

Effects of Freeze-dried Mulberry on Antioxidant Activities and Fermented Characteristics of Yogurt during Refrigerated Storage

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Abstract

This study investigated the effect of added freeze-dried mulberry fruit juice (FDMJ) (1, 3 and 5%) on the antioxidant activity and fermented characteristic of yogurt during refrigerated storage. A decrease in pH of yogurt and increase in acidity was observed during fermentation. The yogurts with FDMJ exhibited faster rate of pH reduction than control. Initial lactic acid bacteria count of yogurt was 6.49-6.94 Log CFU/g and increased above 9 Log CFU/g in control and 1% in FDMJ yogurt for 24 h. The total polyphenol and anthocyanin content of FDMJ yogurt was higher than that of control due to the presence of phytochemical contents in mulberry. Moreover, antioxidant activity such as DPPH and reducing power was highest 5% FDMJ yogurt. During cold storage, pH decreased or remained constant in all yogurts with values ranging from 4.08 to 4.78 units. In sensory evaluation, the score of 1% FDMJ yogurt was ranked higher when compared with other yogurts. It is proposed that mulberry fruit juice powder can be used to improve sensory evaluation and enhance functionality of yogurt.

Keywords: antioxidant activity, freeze dried mulberry, polyphenol content, sensory evaluation, yogurt

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Introduction

Yogurt is a dairy product produced through the fermentation of milk by lactic acid bacteria. Yogurt is rich in nutrients such as potassium, calcium, protein, vitamin B and probiotics that is good for digestion, cholesterol reduction and in prevention of diarrhoea (Gilliland, 1989; Lee and Hong, 2010). In recent years, consumers have become more health conscious and have been attracted towards food that has more health benefit. There has been an increasing interest in the use of natural food additives and incorporation of health-promoting substances into the diet (Varga, 2006). Thus, extensive studies on novel natural sources of food colorant and their potential use as functional ingredients are needed.

Mulberry fruit has been used as a traditional medicine to treat fever, protect liver, strengthen the joints, and in lowering blood pressure. Recently, it has gained an important position in the local soft drink market, although its

biological and pharmacological effects are still poorly defined. Important constituent of mulberry fruits are the anthocyanins (Gerasopoulos and Stavorulakis, 1997). Mulberries contain albaflavan, bergapten, and cyanidin-3-glucosides, which possess antioxidant, antimicrobial, and anti-inflammatory properties (Hong *et al.*, 1997; Teng and Lee, 2014). Anthocyanins are a group of water-soluble natural pigments responsible for the attractive red-blue color of flower and many fruits. Anthocyanins, the major pigment of mulberry are phenolic compounds reported to have various health beneficial effects including antioxidant properties, anti-diabetic effect and anti-bacterial effect (Grace *et al.*, 2009). In addition, mulberry extract contains organic acids, vitamin and minerals such as Ca, K and vitamin C (Jeon *et al.*, 2012). Nevertheless, one of the limitations of employing anthocyanin pigment, as a food colorant in food products, is its poor stability (Yawadio and Morita, 2007).

Therefore, the objective of this study was to investigate the possibility of using mulberry for enhancing nutritional value and in improving quality of yogurt. In addition, the effect on the physicochemical sensory evaluation and stability of yogurt was analyzed during refrigerated storage.

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Materials and Methods

Materials

A powdered yogurt starter in freeze-dried form composed *L. delbrueckii* subsp. *bulgaricus*, *L. casei*, *S. thermophilus*, and *B. longum* (Cell Biotech, Denmark) was used. Skim milk powder (fat 1.0%, protein 35%) and milk (fat 3.2%, protein 3.2%, carbohydrate 4.7%) were used to prepare the yogurt. Fresh mulberry fruits were obtained in a local market (Korea). Mulberry fruits squeezed by using the gauze and freeze-dried.

