

Development of Functional Yogurts Prepared with Mulberries and Mulberry Tree Leaves

An-Cheol Lee and Youn-Ho Hong*

Department of Food and Nutrition, Human Ecology Research Institute, College of Human Ecology,
Chonnam National University, Gwangju 500-757, Korea

Abstract

In order to develop new functional yogurts using mulberries and mulberry leaves, which were cultivated in Hwasun-gun, Jeonnam Province, Korea, the nutritional compositions, fermentation conditions, sensory properties, and storage stabilities of the yogurts were analyzed. The mulberry powder yogurt contained 87.96% moisture, 3.21% carbohydrate, 4.52% protein, 3.63% lipid, and 0.68% ash, and the mulberry leaf yogurt contained 86.36% moisture, 4.13% carbohydrate, 4.87% protein, 3.79% lipid, and 0.85% ash. A yogurt base was fermented for 13 h with 0.01% ABT-5 starter inoculum at 40°C. To prepare the mulberry jam and mulberry leaf yogurts, a variety of mulberry jam and mulberry leaf samples were added to the yogurt base. The sensory evaluation results of the yogurts containing the mulberry jam and mulberry leaves indicated that a product made with 15% mulberry jam was more strongly preferred than other samples. When the mulberry jam and mulberry leaf yogurts were stored at 4°C for 15 d, there were no significant changes in pH, titratable acidity, or viable cell numbers of lactic acid bacteria and *Bifidobacterium bifidum*.

Key words: mulberry, mulberry leaf, yogurt, sensory evaluation

Introduction

Mulberries (*Mulberry bombycis* Koids) and mulberry leaves have been used as traditional medicines and foods since 200 years B. C. (Park *et al.*, 2000). Mulberry fruits are nutritionally rich in flavonoids such as sanggenon, moracin, cyclomulberrin, and kuwanon, which are recognized as antioxidants and anti-aging substances (Hassimoto *et al.*, 2005; Hong *et al.*, 2004). Mulberries also contain anti-bacterial components such as albafluran and anti-inflammatory materials such as bergapten, as well as cyanidin-3-glucosides, which have anti-diabetic and antioxidant properties (Hong, 1997; Kim, 1996; 1998). Mulberry leaves contain protein, vitamins, minerals, fibers, flavones, steroids, and triterpenes, and show various functional properties (Chae *et al.*, 2003). Mulberry leaves have also been applied in anti-diabetic, anti-hyperlipidemia, antioxidant, and anti-heavy metal treatments (Kim *et al.*, 1998; Kim *et al.*, 1998; Kim and Kwon, 1996; Kimura *et al.*, 1995; Yen *et al.*, 1996). The blood sugar

reducing compound 1-deoxynojirimycin (DNJ), the blood pressure reducing compound gamma-aminobutyric acid (GABA), and antioxidant flavonoids are recognized as biologically active substances (Chae *et al.*, 2003).

Yogurt is a fermented milk product made from milk and lactic acid bacteria and may contain different herbs. It is a well-known probiotic food that is good for the digestion and it has anti-bacterial, cholesterol-reducing, and diarrhea-preventive properties. Yogurt is nutritionally rich in protein, protein, calcium, riboflavin, vitamin B₆, and vitamin B₁₂. And it has nutritional benefits beyond those of milk (Gilliand, 1989; Hood and Zottoloa, 1988).

Recently, many kinds of yogurts containing plant components such as bamboo shoots (Park and Jhon, 2006), berry fruits (Seeram, 2008), cherry fruit (Kim *et al.*, 2009), mulberry extract (Suh *et al.* 2006), chlorella (Sung *et al.*, 2005), and green tea (Bang and Park, 2000) have been introduced, and have shown positive biological functions. In this study, we attempted to develop functional yogurts containing mulberries and mulberry leaves, and in order to determine optimal manufacturing conditions, their physicochemical, microbiological, functional, and sensory properties were analyzed.

