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Quality Characteristics and Antioxidant Activity of Yogurt Prepared with Bitter Melon (*Momordica Charanti*) Juice

Eun-Sun Hwang

Department of Nutrition and Culinary Science, Hankyong National University, Anseong, Korea

Email address
ehwang@hknu.ac.kr

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Abstract: The bitter melon juice (1, 3, and 5%) based on the total weight of milk was incorporated into the plain yogurt and incubated for 24 h at 37°C. The quality characteristics and antioxidant activities bitter melon juice containing yogurt has been investigated. The pH of yogurt decreased upto 4.32~4.39 after 24 h incubation as the amount bitter melon juice was increased. The total acidity of control sample was 0.95% and 3 and 5% bitter melon juice containing yogurt had the 0.99 and 1.06%, respectively after 24 h incubation. Sugar content was decreased as incubation time. In chromaticity determination, lightness of yogurt decreased but redness and yellowness were increased with increasing bitter melon juice content. However, by incubation time, lightness, redness and yellowness were increased. The number of lactic acid bacteria in yogurts increased with increasing of incubation time and showed higher lactic acid bacteria number at 12 and 24 h of incubation. However, there are not much differences among the treatments. The total polyphenols and flavonoids contents were proportionally with increasing the levels of bitter melon juice and antioxidant activity of bitter melon yogurt were significantly higher than control and proportionally increased as the bitter melon juice concentration. These results, suggest that bitter melon juice may be useful ingredient in yogurt to improve tastes and antioxidant potential.

Keywords: Bitter Melon, Yogurt, Lactic Acid Bacteria, Sensory Evaluation, Antioxidant Activity

1. Introduction

Yogurt is a widely enjoyed dairy product that is soured and thickened by specific lactic acid producing cultures added to milk [1]. When bacteria such as *bifidobacterium* and *lactobacillus* are added to ferment lactose, the milk protein coagulates to thicken the milk and increase its unique sour taste [2]. Yogurt is an excellent food with high protein, potassium and calcium content and good nutritional value. It provides numerous vitamins and minerals and is relatively low in calories [3]. Yogurt contains probiotics such as *Lactobacillus acidophilus*, *Lactobacillus casei* and *Bifidus*. These probiotics keep the digestive system healthy by balancing intestinal bacteria, keeping the immune system in good condition, and positively affecting cardiovascular and metabolic processes [4, 5]. Recently, fruit such as strawberry, blueberry, raspberry, peach, plums are added to provide bioactive compounds or natural flavor.

Bitter melon (*Momordica charanti*) belongs to the family of *Cucurbitaceae* and it is a climber and bears oblong fruits resembling cucumber in shape; the young fruits are emerald green in color and turn to orange-yellow on ripening [6, 7]. Bitter melon is traditionally cultivated as a vegetable, although it has a bitter taste, and all parts of the plant, such as fruits and leaves, have been used as folk remedies [8]. It has been consumed for various health benefits, including anti-diabetic, anticancer, antioxidant, anti-inflammatory, anti-bacterial, and anti-obesity effects in cell culture, animal and human clinical studies [9, 10]. Bitter melon is a rich source of dietary fiber, minerals, vitamins, glycosides, phenolics, flavonoids, saponins, alkaloids, proteins, and steroids [11]. The bitter constituents have been characterized as momordicosides K and L, and momodicines I and II [12]. The hypoglycaemic effects compounds have been isolated as triterpenoids, monoterpenes, steroids, alkaloids [13, 14].

Bitter melon is good for a person with high blood sugar levels or diabetes because it has possess insulin-like properties [15], increase insulin secretion [16], increase

tissue glucose uptake [16], and decrease glucose absorption [17]. With these health benefits, bitter melon may be used as functional ingredients in yogurt but it has not been studied until now.

The objective of this study was to investigate the quality and sensory characteristics of yogurt prepared with bitter melon juice. The content of the total polyphenols and flavonoids and antioxidant capacity in yogurt were also determined.

2. Material and Methods

2.1. Materials

Gallic acid, catechin, Folin-Ciocalteu's phenol reagent, 2,2-diphenyl-1-picrylhydrazyl radical (DPPH), and 2,2′-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) were purchased from Sigma Chemical (St. Louis, MO). Culture media MRS broth was from BD, USA. All these chemicals are of analytical grade. Skim milk powder and commercial milk were purchased from local store. Kefir starter powder (TSI Inc. Surrey B. C Canada) was used to prepare the yogurt.