Manufacture of yogurt

Milk was mixed with different amounts of freeze-dried mulberry fruit juice powder (1, 3, and 5%) and 10% (w/v) skim milk powder and blended with Lab-blender (MS3040, MTops Misung, Korea) for 5 min. The milk with completely solubilized freeze-dried mulberry fruit juice powder was pasteurized at 90°C for 10 min in water bath. After cooling to 40°C, 0.1% commercial starter culture in freeze-dried form was added and fermented at 36°C for 20 h approximately the acidity reaches from 0.9 to 1.0%. The yogurts were produced in duplicate. After fermentation, all yogurt samples were removed and stored at 4°C for 35 d. The yogurts were produced in duplicate.

pH and acidity

The pH values were measured using a pH meter (AB 15, Fisher Scientific, USA). Yogurt was initially homogenized in water (1: 9 ratio) prior to pH determination. The titratable acidity determined by titration using 0.1 N NaOH to the titration end point of pH 8.2.

Viscosity

Yogurt (100 mL) were placed in a Brookfield viscometer (spindle No. 2, Model LVDV I+, Version 3.0, USA) and measured from 5 to 8 min with 1 min period with 60 rpm. All samples were measured in triplicate.

Sensory evaluation

Sensory evaluations of the yogurts were performed by 20 trained panelists by testing color, flavor, sweet taste, sour taste, and texture. Selection of panelists was performed according to sensory evaluation procedure (Choi *et al.*, 2008). The sensory evaluations of yogurts were rated on a 9-point scale, and high score meant high overall acceptability.

Total phenolic compound and anthocyanin

For total phenolic compounds analysis, the yogurts (1.0

mL) were mixed with 1.0 mL of 95% ethanol and 5 mL of distilled water. These solutions were mixed with 0.5 mL Foline-Ciocalteu reagent (diluted 1:1 with distilled water) and were left to stand for 5 min at room temperature. The one millimeter of 5% Na₂CO₃ was then added to the reaction mixture followed by 60 min incubation at room temperature. Absorbance at 725 nm was converted to total phenolic (mg gallic acid equivalent, mg(GAE)/100 g) using a regression of known concentrations of gallic acid (Sigma Aldrich, Germany) which was determined every time total phenolic content was carried out.

The total anthocyanin contents in yogurt were determined by the pH differential method (Giusti and Wrolstad, 1996) using a UV spectrophotometer. Absorbance of the samples in 0.025 M potassium chloride buffer (pH 1.0) and 0.4 M sodium acetate buffer (pH 4.5) were measured at 520 and 700 nm. The anthocyanin standard, cyanidin-3-glucoside, was used. Data were expressed as mg cyanidin-3-glucoside equivalents per gram of sample (mg CGE/100 g). The absorbance of each sample was calculated as follows:

$$\text{Abs} = (A_{520}B_{700})_{\text{pH}1.0} - (C_{520}D_{700})_{\text{pH}4.5}$$

A : the absorbance at 520 nm of the sample in pH 1.0 buffer solution,

B : the absorbance at 700 nm of the sample in pH 1.0 buffer solution,

C : the absorbance at 510 nm of the sample in pH 4.5 buffer solution,

D : the absorbance at 700 nm of the sample in pH 4.5 buffer solution.

Scavenging activity on DPPH radicals and reducing power

The scavenging activity on DPPH radicals was determined using the method of Blois (1958). The sample (1 g) was homogenized with 10 mL of ethanol. The 0.2 mL of extracted solution was mixed with 0.8 mL of DPPH solution (1.5×10^{-4} M) and left to stand for 30 min in the dark. The absorbance was measured at 517 nm against a blank.

For analysis of reducing power, yogurt (1 g) was mixed with 100 mL of 0.2 mol/L of phosphate buffer, pH 6.5 and 100 mL of 1 g/100 mL of potassium ferricyanide, and, then, incubated at 50°C for 20 min. Two hundred and fifty microlitres of trichloroacetic acid (10 g/100 mL) was added to the mixture and centrifuged at 3000 g for 10 min at room temperature. The resulting supernatant was taken and mixed with 500 mL of H₂O and 100 mL of ferric chlo-

ride (0.1 g/100 mL), and, then, incubated at 37°C for 10 min. The absorbance at 700 nm was measured.

Lactic acid bacteria

The prepared yogurt was diluted with 0.85% sterilized saline by a serial dilution method. Each 1 mL of the diluted solution was plated onto a BCP plate count agar (Eiken Co., Japan) for lactic acid bacteria, according to the pour-plate method. The plates were incubated at 37°C for 48-72 h. The colonies that formed on the plates were counted and expressed as colony-forming units per gram.