*Corresponding author: Youn-Ho Hong, Department of Food and Nutrition, Human Ecology Research Institute, College of Human Ecology, Chonnam National University, Gwangju 500-757, Korea. Tel: 82-62-530-1333; Fax: 82-62-530-1339, E-mail: yhhong@chonnam.ac.kr

Materials and Methods

Materials

The mulberries and mulberry leaves were purchased from a mulberry farm in Hwasun-Gun, Jeonnam, Korea, at the beginning of June and August 2008, respectively. The mulberry leaves were dried and powdered immediately after purchase and stored at -70°C, and the mulberries were stored at -18°C. Milk, skim milk powder, and sugar were purchased from a local market. Pectin was purchased from Kelco (Grossenbrode, Germany). The starter culture ABT-5 (*Lactobacillus acidophilus*, *Bifidobacterium bifidum*, and *Streptococcus thermophilus*) was purchased from Chr. Hansen's Lab (Horsholm, Denmark), and stored at -20°C.

The mulberries and mulberry leaves were dried with a freeze-dryer (Bondiro DC1316, Ilshin Lab Co, Seoul, Korea), pulverized with a Hanil grinder (FM-681C, Seoul, Korea), and screened with a 50 mesh sieve.

Mulberry jam was prepared by mixing 50% of freeze-dried mulberry powder, 50% of sugar, and 1% of pectin.

Analytical-grade chemicals were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

Methods

Composition measurements

The water, carbohydrate, crude fat, crude protein, and crude ash contents of the powdered mulberry and mulberry leaf were analyzed by AOAC methods (AOAC, 2000).

For composition analysis, the concentrations of the mulberry and mulberry leaf yogurts were 15, 10, 1.5, and 0.5%, respectively.

Water content was measured using a drying method at normal pressure, ash was measured by ashing in an electric furnace (JEIO Tech, Seoul, Korea) at 550°C, protein was measured via micro-Kjeldahl analysis, and crude fat was analyzed by the Soxhlet method.

Optimization of yogurt manufacturing conditions

Yogurts containing mulberry jam and mulberry leaves were made from market milk with 5% powdered skim milk by mixing with starter microorganisms at 0.005% (w/v). The mixtures were then kept at 40°C for 16 h, where the pH reached 4.2. The mulberry jam and mulberry leaf powder were added before and after fermentation, respectively. The yogurts were stored at 4°C and analyzed.

The pH values of each of the solutions were adjusted with 1 N HCl or 1 N NaOH solution.

Physicochemical and microbiological analyses

Physicochemical and microbiological changes were analyzed during the manufacture of the mulberry-added yogurts. The pH values of the yogurts were measured by an Orion 3Star bench-top meter (Thermo Electron Co., Beverly, MA, USA) and viable bacterial numbers were counted. To determine viable bacterial numbers in the samples, *Lactobacillus* sp. were counted after incubation at 37°C for 24 h on BCP plate count agar (Eiken Co., Japan) and *Bifidobacterium* sp. were counted after incubation at 37°C for 72 h on BL agar (BBL, Cockeysville, MD, USA).

Sensory evaluation of mulberry and mulberry leaf yogurts

Sensory evaluations of the yogurts were performed by 10 trained graduate student panelists by testing color, flavor, sweet taste, sour taste, and texture. The Sensory evaluations of products were classified on a 10-point scale by giving 1 point for the worst sample, 10 points for the best sample, and those remaining were assigned in-between numbers.

Storage stability of yogurt

The storage stability of the yogurts was assessed by measuring bacterial counts, titratable acidity, and pH.

Statistics

All measurements were performed in triplicate and mean values and standard deviations were calculated. The statistical analysis was performed with the SPSS package (Version 14.0) using Duncan's multiple test.

Results and Discussion

Composition of yogurts

The compositions of the mulberry jam and mulberry leaf yogurts are shown in Table 1. The mulberry jam yogurt contained 83.70% moisture, 7.81% carbohydrate, 4.40% protein, 3.28% lipid, and 0.81% ash, and the mulberry leaf yogurt contained 86.36% moisture, 4.13% carbohydrate, 4.87% protein, 3.79% lipid, and 0.85% ash.

The compositions of the mulberry jam and mulberry leaves were similar to those found in other studies (Lee, 2009; Lee *et al.*, 1998; Park *et al.*, 2000).

