (Korea) and stored at 4 °C until used. Sucrose and D-allulose were purchased from CJ Corp. (Seoul, Korea). The water used to make soymilk was mineral water from DongA-Otsuka Co., Korea.

LAB strains and culture conditions

The LAB strains were isolated from kimchi by using method of Jang (2013). Among them, *Leuconostoc mesenteroides* (LM) and *Lactobacillus plantarum* (LP) were used for the fermentation of soy yogurt. For control group, commercial *Leuconostoc mesenteroides* KCTC13302 and *Lactobacillus plantarum* KCTC33131 were purchased from Korean Collection for Type Cultures. These LAB strains were cultured in MRS broth for 24 h at 30 °C prior to inoculation.

Preparation of soy yogurt

Soybeans were washed and soaked in water for 24 h at 4 °C and then blended with a Halde cutter VCB-61 (Halde Maskiner, Kista, Sweden). Ground soybean mixture was strained through a 200 mesh sieve and then filtered with cotton cloth. Mineral water was added into filtered soymilk until it became 7 °Bx. Soymilk was boiled for 3 min and sweetener was added to it, as indicated in Table 1, and then

cooled until it became 40 °C. Mixed LAB starters were inoculated to 1% soymilk at the level of 10^9 CFU/mL (Lee et al., 2013) and fermented at 30 °C for 96 h and used to analyze the fermentation characteristics every 24 h. The control soy yogurt was prepared by fermenting a soy milk containing only sucrose with commercial LAB (KCTC 13302 and KCTC 33131).

Microbiological analysis

During 96 h fermentation, LAB counts were examined every 24 h. The serially diluted sample (0.1 mL) was inoculated onto de Man Rogosa Sharpe (MRS) agar (Difco., USA) and the plates for LAB were incubated at 30 °C for 48 h. The numbers of LAB strains were counted and presented as log CFU/g.

pH and titratable acidity

Five grams of samples were mixed with 45 mL of distilled water and stirred for 1 min to analyze pH and titratable acidity. The pH of samples was measured 3 times with a digital pH meter (Thermo Orion, USA). In the diluted sample, 2–3 drops of phenolphthalein were added and titrated with 0.1 N NaOH until its pH became to 8.3. The acidity of the soy yogurt was calculated as % lactic acid.

Brix and reducing sugar content

The Brix values of samples were measured 3 times by refractometer (Atago NI, Tokyo, Japan). The reducing sugar content was measured based on di-nitrososalicylic acid (DNS) method (Miller, 1959). One gram of sample was diluted in 49 mL of distilled water and stirred for 1 min. The mixture was centrifuged at $3000\times g$ for 15 min (Vision Sci. Co., Korea) only to use the supernatant of it. One mL aliquot of the supernatant was mixed with 3 mL of DNS solution in the test tube, then heated for 5 min in boiling water bath, and cooled in iced water to stop the reaction. The absorbance was measured at 550 nm using a spectrophotometer (UV-2101, Shimadzu, Kyoto, Japan). The amount of reducing sugar of each sample was calculated from measured absorbance using the glucose standard curve.

DPPH radical scavenging activity

2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity was measured according to the method of Blois with some modifications (Blois, 1958). Two grams of samples were diluted in 2 mL of 80% ethanol and centrifuged at $3000\times g$ for 15 min (Vision Sci. Co., Korea). The 0.5 mL aliquot of the supernatant was mixed with

Table 1 The mixing ratio of ingredients to prepare soy yogurt

Ingredient	Samples ¹					
	A0 ²	A25	A50	A75	A100	CA0
Soymilk (mL)	100	100	100	100	100	100
Sucrose (g)	7	5.25	3.5	1.75	0	7
Allulose (g)	0	1.75	3.5	5.25	7	0

¹A0, A25, A50, A75 and A100 are soy yogurt samples containing allulose with isolated LAB from kimchi (LM and LP). CA0 is a soy yogurt sample containing only sucrose with commercial LAB (KCTC 13302 and KCTC 33131)

²The number means the ratio (%) of allulose in total sweetener amount

1 mL of 0.4 mM DPPH-ethanol solution. The mixture was vigorously shaken and allowed to stand for 30 min at room temperature in the dark. The absorbance was measured at 517 nm using a spectrophotometer (UV-2101, Shimadzu, Kyoto, Japan).

