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Preparation of Yogurt Added with *Angelica keiskei* Juice and Its Quality Characteristics

Hai-Jung Chung

Department of Food Science and Nutrition, Daejin University, Pocheon 487-711, Korea

Abstract

Mixtures prepared from whole milk with added skim milk powder (2.5%, w/v) and *Angelica keiskei* juice (1.5%, w/v) were fermented with lactic acid bacteria (single and mixed culture of *Lactobacillus bulgaricus* and *Strpetococcus thermophilus*) for 24 hours. The fermented mixtures (curd yogurt) were evaluated for acid production (pH and titratable acidity), cell numbers, viscosity, sensory property and keeping quality. Results indicated that the addition of *Angelica keiskei* stimulated the acid production by lactic acid bacteria. The number of viable cells reached $4.5 \sim 7.3 \times 10^9$ CFU/mL for *Angelica keiskei*-added yogurts, while $3.3 \sim 5.1 \times 10^9$ CFU/mL for control yogurts. Viscosity of *Angelica keiskei*-added yogurts was higher (3,609~3,854 centipoises) than that of control yogurts (3,346~3,700 centipoises). Of the microorganism tested, mixed culture of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* was most effective in acid production. The overall sensory score showed that *Angelica keiskei* yogurt fermented with *Streptococcus thermophilus* was evaluated as good as control yogurt. When yogurts were stored at 4°C for 12 days, pH, titratable acidity and viable cells of lactic acid bacteria were not significantly changed ($p<0.05$).

Key words: yogurt, *Angelica keiskei*, *Lactobacillus bulgaricus*, *Streptococcus thermophilus*

INTRODUCTION

Yogurt is a semifluid product made by fermenting pasteurized milk with lactic acid producing bacteria such as *Lactobacillus bulgaricus* and *Streptococcus thermophilus* (1). The milk is usually fortified with powdered milk, which increases viscosity of the product and the total milk solids content to 12~14% (2). Yogurt popularity is growing rapidly in Korea and the reason for this is the concern over nutrition and personal health. Due to its positive nutritional attribute, yogurt has achieved a special prominence and new type yogurt products have been developed (3-13). Starch-added yogurt (3), egg white powder and casein yogurt (4), sweet potato and/or pumpkin-added yogurt (5) and potato-added yogurt (6) were prepared and quality characteristics were investigated by researchers.

Recently, attention has been given to the development of yogurt added with natural plants and herbs. Lim et al. (7) prepared yogurt with medicinal herb extracts to investigate the effect on the growth of lactic acid bacteria. Other researchers developed various yogurt products which were made from box thorn (8), mugwort extract (9), *Platycodon grandiflorum* A.DC. (10) and *Aloe vera* (11).

Angelica keiskei Koidz has been used as a folk remedy for the treatment of hypertensive diseases, liver diseases and neuralgia (14). It is now considered useful for a variety of diseases and consumed mainly as green juice and salads. Green juice of raw leaf and stem is rich in sugars, lipids, proteins, minerals, vitamins and amino acids (15), which is expected to have effects on the growth of yogurt culture and overall quality of yogurt.

This study was conducted to get the fundamental knowledge for the development of a new product, *A. keiskei*-added yogurt. *A. keiskei* green juice was added to the yogurt and the pH, titratable acidity, the level of lactic acid bacteria and viscosity were compared to those of the control yogurt.

MATERIALS AND METHODS

Bacterial strains and culture conditions

Yogurt cultures used in this study were *Lactobacillus bulgaricus* (ATCC 33409) and *Streptococcus thermophilus* (KCTC 2185). They were grown separately at 37°C in MRS broth.

Preparation of yogurt

Fresh leaves and stems of *A. keiskei* were washed with running tap water several times, drained and extracted with a juicer (BKJ-200, Korea). Pasteurized whole milk, enriched with 2.5% (w/v) nonfat dried skim milk powder and 1.5% (w/v) *A. keiskei* juice was heated at 85°C for 10 min. After cooling to 40°C, mixes were then inoculated with 3% (v/v) culture (single and 1:1 mixture of *L. bulgaricus* and *S. thermophilus*) and incubated at 37°C for 24 hours. During fermentation, yogurt samples were taken and analyzed every 6 hours.

pH and titratable acidity determination

Samples of each yogurt preparation were measured for pH and titratable acidity at 6 hour intervals for 24 hours. The

E-mail: haijung@road.daejin.ac.kr
Phone: 82-31-539-1861, Fax: 82-31-539-1860

pH of yogurt was measured directly with a pH meter (Inolab level II, Germany). Titratable acidity was determined by titrating with 0.1 N NaOH to pH 8.1 using a digital burette (Jencons, UK) and expressed in terms of lactic acid content (%).