Total sugars

To evaluate total sugars, the phenol-sulfuric acid method was used (Dubois *et al.*, 1956). Briefly, 0.5 mL of diluted yogurt was added to 0.5 mL of 5% phenol (Shinyo Pure Chemicals Co., Ltd., Japan). After shaking, 2.5 mL of concentrated H₂SO₄ (Deajung Chemicals & METALS Co., Ltd., Korea) was added. The mixture was left to stand for 30 min and absorbance was read at 480 nm using distilled water as blank in spectrophotometer (V-530, Jasco Co., Japan). D-glucose was employed as standard.

Color values

Color values were measured with color meter (CR-200, Minolta Co., Japan). Data was expressed by Hunter L (lightness), a (redness) and b (yellowness) and ΔE value was calculated with the following equation:

$$\Delta E = (\Delta L^2 + \Delta a^2 + \Delta b^2)^{1/2}$$

Statistical analysis

The physicochemical results are presented as means \pm standard deviation (SD) of triplicate determinations. The lactic acid bacteria experiments were performed twice, and the data were expressed as Log CFU/g for statistical analyses using SAS version 8.01 (2002, Statistical Analysis System, USA). Data were analyzed by one-way analysis of variance. Differences between companies and processes were tested for significance by means of Duncan's multi-range tests. Tests were considered significant at $p < 0.05$.

Results and Discussion

Effects of FDMJ on yogurt fermentation

The pH value and acidity of yogurts with different ratio of FDMJ during fermentation period are shown in Fig. 1. Initial pH of yogurt were 6.29-6.46, and the pH of control

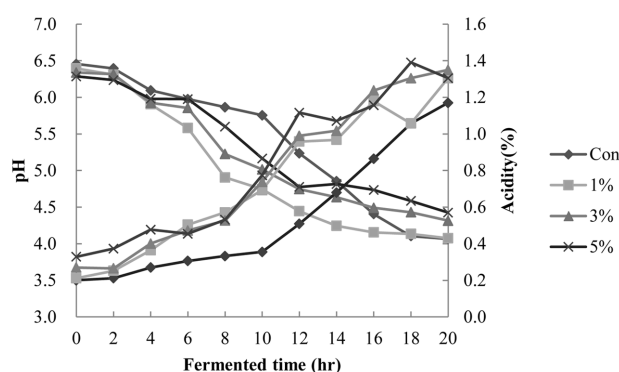


Fig. 1. Changes of pH and acidity in yogurt added with freeze-dried mulberry fruit juice during fermented periods.

decreased during fermentation period. Increasing the period of fermentation from 0 to 20 h, resulted in decrease in the pH values from 6.46 to 4.07. The pH of control was 6.46 and that of 5% treatment was 6.29, pH of yogurt supplemented with FDMJ tended to be lower than control and was found to be dependent on different ratios of FDMJ ($p < 0.05$). During fermentation, decreasing rate of pH in 1% treatment was higher than 5% treatment due to neutralization of lactic acid due to addition of FDMJ (Sung *et al.*, 2005). Lee and Lee reported that pH of yogurt supplemented with 20% barley flour was higher than control (Lee and Lee, 2014). Titratable acidity of yogurt increased by addition of FDMJ due to presence of organic acid in FDMJ. Titratable acid of initial yogurt was 0.20-0.33% and increased during fermentation. Acidity of control increased rapidly after 10 h and in that of treatments was after 8 h. On 18 h of fermentation, acidity value of yogurts was 1.06% (control), 1.06% (1%), 1.31% (3%), and 1.39% (5%), respectively. Production of organic acids such as lactic acid, citric acid, formic acid, acetic acid and butyric acid in yogurt has been reported to be related with accumulation of acid (Billard *et al.*, 2007; Ostlie, 2003). This study supported the fact that addition of FDMJ is effective in shortening fermentation time for yogurt.