Sensory evaluation

This study was reviewed and approved by the institutional review board (IRB) of the Kyung Hee University (KHSIRB-18-011) to follow bioethics. The sensory evaluation of soy yogurt was conducted in accordance with the rules of IRB. The soymilk was fermented at 30 °C for 48 h and homogenized and kept in a refrigerator at 4 °C for sensory evaluation. The 37 general panelists were recruited and the test was carried out to evaluate color, flavor, sweet taste, sour taste, mouth feel and overall preference of soy yogurt samples using a 7-point hedonic scale (1 = dislike very much; 7 = like very much).

Statistics analysis

All experiments were performed three times and statistical analysis of the data was conducted with SAS 9.4 program. The data were showed as mean $\pm$ standard deviation. One way analysis of variance (ANOVA) was performed to compare multiple group means, followed by Duncan's multiple-range test to determine statistically significant ($p < 0.05$) differences.

Results and discussions

Microbiological analysis

The number of LAB grown in the soy yogurt prepared with different content of D-allulose and sucrose was counted (Fig. 1). The initial LAB counts of the soy yogurt had the range of 6.90–7.04 log CFU/g. After 24 h fermentation, the LAB counts considerably increased in all samples and had the range of 9.27–9.49 log CFU/g. Among all samples, A50 had the highest LAB contents at 24 h of fermentation. And then the LAB counts constantly decreased during 96 h of fermentation. At 96 h, the LAB counts of fermented soy-milk had the range of 7.93–8.50 log CFU/g.

Lee et al. (2013) reported that the number of LAB in soy yogurt was highest at 24 h-fermentation: it was 8.53 log CFU/mL. Similarly, in this study, all samples showed the highest number of LAB at 24 h-fermentation. According to Korean Ministry of Food and Drug Safety (2016), the LAB counts of fermented dairy products have to be above 10^7 CFU/mL. Therefore, it is suggested that the LAB counts of the soy yogurt of this study have

Fig. 1 LAB counts of soy yogurt with different content of D-allulose during 96 h fermentation

suitable numbers during 96 h fermentation. Moreover, the isolated LAB, LM and LP, could be used as an appropriate starter.

pH and titratable acidity

The changes in pH of the soy yogurt during 96 h fermentation are shown in Fig. 2(A). The initial pH of the soy yogurt had the range of 6.83–6.92 and significantly decreased to 4.41–4.57 at 24 h. The pH of all other samples except A100 decreased gradually until 96 h. At 48 h, the pH of A100 was 4.52 and pH of other samples had the range of 4.18–4.31. After 72 h fermentation, the pH of the soy yogurt had the range of 4.11–4.51, and at 96 h, the pH had the range of 4.04–4.56. During 96 h-fermentation, the pH of the soy yogurt was generally high as the content of D-allulose increased. Kimoto-Nira et al. (2016) reported that acid production by LAB strains in milk was suppressed by D-allulose. These results suggest that LAB are not capable of fermenting D-allulose added in soymilk. Chang (2010) reported that pH of the soy yogurt fermented by *Bifidobacterium breve*, *Streptococcus thermophiles* and *Lactobacillus acidophilus* were 4.72, 4.66, and 4.29 at 36 h-fermentation. Jang et al. (2002) mentioned that the suitable pH of yogurt has the range of 3.9–4.2. It is suggested that 48 h-fermentation of our samples showed desirably decreased pH except A75 and A100.

The changes in titratable acidity of the soy yogurt for 96 h-fermentation are in Fig. 2(B). After 48 h-fermentation, the titratable acidity of the soy yogurt had the range of 0.56–1.00%. The titratable acidity of A0, A25, A50, A75 and CA0 increased to 1.02–1.17% at 96 h-fermentation. However, the titratable acidity of A100 was 0.60% at 96 h-

Fig. 2 pH (A) and titratable acidity (B) of soy yogurt with different content of D-allulose during 96 h fermentation

fermentation. These results suggest that D-allulose should be a non-fermentable sugar for LAB and soy milk contain soy oligosaccharide. Overall, as much as the content of D-allulose increases, the titratable acidity of samples showed lower values. Morazza et al. (2009) reported that sucrose can be utilized by LAB because glucose and fructose are the products of sucrose hydrolysis. Bae et al. (2004) suggested that the suitable titratable acidity of yogurt is about 1%. Based on these findings, the soymilk has to be fermented at least for 48 h to be appropriately ingested.