Microbiological analysis

Each yogurt sample of 0.1 g was weighed and blended with 9.9 mL of sterilized saline solution (0.85%) followed by serial dilution using the same sterile diluent. Each diluted sample (1.0 mL) was poured on Plate Count Agar (Difco Co., USA) containing 0.04% of brom cresol purple. The plates were incubated at 37°C for 48~72 hours and colonies were counted and expressed as number of colony forming units (CFU) per mL sample.

Viscosity measurement

Viscosity was measured with a viscometer (Viscostar L, Spain). Prior to viscosity measurements yogurt was stirred for 30 s. Data were recorded 30 s intervals up to 3 min and average values were expressed as centipoise (cp) units. All viscosity readings were taken at 15±1°C.

Sensory evaluation

After fermenting for 24 hours, yogurt samples were kept in the refrigerator (4±1°C) for five hours before served. Control yogurt was prepared by fermenting with *L. bulgaricus*. Sensory evaluation was made by 8 trained panelists. Products were evaluated for appearance, odor, acidic taste, texture and overall quality using a 9-point scale. Acidic taste was rated as extremely weak (1) to extremely strong (9); texture was rated as sandy (1) to smooth (9); appearance, odor and overall quality were rated as dislike very much (1) to like very much (9).

Changes in pH, titratable and viable cell counts during storage

In order to examine any quality changes during storage, both the control and *A. keiskei*-added yogurts were stored at 4±1°C for 12 days and pH, titratable acidity and cell numbers were monitored every three days.

Statistical analysis

All experiments were replicated three times. The data were analyzed by analysis of variance (ANOVA) with the software of SPSS (version 7.5) with significance defined at $p<0.05$. Significant differences between treatment means were determined by using Duncan's multiple range test.

RESULTS AND DISCUSSION

pH and titratable acidity

1.5% (w/v) *A. keiskei* juice was added to the yogurt mix and inoculated with single and mixed culture (1:1 ratio) of *L. bulgaricus* and *S. thermophilus*. Preliminary tests to determine an optimal level of *A. keiskei* revealed that there was no discernible difference in acid production when three levels of (1.5, 3.0 and 4.5%, w/v) *A. keiskei* juice were added to the yogurt (data not shown). Hence the lowest level was added to the yogurt for these experiments. Samples of each yogurt preparation were measured for pH and titratable acidity at 6 hour intervals over 24 hours. As shown in Table 1, the initial pH of the yogurt mixes ranged from 6.56~6.58 and after 12 hour incubation, pH values of all mixes were decreased to around 5.0 and *A. keiskei* juice significantly lowered pH ($p<0.05$). After 24 hours of incubation, the final pH values of control yogurts and those of *A. keiskei*-added yogurts showed no significant difference. As shown in Table 2, the initial titratable acidity of all samples were 0.151~0.158%. It gradually increased during fermentation and the final acidity values of *A. keiskei*-added yogurts were significantly higher (1.266~1.278%) ($p<0.05$) than those of control yogurts (1.121~1.129%). Shin et al. (11) reported that the addition of *Aloe vera* to yogurt remarkably stimulated acid production, resulting in 1.293~1.407%. Lee et al. (10) reported that *Platycodon grandiflorum* A.DC. yogurt showed the pH value of 4.1 and the titratable acidity value of 1.15%. Other researchers (6) reported that yogurts added with potato puree exhibited a pH of 3.86~3.89. Kim et al. (16) reported that the pH of commercial yogurt in Korea was 3.87~4.19, and titratable acidity ranged from 0.97~1.43%, which is in accordance with our data. We chose *L. bulgaricus* and *S. thermophilus* as yogurt starters because they are the principal starter culture organisms that contribute to acid production and flavor development. *S. thermophilus* primarily contri-

Table 1. Changes in pH of yogurts during fermentation at 37°C

Strain	*AK*[1] added amount (%)	Incubation time (hour)				
		0	6	12	18	24
Lac.	0	6.56[2)a3)]	6.17[a]	5.09[a]	4.38[b]	4.00[a]
bulgaricus	1.5	6.56[a]	5.98[b]	4.93[c]	4.21[c]	3.92[a]
Str.	0	6.58[a]	6.21[a]	5.15[a]	4.36[a]	4.07[a]
thermophilus	1.5	6.56[a]	6.03[b]	5.00[bc]	4.21[c]	3.98[a]
Mixed	0	6.58[a]	6.23[a]	5.01[b]	4.36[b]	3.97[a]
strain[4)]	1.5	6.57[a]	5.92[b]	4.94[bc]	4.19[d]	3.82[a]