Lactic acid bacteria count of yogurts with different ratios of FDMJ during fermentation period is shown in Fig. 2. Initial lactic acid bacteria count was 6.79-6.94 Log CFU/g, the growth rate of lactic acid bacteria in FDMJ added yogurt was higher than control ($p < 0.05$). The count of 5% treatment was 8.58 Log CFU/g at 8 h while control reached 8.91 Log CFU/g at 20 h. The main aim of addition of FDMJ was to promote proliferation of lactic acid bacteria in yogurt. Lee and Hwang (2006) reported that the addition of *Rubus coreanum miquel* was effective in

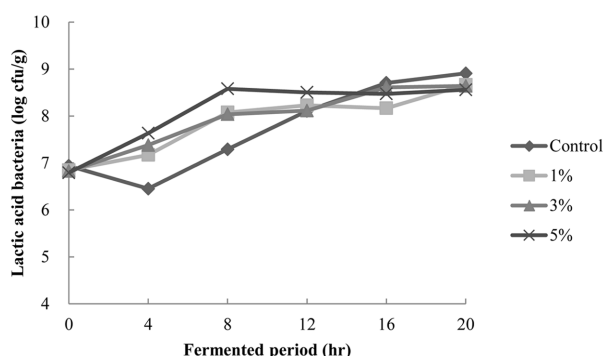


Fig. 2. Changes of lactic acid bacteria count in yogurt added with freeze-dried mulberry fruit during fermented periods.

stimulating the growth of lactic acid bacteria in yogurt due to high content of vitamins, minerals and carbohydrates (Madigan *et al.*, 2003).

Viscosity of yogurts with different ratios of FDMJ is shown in Fig 3. Viscosity development is related to the aggregation of casein micelles and gel formation is attributed to the biochemical and physicochemical changes during fermentation of milk (Dalglish and Law, 1988). In this study, addition of FDMJ reduced viscosity of yogurt and control had the highest value of 186.30 cP, while 5% treatment exhibited value of 101.75 cP ($p < 0.05$). Bae *et al.* (2000) reported that the viscosity of yogurt decreased by adding mugwort extract because high concentrations of supplement in yogurt broke down the coagulated milk (Sung *et al.*, 2005; Tseng and Zhao, 2013).

Sensory evaluation of yogurts with different ratios of FDMJ is shown in Fig. 4. Color score of control was 4.21

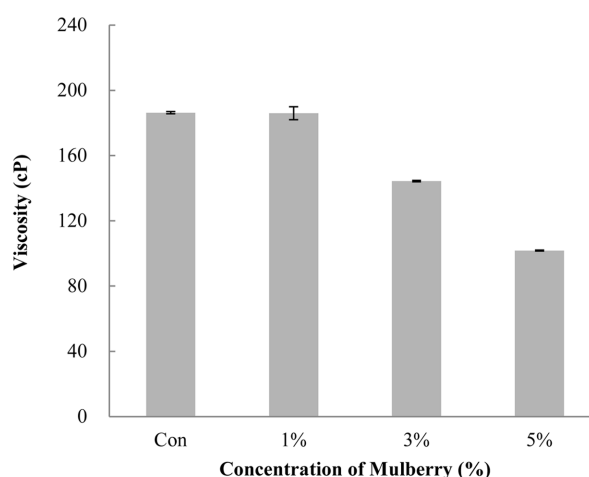


Fig. 3. Viscosity of yogurts with freeze-dried mulberry fruit.

while it was 7.71 in 3% treatment. The best score of texture and taste was observed in 3% and 1% treatments, respectively. In taste evaluation, it was revealed that addition of FDMJ into yogurt was related with sour and sweet taste and high score was obtained by adding FDMJ. Overall acceptability score was the highest in 1% treatment and no difference was seen between control and 3% treatment ($p < 0.05$).

Antioxidant compounds and antioxidant activity

Total phenolic content of yogurts with different ratios of FDMJ during refrigerated storage for the duration of 35 d is shown in Table 1. Initial total phenolic content of FDMJ added yogurt was 51.66, 92.23 and, 132.87 mg