Brix and reducing sugar content

The changes in Brix of the soy yogurt during 96 h fermentation are shown in Fig. 3(A). The Brix of the soy

Fig. 3 Brix (A) and reducing sugar content (B) of soy yogurt with different content of allulose during 96 h fermentation

yogurt decreased from 13.65 to 13.95 °Bx at the beginning to 9.14–9.62 °Bx after 24 h fermentation. Then the Brix had the range of 8.99–9.34 °Bx at 48 h, 8.98–9.24 °Bx at 72 h, and 8.95–9.19 °Bx at 96 h. After 48 h fermentation, there were no significant differences across the content of D-allulose.

The changes in reducing sugar content of the soy yogurt during 96 h fermentation are shown in Fig. 3(B). From the beginning to the end of fermentation, the more D-allulose are contained in the soy yogurt, the higher value of reducing sugar content appeared. Also, as the fermentation progressed, A0, A25, A50, A75, A100, and CA0 had the highest reducing sugar content at 24 h, and the range was 1.41–6.24%. Then their values constantly decreased to 0.83–6.12% at 96 h. However, the reducing sugar content

of A100 was weakly, but not significantly, increased 24 h after LAB fermentation and then slightly decreased. Moreover, A0 and CA0, which are containing only sucrose as a sweetener, had 0.35% and 0.33% of reducing sugar at the beginning, but revealed different tendency of the reducing sugar content as fermentation time. The reducing sugar content of A0 increased to 3.51% at 24 h, and then constantly decreased to 2.51% at 96 h. In contrast, the reducing sugar content of CA0 increased to 1.41% at 24 h and then constantly decreased. In addition, D-allulose, which contains one ketone group, is a reducing sugar (Zhang et al., 2016). Kimoto-Nira et al. (2016) found that D-allulose is not metabolized by LAB. However, sucrose can be utilized by LAB to produce lactic acid (Morazza et al., 2009). These results suggest that the isolated LAB could produce reducing sugar from sucrose and soy milk ingredients than commercial control LAB and the reducing sugar be constantly degraded to short chain fatty acids.

DPPH radical scavenging activity

DPPH is a stable free radical which has an unpaired valence electron at one atom of nitrogen bridge and scavenging of DPPH radical is the basis of the popular DPPH antioxidant assay. The changes in DPPH radical scavenging activity of the soy yogurt during 96 h fermentation are shown in Fig. 4. DPPH radical scavenging activities of the soy yogurt were significantly increased as the fermentation progressed, from initial 36.70–39.86% to 46.96–56.67% at 48 h. Among all samples, CA0 (56.67%) had the highest DPPH radical scavenging activity at 48 h, A0 (54.44%), A25 (54.18%), A50 (53.28%) followed and A100 (46.96%) had the lowest DPPH radical scavenging activity. Although

there was an increasing tendency of DPPH radical scavenging activity as the content of D-allulose decreased, there were no significant differences among A0, A25 and A50. According to Hwang et al. (2014), DPPH radical scavenging activity of soy yogurt and soybean powder yogurt were 28.10% and 34.40%. And after 48 h fermentation, they increased to 36.53% and 41.61%. Yang et al. (2013) reported that DPPH radical scavenging activity of soybean yogurt fermented by *Lactobacillus plantarum* was 36.35% after 24 h fermentation. In accordance with these studies, it is supposed that the soy yogurt in our study had relatively high antioxidant activity. But because the methods to extract and to prepare samples are different, it could impact on DPPH radical scavenging activity.