2.2. Preparation of Bitter Melon Juice

Bitter melon (*Momordica charanti*) was purchased from a local farm in Cheonan, Korea in July of 2015 before ripeness. The bitter melon was washed with tap water, removed moisture and grind with food grinder (Black & Decker, Seoul, Korea), and then filtered through a double layer of cheese cloth to obtain the juice, which was used for yogurt making.

2.3. Preparation of Yogurt

For preparation of bitter melon containing yogurt, market milk (Seoul milk, Korea) and skim milk (Seoul milk, Korea) mixture was heated to the boiling point, then cooled down gradually until reached about 30°C. After that, bitter melon juice and oligosaccharide were added due to the property percentages (0, 1, 3 and 5%). The well-mixed mixtures were inoculated with 0.1% of yogurt culture (YO-MIX^{TM}, Danisco Co., Ltd., Copenhagen, Denmark) which was constituted by *Streptococcus thermophilus*, *Lactobacillus casei, L. bulgaricus*, *Bifidobacterium longum* as a starter and incubated at 37°C for 24 h. The yogurt samples were collected and analyzed for 0, 3, 6, 12 and 24 h during fermentation. For the sensory evaluation, yogurts stored at 4°C after 24 h incubation were used. The formula for yogurt incorporated with different amount of bitter melon juice is presented in Table 1.

Table 1. *Formula for yogurt incorporated with various amount of bitter melon juice.*

	Bitter melon juice (%)			
	0	**1**	**3**	**5**
Bitter melon juice (g)	0	5	15	25
Milk (g)	450	445	430	425
Skim milk (g)	50	50	50	50
Starter (g)	2	2	2	2
Oligosaccharide (g)	5	5	5	5

2.4. pH, Total Acidity and Sugar Content Changes in Yogurt During Incubation

The pH of yogurt during fermentation were measured by using a pH meter (420 Benchtop, Orion Research, Beverly, MA, USA. Total acidity determined by titrating 10 mL of diluted yogurt with 0.1 M sodium hydroxide solution until the pH of yogurt reaches at 8.3. The total acidity (%) was calculated using the following equation was expressed as lactic acid amount in yogurt.

Total acidity = {(V × NaOH factor × A × D) 100} / (volume of sample)

V: volume of added NaOH (mL), A: conversion factor = 0.009 (for lactic acid), D: dilution factor, F: factor of 0.1 N NaOH

The sugar content was measured using the refractometer (PR-201α, ATAGO, Japan) and expressed as °Brix.

2.5. Color Determination

Color changes of yogurt during the fermentation were measured using a colorimeter (Chrome meter CR-400, Minolta, Tokyo, Japan) calibrated with standard calibration scale of L^*=97.10, a^*=0.24 and b^*=1.75. The color values were expressed as L^* (lightness), a^* (redness), and b^* (yellowness).

2.6. LAB Counting

MRS broth (Difco, Detroit, MI, USA) agar was used to quantify the LAB viable in the yogurt. The 10 g of yogurt were 10 times diluted with 0.85% saline solution and homogenized with stomacher (Bagmixer R400, Interscience, Saint Nom, France) for 2 min at speed 4. After further dilution, 1 mL of samples was added to MRS broth in petri dishes and then incubated for 48 hr at 37°C. Characteristic colonies appearing on the respective media were counted and multiplied by the dilution factor and expressed as log colony forming units per milliliter (log CFU/mL).

2.7. Total Phenolic and Flavonoid Contents Determination

Total polyphenol content was measured with Folin-Ciocalteu's phenol reagent using the method of Nguyen and Hwang [18]. The sample (200 µL) was mixed with 400 µL of 2% 2N-Folin-Ciocalteu's phenol reagent and reacted at room temperature for 3 min. After 3 min, 800 µL of 10% Na_2CO_3 was added and reacted at room temperature for 1 hr. The mixture was mixed well and absorbance was determined with a microplate reader (Infinite M200 Pro, Tecan Group Ltd., San Jose, CA) at 750 nm. The total polyphenol content in the sample was expressed as the gallic acid equivalents (GAE) contained per gram of the sample using the standard curve of gallic acid.

The total flavonoid concentration in the sample was determined by referring to the method of Nguyen and Hwang [18]. Briefly, 500 µL of sample was mixed with 30 µL of 5% $NaNO_2$ and allowed to react at 25°C for 6 min. Then, 60 µL

of 10% $AlCl_3 \cdot 6H_2O$ was added, and the mixture was kept at room temperature for 6 min. NaOH (1.0 M, 200 µL) was added to the mixture. Finally, 110 µL of distilled water was added, mixed well and then the absorbance at 510 nm was measured with a microplate reader to calculate the total flavonoid concentration in the sample.