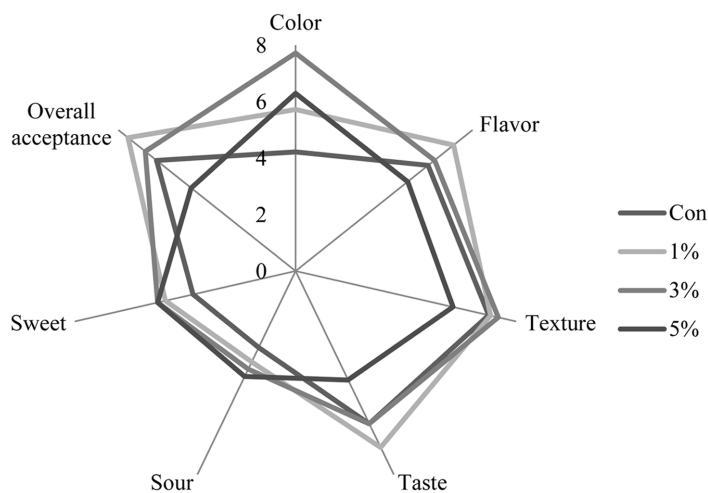


Fig. 4. Sensory evaluation of yogurt added with freeze-dried mulberry fruit juice.

GAE/100 g for 1, 3, and 5% treatments respectively, while that of control was 37.23 mg GAE/100 g. The highest total phenolic content in yogurt was found in 5% treatment because of addition of mulberry ($p<0.05$). The total phenolic content of FDMJ supplemented yogurt increased gradually during storage with maximum values reaching to 64.36, 106.50 and 144.71 mg GAE/100 g in 1, 3, and 5% treatment. Since control contained no FDMJ, the total phenolic content reflected the presence of phenolic compounds related to milk protein breakdown (Shaboo and Ahmad, 2011). The amino acid, tyrosine, has phenolic side chain, which raised the total phenolic content in yogurt (Shah, 2000). Anthocyanin contents also 5% FDMJ supplemented yogurt contained the highest value among all treatments. Anthocyanins are group of natural phenolic compounds responsible for the coloring of many plants, flowers, and fruits (Chena *et al.*, 2006). During storage, an increase in anthocyanin content in yogurts was

observed except for control and the highest level was observed in 5% treatment.

All FDMJ added yogurt had higher antioxidant activity than control throughout the storage period. The higher antioxidant activities in FDMJ added yogurt than in control were most likely contributed by individual mulberry phytochemical contents and because of microbial metabolic activities (Thomson *et al.*, 2007). The antioxidant activities of natural components may have a reciprocal correlation with their reducing powers. In this study, the highest reducing power in yogurt was 1.60 for 5% treatment, while that of control was 0.64. During refrigerated storage of yogurt, reduction in antioxidant activities is attributed to degradation of phenolic compounds with antioxidant activities (Yildiz and Eyduran, 2009) or increasing milk protein-polyphenol interaction (Yuksel *et al.*, 2010).

Table 1. Antioxidant compounds of yogurt added with freeze-dried mulberry fruit during storage at 4°C

| | Time (d) | Con | 1% | 3% | 5% |
|---------------------------------------|----------|--------------|---------------|----------------|---------------|
| Total phenolic content (mg GAE/100 g) | 0 | 37.23±1.52Dc | 51.66±1.62Cb | 92.23±2.09Bc | 132.87±3.11Ad |
| | 7 | 34.41±0.38Db | 53.92±0.75Cb | 99.81±1.49Bb | 137.94±0.27Ac |
| | 14 | 36.44±0.18Da | 64.36±15.99Ca | 104.57±1.23Ba | 140.41±0.03Ab |
| | 21 | 37.47±1.42Da | 56.39±1.26Cab | 105.24±3.93Ba | 145.86±0.12Aa |
| | 28 | 37.62±0.81Da | 57.90±0.09Cab | 102.20±0.85Bab | 144.60±0.62Aa |
| | 35 | 36.71±0.33Da | 59.56±1.12Cab | 106.50±2.64Ba | 144.71±1.15Aa |
| Anthocyanin (mg CGE/100 g) | 0 | 0.00±0.00D | 4.88±0.23Cc | 21.03±1.59Bab | 36.49±0.81Acd |
| | 7 | 0.00±0.00D | 5.32±0.23Cab | 21.57±0.27Bab | 39.85±0.56Aab |
| | 14 | 0.00±0.00D | 5.52±0.13Ca | 22.30±0.63Ba | 37.12±1.67Acd |
| | 21 | 0.00±0.00D | 5.17±0.08Cabc | 20.25±0.38Bb | 34.97±2.54Ad |
| | 28 | 0.00±0.00D | 4.95±0.04Cbc | 20.97±1.53Bab | 37.77±0.80Abc |
| | 35 | 0.00±0.00D | 5.07±0.34Cbc | 21.58±0.64Bab | 40.65±0.03Aa |

GAE, gallic acid equivalent; CGE, cyanidin-3-glucoside equivalent.