Sensory evaluation

The sensory evaluation results of the soy yogurt are shown in Table 2. In the color evaluation, there were no significant differences in 6 samples and it was the range of 5.22–5.41 points. The flavor of soy yogurt was higher in the order of A50 (4.30), A75 (4.24), A25 (4.16), A0 (3.97) and CA0 (3.57) at $p = 0.0551$ level. The sweet taste of soy yogurt with A100 (4.97) was significantly higher than those of A0 (4.11), A25 (4.30), A50 (4.32), and CA0 (3.95). The preference of sour taste of soy yogurt with A100 (5.03) was significantly higher than those A0 (4.00) and CA0 (4.22), but A25 (4.54), A50 (4.51) and A75 (4.62) showed no significant difference. The mouthfeel of soy yogurt were no significant differences. The overall preference of soy yogurt with A100 (5.03) was significantly higher than those A0 (4.27) and CA0 (3.97), but A25 (4.43), A50 (4.51) and A75 (4.59) showed no significant difference. It is observed that the sweet taste and the sour taste greatly involved with the overall preference. The soy yogurt with A100 showed the highest scores in all rating categories. It seems that these results are because the soy yogurt with higher amount of D-allulose had higher pH values, higher sweet taste and lower sour taste. At 48 h, the pH of A100 was 4.52 and pH of other samples had the range of 4.18–4.31. Therefore the pH of A100 was not suitable pH of yogurt. On the basis of these results, it is suggested that 50% D-allulose which had pH 4.22 can be used instead of sucrose to produce soy yogurt.

In conclusion, the acid production in LAB was suppressed by D-allulose. Meanwhile the LAB counts of soy yogurt with D-allulose had suitable range during 96 h fermentation. Also, it was observed that the soy yogurt fermented by isolated LAB from kimchi had significantly superior scores in sensory evaluation than the sample with commercial LAB. Also, the more D-allulose was contained in the soy yogurt, the higher scores of overall preference appeared. A100 got the highest scores in overall

Fig. 4 DPPH radical scavenging activity of soy yogurt with different content of D-allulose during 96 h fermentation

Table 2 Sensory scores of soy yogurt with different content of allulose fermented for 48 h

Sample ²	Sensory scores ¹					
	Color	Flavor	Sweet taste	Sour taste	Mouthfeel test	Overall preference
A0	5.41 ± 1.07	3.97 ± 1.14	4.11 ± 1.37 ^b	4.03 ± 1.50 ^b	4.73 ± 1.33	4.27 ± 1.41 ^b
A25	5.38 ± 1.19	4.16 ± 1.40	4.30 ± 1.35 ^b	4.54 ± 1.41 ^{ab}	4.43 ± 1.34	4.43 ± 1.17 ^{ab}
A50	5.30 ± 1.10	4.30 ± 1.00	4.32 ± 1.42 ^b	4.51 ± 4.41 ^{ab}	4.70 ± 1.05	4.51 ± 1.39 ^{ab}
A75	5.22 ± 1.23	4.24 ± 1.06	4.46 ± 4.24 ^{ab}	4.62 ± 1.21 ^{ab}	4.46 ± 1.35	4.59 ± 1.19 ^{ab}
A100	5.38 ± 1.21	4.32 ± 1.38	4.97 ± 1.32 ^a	5.03 ± 1.36 ^a	4.84 ± 1.32	5.03 ± 1.14 ^a
CA0	5.32 ± 1.25	3.57 ± 1.01	3.95 ± 1.27 ^b	4.22 ± 1.32 ^b	4.59 ± 1.21	3.97 ± 1.24 ^b
F value	0.13 ($p = 0.9853$)	2.20 ($p = 0.0551$)	2.62 ($p = 0.0253$)	2.36 ($p = 0.0413$)	0.58 ($p = 0.7119$)	2.88 ($p = 0.0155$)

¹Sensory scores are indicated as follows: 1 = dislike very much and 7 = like very much

²A0–A100 are soy yogurt samples containing allulose with isolate LAB, LM and LP. Also, each number means the ratio (%) of allulose in total sweetener amount. CA0 is a soy yogurt sample containing only sucrose with commercial LAB, KCTC13302 and KCTC33131

^{a,b}Means with different superscript letters in a column are significantly different at the $p < 0.05$ level

preference, A25, A50 and A75 were high as well and there was no significant difference among A25–A100. However, the pH and the titratable acidity of A100 are relatively unsuitable to soy yogurt in terms of the food safety and the storage quality. Hence, A50, which had high score in overall preference, revealed moderate pH, titratable acidity and DPPH radical scavenging activity, could be developed to an D-allulose-contained soy yogurt with lower calories.

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