[1)] *Angelica keiskei* juice
[2)] Mean values of three replications
[3)] Mean values within columns followed by the same letters are not significantly different ($p<0.05$).
[4)] *Lactobacillus bulgaricus*+*Strpetococcus thermophilus* (1:1)

Table 2. Changes in titratable acidity of yogurts during fermentation at 37°C (unit : %)

Strain	*AK*[1] added amount (%)	Incubation time (hour)				
		0	6	12	18	24
Lac.	0	0.158[2)a3)]	0.263[a]	0.578[a]	0.919[a]	1.123[a]
bulgaricus	1.5	0.158[a]	0.277[ab]	0.678[b]	1.057[b]	1.268[b]
Str.	0	0.158[a]	0.220[a]	0.578[a]	0.901[a]	1.121[a]
thermophilus	1.5	0.157[a]	0.261[ab]	0.654[b]	1.050[cb]	1.266[b]
Mixed	0	0.157[a]	0.220[a]	0.542[a]	0.920[a]	1.129[a]
strain[4)]	1.5	0.151[a]	0.286[b]	0.690[b]	1.075[b]	1.278[b]

[1)] *Angelica keiskei* juice
[2)] Mean values of three replications
[3)] Mean values within columns followed by the same letters are not significantly different ($p<0.05$).
[4)] *Lactobacillus bulgaricus*+*Strpetococcus thermophilus* (1:1)

butues to lactic acid production, while *L. bulgaricus* produces flavor (17).

Microbiological changes

Results of microbiological changes during 24 hour fermentation are summarized in Table 3. The initial starter bacterial numbers in yogurts were $2.4\sim3.9\times10^6$ CFU/mL. After 12 hours of fermentation, viable cells increased markedly up to $2.5\sim4.6\times10^9$ CFU/mL, but slowed down thereafter. At 24 hours, the cell numbers ranged from $3.3\sim7.3\times10^9$ CFU/mL, which meets the regulatory level and the result is in good agreement with other reports (5,8,10,16). Addition of *A. keiskei* resulted in stimulation of acid production and this may be explained by the fact that *A. keiskei* is rich in nutrients such as lipids, proteins, minerals, vitamins and amino acids which are essential to the growth of lactic acid bacteria, hence accelerating acid production. Of the organisms tested, mixed culture of *L. bulgaricus* and *S. thermophilus* exhibited maximum population in *A. keiskei*-added yogurt (7.3×10^9 CFU/mL).

Viscosity of *Angelica keiskei* yogurt

The influence of *A. keiskei* on the viscosity is shown in Table 4. The curd was not formed until after 12 hours of fermentation, except those treated with *A. keiskei* juice. This suggests that *A. keiskei* reacted more favorably with milk, resulting in a more rapid development of viscosity. After 18 hours fermentation, the viscosity was dramatically increased and the result confirmed those of Kroger (18) that yogurt milk became solid at an acidity (lactic acid) of about 0.6% and pH of about 5.3. Our result was that for control yogurts, titratable acidity was below 0.6%, whereas for *A. keiskei*-added yogurts, titratable acidity was above 0.65% at 12 hours of fermentation. At the end of 24 hours of fermentation, *A. keiskei*-added yogurts had higher viscosity (3,609~3,854 cps) than the controls (3,346~3,700 cps). Similar results have been observed by *Aloe vera*-added yogurts and box thorn-added yogurts, which showed the viscosity values of 3,860~4,300 cps (11) and 2,250~2,235 cps (8), respectively. Kim et al. (16) reported the viscosity of commercial yogurts as 7,850~21,000 cps, which is in disagreement with our result. This may be explained by the differences in total solids contents and ingredients used (6,11). And the viscosity modifiers such as starches, gums, gelatins and pectins could be used to increase the viscosity (12). Increase in acidity during fermentation enhanced curd stability because of the increase in water-binding capacity of proteins. This supports our findings that *A. keiskei* which accelerated acid production tended to yield a finished product with higher viscosity than the controls.