^{A-D}Means within row with different superscripts are significantly different by mulberry levels ($p<0.05$).

^{a-d}Means within column with different superscripts are significantly different by periods ($p<0.05$).

Table 2. Antioxidant activity of yogurt added with freeze-dried mulberry fruit during storage at 4°C

| | Time (d) | Con | 1% | 3% | 5% |
|----------------|----------|-------------|-------------|--------------|--------------|
| DPPH (%) | 0 | 16.01±0.32D | 26.28±0.51C | 52.77±1.41B | 74.21±0.45A |
| | 7 | 17.21±0.15D | 25.69±1.33C | 52.89±0.35B | 74.76±0.54A |
| | 14 | 17.84±0.09D | 25.68±1.15C | 52.54±0.39B | 72.81±0.40A |
| | 21 | 14.87±1.11D | 26.82±0.69C | 53.82±0.36B | 73.72±0.93A |
| | 28 | 17.59±0.22D | 26.19±0.10C | 51.59±1.88B | 74.77±0.06A |
| | 35 | 17.10±0.14D | 26.50±0.03C | 52.60±0.15B | 74.35±0.36A |
| Reducing power | 0 | 0.64±0.01Db | 0.84±0.02C | 1.25±0.00Bab | 1.60±0.03Aab |
| | 7 | 0.65±0.00Db | 0.83±0.02C | 1.27±0.01Ba | 1.61±0.02Aa |
| | 14 | 0.66±0.00Da | 0.84±0.02C | 1.24±0.00Bab | 1.59±0.01Aab |
| | 21 | 0.60±0.00Dc | 0.79±0.00C | 1.15±0.02Bc | 1.46±0.00Ab |
| | 28 | 0.62±0.01Dc | 0.81±0.00C | 1.18±0.00Bc | 1.55±0.04Aab |
| | 35 | 0.61±0.00Dc | 0.82±0.00C | 1.22±0.02Bbc | 1.57±0.02Aab |

^{A-D}Means within row with different superscripts are significantly different by mulberry levels ($p<0.05$).

^{a-c}Means within column with different superscripts are significantly different by periods ($p<0.05$).

Table 3. pH and acidity of yogurt added with freeze-dried mulberry fruit during storage at 4°C

| | Time (d) | Con | 1% | 3% | 5% |
|---------|----------|---------------|----------------|----------------|----------------|
| pH | 0 | 4.11 ± 0.01Dc | 4.45 ± 0.00Ca | 4.75 ± 0.00Ba | 4.78 ± 0.01Aa |
| | 7 | 4.11 ± 0.01Dc | 4.26 ± 0.00Ce | 4.62 ± 0.04Bbc | 4.73 ± 0.01Ac |
| | 14 | 4.12 ± 0.00Dc | 4.32 ± 0.01Cc | 4.64 ± 0.01Bb | 4.76 ± 0.00Ab |
| | 21 | 4.16 ± 0.00Db | 4.36 ± 0.01Cb | 4.60 ± 0.00Bc | 4.69 ± 0.01Ad |
| | 28 | 4.17 ± 0.01Da | 4.27 ± 0.01Ce | 4.53 ± 0.03Bd | 4.75 ± 0.00Ab |
| | 35 | 4.08 ± 0.01Dd | 4.30 ± 0.00Cd | 4.59 ± 0.00Bc | 4.69 ± 0.01Ad |
| Acidity | 0 | 0.92 ± 0.05Bb | 0.96 ± 0.01Bc | 0.97 ± 0.01B | 1.07 ± 0.01Ab |
| | 7 | 1.14 ± 0.06a | 1.13 ± 0.08ab | 1.14 ± 0.02 | 1.13 ± 0.11b |
| | 14 | 1.27 ± 0.04Aa | 1.04 ± 0.05Bbc | 1.11 ± 0.01AB | 1.31 ± 0.07Aab |
| | 21 | 1.31 ± 0.09a | 1.25 ± 0.08a | 1.15 ± 0.07 | 1.40 ± 0.03a |
| | 28 | 1.13 ± 0.04a | 1.17 ± 0.01ab | 1.15 ± 0.03 | 1.06 ± 0.07b |
| | 35 | 1.18 ± 0.13a | 1.18 ± 0.03ab | 1.14 ± 0.14 | 1.28 ± 0.09ab |

^{A-D}Means within row with different superscripts are significantly different by mulberry levels ($p < 0.05$).