Table 1. Compositional analyses of yogurts containing mulberry jam and mulberry leaf powder

Samples	Moisture (%)	Carbohydrate (%)	Crude lipid (%)	Crude protein (%)	Crude ash (%)
15% MJAF	83.70±0.58 ^a	7.81±0.58 ^b	3.28±0.09 ^a	4.40±0.03 ^a	0.81±0.06 ^b
10% MJBF	85.27±0.33 ^{ab}	6.16±0.37 ^b	3.36±0.05 ^a	4.43±0.02 ^a	0.78±0.03 ^{ab}
0.5% MPBF	87.96±0.72 ^b	3.21±0.68 ^a	3.63±0.10 ^a	4.52±0.02 ^a	0.68±0.05 ^a
1.5% MLPAF	86.36±0.37 ^{ab}	4.13±0.27 ^{ab}	3.79±0.07 ^a	4.87±0.03 ^a	0.85±0.02 ^c

MJAF: mulberry jam added after fermentation, MJBF: mulberry jam added before fermentation, MPBF: mulberry powder added before fermentation, MLPAF: mulberry leaf powder added after fermentation.

Values are mean ± SD.

Data were analyzed by Duncan's multiple range test.

^{a-c}Values with different superscripts within a column indicate significant difference ($p < 0.05$).

Optimization of yogurt manufacturing conditions

To optimize the yogurt manufacturing conditions, ABT-5 microbial granules were added to saline at 0.005, 0.01, 0.02, 0.05, and 0.1%, and then inoculated into a diluted liquid of 5% added-skim milk and fermented at 40°C. The fermentation was stopped at pH 4.2 and a titratable acidity of 0.90-1.00%. Viable lactic acid bacteria colonies and fermentation time were determined. According to the results, the viable lactic acid bacteria colonies were suitable to the standards of the Food Codex (Korea Food Industry Association, 2008), and fermentation time was between 9 and 16 h, which was reduced as the inoculation concentration increased (Fig. 1). In this study, for the optimum conditions of yogurt manufacture, we used starter microorganism granules at 0.01%, a fermentation temperature of 40°C, and a fermentation time of 13 h. The carbohydrate content of the mulberry jam-added yogurts was lower after fermentation than before fermentation. Carbohydrate seemed to be used as an energy source during fermentation.

Physicochemical and microbiological changes during the manufacture of mulberry-added yogurts

The pH values of the yogurts decreased as the added

amounts of mulberry jam or mulberry leaf increased, and showed similar tendencies. Viable bacterial numbers after fermentation were slightly higher than the control, but no significant differences were found as shown in Fig. 2 and Fig. 3.

Sensory evaluations of mulberry and mulberry leaf yogurts

The sensory evaluation results of the yogurts containing

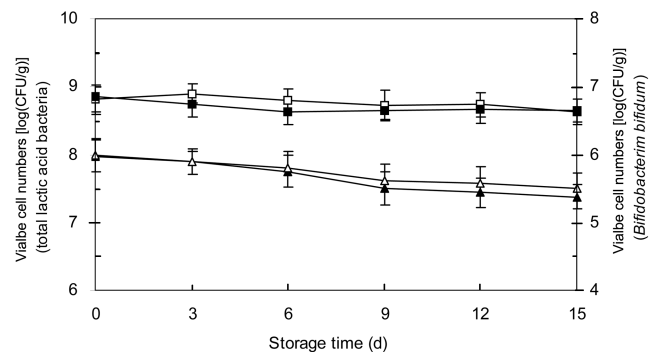


Fig. 2. Changes in viable cell numbers of total lactic acid bacteria (□ : control, ■ : mulberry jam) and *Bifidobacterium bifidum* (△ : control, ▲ : mulberry jam) during storage of yogurt with mulberry jam added after fermentation.