The total flavonoid content in the sample was calculated using the catechin standard curve and the flavonoid contained in 1 g of the sample was expressed as catechin equivalents (CE).

2.8. Antioxidant Activity

Antioxidant activities of yogurt were measured with DPPH and ABTS radical-scavenging activity as described by Thi and Hwang [19]. Various concentrations of 48 µL samples and 192 µL of 50 µM DPPH solution were mixed and reacted in the dark for 30 min at temperature. The absorbance at 517 nm was measured with a microplate reader and the DPPH radical-scavenging activity of the sample was calculated by the following equation.

Inhibitory activity (%) = $[(Absorbane_{blank} - Absorbane_{sample})/ Absorbane_{blank}] \times 100$

For ABTS assay, aqueous solution of 7 mM ABTS and 2.45 mM $K_2S_2O_8$ were mixed at a ratio of 1:2 and reacted in the dark for 24 h to produce the ABTS radicals. This solution was diluted with 94% ethanol and adjusted to an absorbance of 0.17 ± 0.03 at 734 nm. The samples (50 µL) and 950 µL of ABTS solution were mixed and reacted in the dark for 10 min at room temperature. The abosorbance at 734 nm was measured with a microplate reader and the ABTS radical-scavenging activity of the sample was calculated by the following equation.

Inhibitory activity (%) = $[(Absorbane_{blank} - Absorbane_{sample})/ Absorbane_{blank}] \times 100$

2.9. Sensory Evaluation

All types of yogurt were subjected to sensory evaluation using 40 panelists after the 24 h of fermentation stored at 4°C. Each panelists were asked to rank the 4 samples for color, flavor, taste, mouth feel, thickness and overall acceptance using 9-point hedonic scale with very poor (score 1) and excellent (score 9). All samples were placed into 50 mL white plastic cups with blinded by coding and were served to the panels in a random order. Water was provided for rinsing of their moth between the sample testing.

3. Statistical Analysis

The results are presented as means ± standard deviation. Statistical analysis was performed by using the statistical analysis system (SPSS software package, version 22.0). Data were compared using one-way analysis of variance; $p<0.05$ was considered significant.

4. Results and Discussion

4.1. pH, Total Acidity, and Sugar Content Changes in Yogurt During Incubation

The pH values, total acidity, and sugar contents of yogurt during incubation for 24 hr at 37°C are presented in Table 2. An overall decreased of pH of yogurts occurred during incubation for 24 h. The initial pH for all yogurts was 6.50~6.68 in control and bitter melon juice treated yogurt at 0 incubation time and pH of control and 1% bitter melon containing yogurt showed higher pH than 3 or 5% bitter melon juice treated yogurts. The pH decreased gradually from 3 hr of incubation. The pH was reached at 4.32~4.39 in both control and 1~5% bitter melon juice added yogurts after 24 h incubation. In the yogurts control and 1% bitter melon juice containing yogurt, the values of pH are higher than the 3 or 5% bitter melon juice containing yogurts. Besides, the reduction of pH almost followed the manner of bitter melon juice concentration. Higher bitter melon juice containing yogurt had faster rates of pH reduction than control and lower amount of bitter melon juice containing yogurt. The yogurt containing 5% of bitter melon juice reached pH at 4.32 while control and 1% bitter melon juice containing yogurt without bitter melon juice showed pH of 4.39 at 24 h incubation. The lactose in the milk is converted to lactic acid when pH drops below pH 5 and the production of lactic acid forms the basic structure and texture of yogurt [20]. The micelles of caseins, a hydrophobic protein, lose their tertiary structure and allows for the semisolid texture of yogurt at below pH 5 [21]. So, dropping of pH is very important in yogurt making and bitter melon juice didn't interfered lowering pH during the fermentation.

Table 2. *Changes in pH, total acidity, and sugar content of yogurt incorporated with various amount of bitter melon juice during incubation.*

Incubation time (hr)	Bitter melon juice (%)			
	0	1	3	5
pH				
0	6.66 ± 0.01^{cE}	6.68 ± 0.01^{dE}	6.57 ± 0.01^{bE}	6.50 ± 0.00^{aE}
3	6.41 ± 0.01^{bD}	6.42 ± 0.01^{bD}	6.40 ± 0.01^{abD}	6.35 ± 0.03^{aD}
6	5.84 ± 0.00^{dC}	5.79 ± 0.00^{cC}	5.75 ± 0.01^{bC}	5.70 ± 0.00^{aC}
12	5.05 ± 0.01^{cB}	5.05 ± 0.01^{cB}	4.99 ± 0.00^{bB}	4.96 ± 0.01^{aB}
24	4.39 ± 0.00^{cA}	4.39 ± 0.01^{cA}	4.34 ± 0.00^{bA}	4.32 ± 0.00^{aA}
Total acidity (%)				
0	0.05 ± 0.01^{aA}	0.05 ± 0.00^{aA}	0.08 ± 0.01^{abA}	0.10 ± 0.02^{bA}
3	0.12 ± 0.01^{abB}	0.10 ± 0.01^{aB}	0.13 ± 0.00^{bB}	0.12 ± 0.00^{abA}
6	0.38 ± 0.00^{aC}	0.39 ± 0.01^{bC}	0.41 ± 0.00^{cC}	0.42 ± 0.01^{cB}