^{a-d}Means within column with different superscripts are significantly different by periods ($p < 0.05$).

pH and acidity

During storage period, pH and acidity value of yogurt are shown in Table 3. Initial pH was from 4.11 to 4.78 and a decrease for all yogurts was observed during storage period. On day 35, pH of yogurts were 4.08-4.69. Michael (2010) had previously reported decrease in yogurt pH during storage period. The pH value of 3% treatment exhibited about 0.2 unit reduction after 28 d, which could be explained based on high rate of production of lactic acid due to bacterial metabolic activity along with consumption of lactose (Tseng and Zhao, 2013). Initial acidity of yogurts was 0.92-1.07% and total acidity increased in all yogurts during storage period with values reaching to 1.14-1.28%.

Lactic acid bacteria

Lactic acid bacteria counts in yogurt during storage period is shown in Fig. 4. Initial lactic acid bacteria count

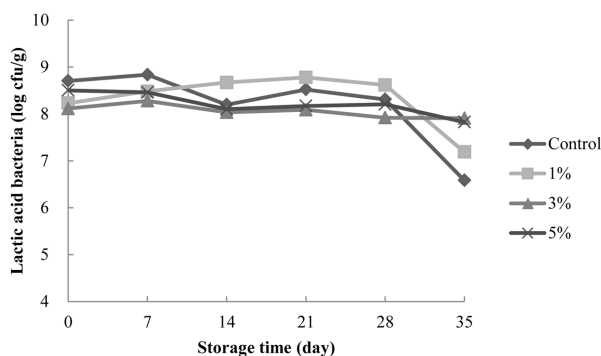


Fig. 5. Changes of lactic acid bacteria count in yogurt added with freeze-dried mulberry fruit during storage at 4°C.

was 8.12-8.70 Log CFU/g. During storage, the value of yogurts decreased by 6.5-7.9 Log CFU/g on 35th d. Jaziri (2009) reported the yogurt microflora was found to be present at sufficiently high levels (8.28-9 Log CFU/g) both at the beginning and the end of the six-week storage period. On 35th d, further decrease in lactic acid bacteria count in control and 1% treatment was observed when compared to 3, 5% treatments. Michael *et al.* (2010) reported that the reduction in *L. bulgaricus* was less plant extract added yogurt compared with the non-added yogurt and *S. thermophilus* counts in all yogurt remained >6 Log CFU/mL for 50 d of storage.

Total sugar

Total sugar content in yogurt during storage are shown in Fig. 5. Initial total sugar contents of control were 4.69% and that of 5% treatment was 6.5%. It has been reported that the main sugars of mulberry were glucose

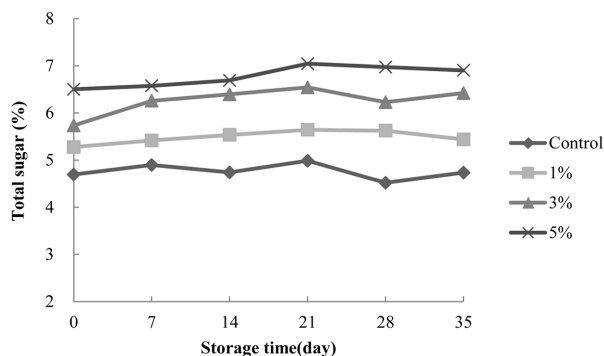


Fig. 6. Changes of total sugar content in yogurt added with freeze-dried mulberry fruit during storage at 4°C.