Table 4. Changes in viscosity of yogurts during fermentation at 37°C (unit : cps)

Strain	*AK*[1] added amount (%)	Incubation time (hour) 0	6	12	18	24
Lac. bulgaricus	0	-	-	-	3164[a]	3346[a]
	1.5	-	-	898[2)a3)]	3341[a]	3749[a]
Str. thermophilus	0	-	-	-	3116[a]	3402[a]
	1.5	-	-	704[b]	3076[a]	3609[a]
Mixed strain	0	-	-	-	3316[a]	3700[a]
	1.5	-	-	868[a]	3266[a]	3854[a]

[1)] *Angelica keiskei* juice
[2)] Mean values of three replications
[3)] Mean values within columns followed by the same letters are not significantly different ($p<0.05$).
[4)] *Lactobacillus bulgaricus*+*Strpetococcus thermophilus* (1:1)

Sensory evaluation of yogurt products

Sensory quality of *A. keiskei*-added yogurt fermented with single and mixed culture of *L. bulgaricus* and *S. thermophilus* was compared to control yogurt fermented with *L. Bulgaricus* and the results are presented in Table 5. *A. keiskei*-added yogurts fermented with mixed culture had the highest acidity score, whereas *S. thermophilus* had the lowest. This was in agreement with pH and titratable acidity values presented in Table 1 and 2. Appearance values for the *A. keiskei* products were lower than for the control yogurt, because panelists are unfamiliar with the green color of the products. There were no significant differences in odor scores among yogurts tested. This would indicate that *A. keiskei* added yogurt possess the same desirable odor as milk yogurt. It is because a low level of *A. keiskei* juice was added to the yogurt, so any typical odor derived from it were not detected by the panelists. Texture score of the *A. keiskei* yogurt products was lower than that of the control, which may be due to the uneven homogenization of *A. keiskei* components. Texture is a critical aspect of consumer acceptability of yogurt and various factors such as total solids, homogenization, type of culture, acidity, and heat pretreatment of milk influence the textural properties

Table 3. Changes in viable cell counts of yogurts during fermentation at 37°C (unit : CFU/mL)

Strain	*AK*[1] added amount (%)	Incubation time (hour) 0	6	12	18	24
Lac. bulgaricus	0	2.8×10^6	3.4×10^6	2.6×10^9	3.8×10^9	4.1×10^9
	1.5	2.4×10^6	4.5×10^6	4.4×10^9	5.5×10^9	6.5×10^9
Str. thermophilus	0	2.9×10^6	3.3×10^6	2.5×10^9	2.6×10^9	3.3×10^9
	1.5	3.9×0^6	4.9×10^6	3.4×10^9	3.8×10^9	4.5×10^9
Mixed strain[2)]	0	3.3×10^6	4.3×10^6	3.9×10^9	4.9×10^9	5.1×10^9
	1.5	3.4×10^6	4.8×10^6	4.6×10^9	5.9×10^9	7.3×10^9

[1)] *Angelica keiskei* juice
[2)] *Lactobacillus bulgaricus*+*Strpetococcus thermophilus* (1:1)

Table 5. Sensory evaluation scores of yogurts prepared with *Angelica keiskei* juice

Sample[1)]	Appearance	Odor	Acidic taste	Texture	Overall quality
A	6.25[2)a3)]	5.43[a]	5.04[a]	6.67[a]	5.29[a]
B	5.46[b]	5.00[a]	5.17[a]	4.76[b]	4.30[b]
C	5.54[ab]	5.29[a]	4.93[a]	4.46[b]	5.29[a]
D	5.58[ab]	4.71[a]	5.38[a]	5.17[b]	5.04[a]

[1)]A : control with *Lac. bulgaricus*
B : 1.5% *Angelica keiskei*-added yogurt with *Lac. bulgaricus*
C : 1.5% *Angelica keiskei*-added yogurt with *Str. thermophilus*
D : 1.5% *Angelica keiskei*-added yogurt mixed culture of *Lactobacillus bulgaricus*+*Streptococcus thermophilus* (1:1)
[2)]Mean values of three replications
[3)]Mean values within columns followed by the same letters are not significantly different ($p<0.05$).

of yogurt (19). Among the products, *A. keiskei*-added yogurt fermented with *S. thermophilus* and mixed culture was evaluated as good as control, while *A. keiskei* yogurt with *L. bulgaricus* was inferior.

Changes in pH, titratable acidity and viable cell counts during storage

Changes in pH, titratable acidity and the number of viable cells are shown in Fig. 1, 2 and 3, respectively. The pH of each product decreased gradually with the exception of 1.5% *A. keiskei* yogurt fermented with *S. thermophilus*. The titratable acidity and the number of viable cells increased, but slowed down after 6 days of storage and remained nearly constant. It has been reported that keeping qualities of yogurts added with potato-added yogurt (6) and box thorn-added yogurt (8) were not changed much for 15 days at 5°C and the result is consistent with our findings.