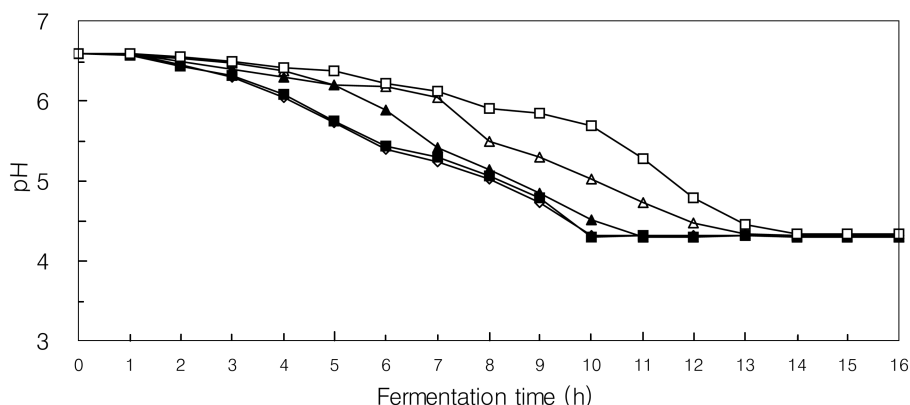


Fig. 1. Effects of starter concentration (◇ : 0.10%, ■ : 0.05%, ▲ : 0.02%, △ : 0.01%, □ : 0.005%, w/v) on pH during fermentation.

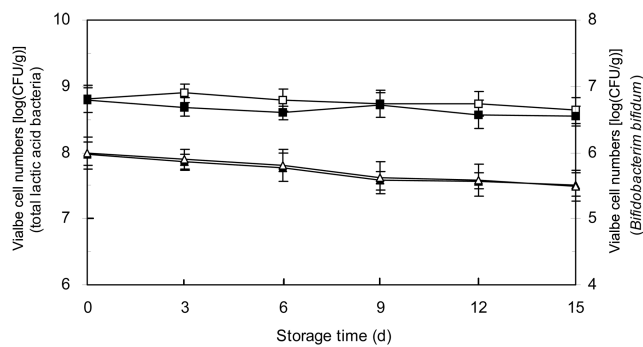


Fig. 3. Changes in viable cell numbers of total lactic acid bacteria (□ : control, ■ : mulberry leaf powder) and *Bifidobacterium bifidum* (△ : control, ▲ : mulberry leaf powder) during storage of yogurt with mulberry leaf powder added after fermentation.

various concentrations of mulberry jam and mulberry leaf are shown in Tables 2, 3, 4, and 5, respectively. The yogurt samples with 15% of mulberry jam added after fermentation had significantly higher sensory values than samples

with mulberry jam added before fermentation. In addition, the yogurt samples with 10% of mulberry jam added before fermentation had significantly higher sensory values than samples with additions after fermentation (Table 2 and 3). The yogurts with 0.5-1.5% mulberry leaf added after fermentation did not show significant differences in sensory values. However, the yogurt samples with 1.5% mulberry leaf added before fermentation had significantly higher sensory values than those with mulberry leaf added after fermentation (Table 4 and 5).

The overall acceptability of the yogurt with mulberry jam added after fermentation was higher than that of yogurt with mulberry jam added before fermentation. This suggests that higher mulberry jam content improved the sensory quality of products (Table 2 and 3). The sensory evaluations of yogurts with added mulberry leaf were inferior to those with added mulberry jam. This suggests that added sugar in the jam garnered more points.

Table 2. Sensory evaluations of yogurts with additions of various amounts of mulberry jam before fermentation

Concentration (%)	Color	Flavor	Sweet taste	Sour taste	Flesh amount	Texture	Overall acceptability
5	6.00±2.10 ^a	5.55±1.86 ^{ab}	4.09±1.76 ^a	5.45±2.46 ^b	3.00±1.18 ^a	5.82±2.09 ^{ab}	5.73±2.00 ^b
10	6.18±1.17 ^a	6.73±1.85 ^c	6.40±2.14 ^b	5.50±2.38 ^b	5.15±1.97 ^b	6.55±1.57 ^b	7.64±0.92 ^c
15	6.36±1.86 ^a	5.91±1.87 ^b	6.09±2.47 ^b	4.82±1.94 ^{ab}	5.18±2.14 ^b	6.09±2.12 ^b	6.35±1.24 ^{bc}
20	6.18±2.40 ^a	4.00±1.67 ^a	4.09±1.92 ^a	3.45±1.75 ^a	4.73±2.94 ^{ab}	3.45±2.16 ^a	2.91±1.45 ^a

Value are mean ± SD.

Data were analyzed by Duncan's multiple range test.

^{a-c}Values with different superscripts within a column indicate significant difference ($p < 0.05$).