Table 4. Change of color value on yogurt added with freeze-dried Mulberry fruit during storage at 4°C

| | Time (d) | Con | 1% | 3% | 5% |
|----------|----------|---------------|--------------|--------------|--------------|
| L*-value | 0 | 80.45±0.01Ab | 65.83±0.08Bc | 54.77±0.07Cb | 50.28±0.06Dd |
| | 7 | 81.06±0.05Aa | 68.49±0.02Ba | 58.14±0.04Ca | 51.47±0.01Db |
| | 14 | 79.46±0.19Ac | 65.09±0.40Bd | 54.84±0.11Cb | 52.24±0.00Da |
| | 21 | 80.56±0.06Ab | 66.96±0.01Bb | 55.03±0.10Cb | 51.12±0.06Dc |
| | 28 | 80.45±0.01Ab | 65.86±0.13Bc | 54.94±0.08Cb | 50.93±0.27Dc |
| | 35 | 81.00±0.01Aa | 64.80±0.35Bd | 54.78±0.16Cb | 51.47±0.18Db |
| a*-value | 0 | 0.93±0.01Da | 6.83±0.02Ca | 8.74±0.02Ab | 8.47±0.08Bb |
| | 7 | 0.89±0.01Dab | 6.12±0.01Cb | 8.47±0.00Bd | 8.85±0.05Aa |
| | 14 | 0.72±0.16Dbcd | 6.04±0.03Cc | 8.92±0.03Aa | 8.18±0.16Bc |
| | 21 | 0.79±0.05Dabc | 5.90±0.04Cd | 8.81±0.01Aab | 8.17±0.02Bc |
| | 28 | 0.62±0.05Dcd | 5.90±0.00Cd | 8.61±0.05Ac | 8.03±0.12Bcd |
| | 35 | 0.57±0.01Dd | 5.82±0.04Ce | 8.39±0.10Ad | 7.86±0.13Bd |
| b*-value | 0 | 15.80±0.04Aab | 4.57±0.01Bf | 1.10±0.03Cf | -0.83±0.02De |
| | 7 | 15.59±0.04Ab | 6.27±0.01Ba | 3.37±0.01Ca | 0.16±0.00Dd |
| | 14 | 14.80±0.19Ac | 5.25±0.01Be | 1.34±0.03Ce | 0.38±0.08Dc |
| | 21 | 15.07±0.26Ac | 5.64±0.05Bd | 1.77±0.01Cb | 1.23±0.09Da |
| | 28 | 15.57±0.02Ab | 6.18±0.01Bb | 1.66±0.05Cc | 0.98±0.01Db |
| | 35 | 15.99±0.01Aa | 5.96±0.06Bc | 1.57±0.04Cd | 1.29±0.06Da |

^{A-D}Means within row with different superscripts are significantly different by mulberry levels ($p<0.05$).

^{a-f}Means within column with different superscripts are significantly different by periods ($p<0.05$).

and fructose (Kim *et al.*, 2010; Lee *et al.*, 1998). Yang *et al.* (2014) reported that reducing sugar contents of fresh mulberry was observed to be 6.42%. The 5% treatment contained more reducing sugar than control because of mulberry with lots of sugar content. Most commercial yogurt added flavor and sweeter because its acid taste was not popular and shunned by most consumers. Mulberry yogurt is recommended because mulberry is a natural sweetener with beneficial nutritional properties.

Color value

Color value L, a, and b value of yogurt are shown in Table 4. Increased concentration of FDMJ powder, led to a significant decrease in the L and b values of all yogurts from 50.28 to 90.45 and 15.80 to -0.83, respectively. The a-value increased significantly by increasing the concentration of FDMJ powder in yogurt due to the presence of anthocyanin. Anthocyanins are becoming increasingly important not only as antioxidants, but also as food colorants. In addition, color is a very important sensory evaluation. Because of sensory evaluation of FDMJ, yogurts supplemented with FDMJ had higher sensory values than that of control. A decrease in a value was observed during storage.

Conclusion

As a result of studying a possibility of adding freeze-

dried mulberry into yogurt. antioxidant activity in mulberry added yogurt was higher than that in control. During cold storage DPPH and reducing power of yogurt was maintained like initial yogurts. Mulberry contains a number of polyphenol, which have various health beneficial effects including antioxidant properties, anti-diabetic effect and anti-bacterial properties. However, raw mulberries are extremely perishable due to their high moisture content. Therefore, we produced yogurts with freeze-dried mulberry fruit powder. As the results of this study, we state that freeze-dried mulberry fruit can be added to yogurt for enhancing nutritional value, improving quality of yogurt, improving sensory evaluation and for enhancement of functionality of yogurt.

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