The increase in acidity of yogurt during storage and delivery has been a problem (19,20), since high acidity is not appealing to consumers. Therefore, monitoring acidity changes during storage would be helpful to yogurt manufacturers in estimating the acidity at point of sale.

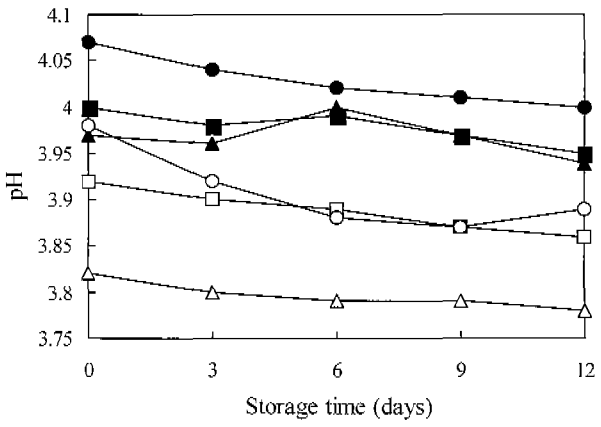

Fig. 1. Changes in pH of yogurts during storage at 4°C. ■-■ : control with *L. bulgaricus*, ●-● : control with *S. thermophilus*, ▲-▲ : control with mixed culture, □-□ : 1.5% *Angelica keiskei* yogurt with *L. bulgaricus*, ○-○ : 1.5% *Angelica keiskei* yogurt with *S. thermophilus*, △-△ : 1.5% *Angelica keiskei* yogurt with mixed culture.

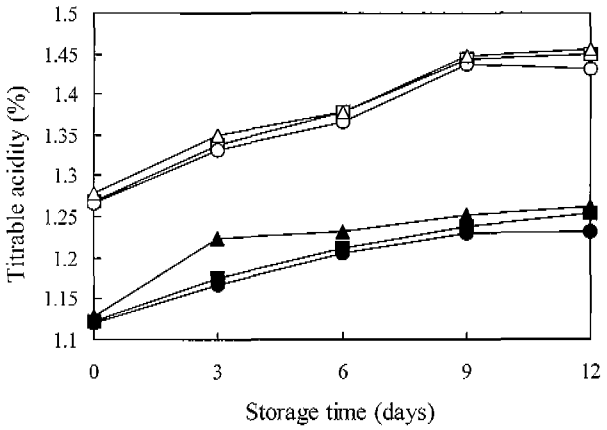

Fig. 2. Changes in titratable acidity of yogurts during storage at 4°C. ■-■ : control with *L. bulgaricus*, ●-● : control with *S. thermophilus*, ▲-▲ : control with mixed culture, □-□ : 1.5% *Angelica keiskei* yogurt with *L. bulgaricus*, ○-○ : 1.5% *Angelica keiskei* yogurt with *S. thermophilus*, △-△ : 1.5% *Angelica keiskei* yogurt with mixed culture.

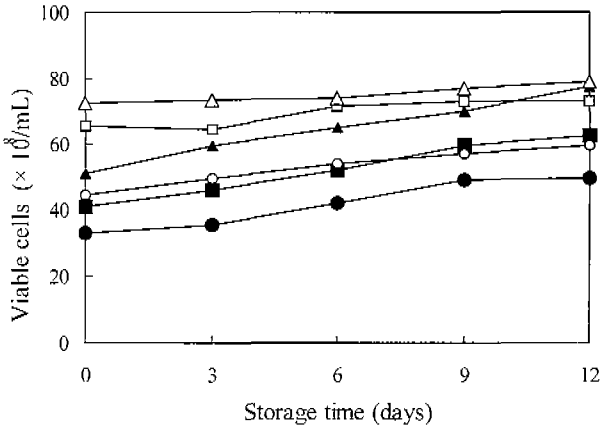

Fig. 3. Changes in viable cell counts of yogurts during storage at 4°C. ■-■ : control with *L. bulgaricus*, ●-● : control with *S. thermophilus*, ▲-▲ : control with mixed culture, □-□ : 1.5% *Angelica keiskei* yogurt with *L. bulgaricus*, ○-○ : 1.5% *Angelica keiskei* yogurt with *S. thermophilus*, △-△ : 1.5% *Angelica keiskei* yogurt with mixed culture.

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