Table 3. Sensory evaluations of yogurts with additions of various amounts of mulberry jam after fermentation

Concentration (%)	Color	Flavor	Sweet taste	Sour taste	Flesh amount	Texture	Overall acceptability
5	5.55±2.30 ^a	5.64±1.96 ^a	4.45±1.92 ^a	5.00±2.53 ^{ab}	5.18±1.40 ^a	5.45±1.21 ^a	4.27±2.15 ^a
10	7.45±0.82 ^{ab}	7.09±1.38 ^b	5.91±1.45 ^a	4.91±1.87 ^a	6.45±1.63 ^{ab}	6.45±1.37 ^a	6.55±1.69 ^{ab}
15	8.36±0.50 ^b	7.55±0.93 ^b	7.82±1.17 ^b	6.00±1.95 ^b	7.73±1.62 ^b	6.82±1.33 ^a	7.82±1.33 ^c
20	6.18±2.09 ^a	7.00±2.05 ^b	7.18±1.25 ^b	4.73±2.24 ^a	7.36±2.16 ^b	6.81±1.81 ^a	7.18±1.04 ^{bc}

Values are mean ± standard deviation.

Data were analyzed by Duncan's multiple range test.

^{a-c}Values with different superscripts within a column indicate significant difference ($p < 0.05$).

Table 4. Sensory evaluations of yogurts with additions of various amounts of mulberry leaf powder before fermentation

Concentration (%)	Color	Flavor	Sweet taste	Sour taste	Texture	Overall acceptability
0.5	5.36±2.62 ^b	4.55±1.37 ^b	2.55±1.44 ^a	5.45±3.23 ^b	5.64±1.80 ^b	4.82±1.94 ^b
1.0	5.45±1.69 ^b	3.45±1.75 ^{ab}	2.55±1.37 ^a	5.09±2.91 ^b	4.64±2.06 ^a	3.55±2.11 ^{ab}
1.5	4.55±2.02 ^{ab}	2.73±1.49 ^a	2.45±1.21 ^a	4.09±2.39 ^a	4.27±2.24 ^a	2.91±1.70 ^a
2.0	4.00±1.90 ^a	1.91±0.93 ^a	2.36±1.69 ^a	3.64±2.62 ^a	3.73±2.45 ^a	2.55±1.57 ^a

Values are mean ± standard deviation.

Data were analyzed by Duncan's multiple range test.

^{a-c}Values with different superscripts within a column indicate significant difference ($p < 0.05$).

Table 5. Sensory evaluations of yogurts with additions of various amounts of mulberry leaf powder after fermentation

Concentration (%)	Color	Flavor	Sweet taste	Sour taste	Texture	Overall acceptability
0.5	5.73±2.69 ^a	4.91±2.17 ^{ab}	3.22±2.20 ^a	4.73±3.13 ^a	5.55±2.34 ^b	4.82±2.86 ^{ab}
1.0	6.45±1.69 ^a	4.64±1.80 ^a	3.18±1.47 ^a	4.73±2.57 ^a	5.09±1.86 ^b	4.45±1.86 ^{ab}
1.5	6.73±2.24 ^a	5.67±2.06 ^b	3.09±1.45 ^a	4.73±1.95 ^a	5.85±2.32 ^b	5.85±2.32 ^b
2.0	5.55±2.62 ^a	4.55±2.30 ^a	3.18±1.54 ^a	4.91±1.81 ^a	3.73±1.90 ^a	3.55±2.21 ^a

Values are mean ± standard deviation.

Data were analyzed by Duncan's multiple range test.

^{a-c}Values with different superscripts within a column indicate significant difference ($p < 0.05$).

Storage stability of yogurt

The different kinds of yogurt were stored at 4°C in a refrigerator for 15 d, and pH values, titratable acidity, and viable bacterial numbers were measured. The pH values of the samples were 4.18-4.39 and their titratable acidities were 0.96-1.09%, showing almost no change over the storage time. Chamber *et al.* (1979) reported that the optimum pH of yogurt is in a range of 3.27-4.53.

Numbers of lactic acid bacteria and *bifidus* bacteria decreased slowly and similar to the control over the 15 days of storage time (Fig. 2 and 3). This offered evidence that the mulberry and mulberry leaf ingredients did not affect bacterial numbers at the cold storage temperature.

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