Incubation time (hr)	Bitter melon juice (%)			
	0	1	3	5
12	0.65±0.00^{aD}	0.63±0.03^{aD}	0.65±0.00^{aD}	0.72±0.00^{bC}
24	0.95±0.03^{aE}	0.99±0.03^{abE}	0.99±0.03^{abE}	1.06±0.03^{bD}
Sugar content (°Brix)				
0	23.60±0.14^{aC}	23.15±0.07^{aC}	23.35±0.49^{aC}	23.30±0.28^{aD}
3	23.65±0.07^{cC}	23.30±0.00^{bC}	23.20±0.14^{bC}	23.05±0.0.35^{aD}
6	23.60±0.00^{bC}	23.60±0.00^{bC}	23.50±0.14^{abC}	22.50±0.00^{aC}
12	16.20±0.85^{aB}	16.60±0.85^{aB}	15.40±0.28^{aB}	15.60±0.00^{aB}
24	14.80±0.57^{aA}	14.40±0.00^{aA}	14.40±0.00^{aA}	14.00±0.00^{aA}

Data were the mean ± SD of three separate experiments.
$^{a-d}$Values with the different superscript within the same row are significantly different at $p<0.05$.
$^{A-E}$Values with different superscript letters within the same column are significantly different at $p < 0.05$.

In general, the total acidity increased during incubation time and this going-up trend was almost linear. An overall increased of total acidity of yogurts occurred during incubation for 24 h. The initial total acidity for all yogurts was 0.05~0.10 in control and bitter melon juice treated yogurt at 0 incubation time and 3 and 5% bitter melon containing yogurt showed higher total acidity than control or 1% bitter melon juice treated yogurts. The total acidity of control sample was 0.95% and 3 and 5% bitter melon juice containing yogurt had the 0.99 and 1.06%, respectively after 24 h incubation.

The initial sugar contents in control and bitter melon juice added yogurt was 23.15~23.60. There were no statistical differences in °Brix between control and bitter melon juice added samples during 24 h incubation time. Sugar contents in each yogurt samples reduced following fermented time. After 12 h of incubation time, the sugar content dropped more significantly. The sugar contents in control was 14.80 °Brix and 3 and 5% bitter melon juice containing yogurt had the 14.40 and 14.00 °Brix both at 24 h incubation time.

4.2. Color Evaluation

Color properties of the yogurts during 24 h fermentation are shown in Table 3. L^* values, indicating the brightness, ranged from 49.35 to 52.47 in control and three bitter melon juice added yogurt at 0 incubation time. Control sample showed the highest L^* values and L^* value was decreased in more bitter melon juice added yogurt and 5% bitter melon juice added yogurt showed the lowest L^* value among the samples. By fermentation, L^* values were increased than initial value. L^* value in control sample was increased from 52.47 to 58.53 after 24 h fermentation. L^* value in 3 and 5% bitter melon juice incorporated yogurt was increased from 50.70 to 56.40 and 49.35 to 53.84, respectively after 24 h fermentation. Redness, indicating the a^* values ranged from -2.18 to -1.38 in control and three treatment samples and control and all samples showed negative value from 0 to 24 hr incubation time. Control sample showed the lowest a^* values and a^* value were increased in more bitter melon juice added yogurt and 5% bitter melon juice added yogurt showed the highest a^* value. By fermentation, a^* values were increased than initial value. a^* value in 1% bitter melon juice added yogurt was increased from -1.77 to -1.59 after 24 h fermentation. a^* value in 5% bitter melon juice incorporated yogurt was increased from -1.38 to -1.30 after 24 h fermentation. Yellowness, indicating the b^* values ranged from 3.50 to 4.07 in control and three treated samples at 0 time point. Control sample showed the lowest b^* values and the value were increased in more bitter melon juice added yogurt and 5% bitter melon juice added yogurt showed the highest b^* value among the treatments. By fermentation, b^* values were increased than initial value. b^* value in control sample was increased from 3.50 to 4.53 after 24 h fermentation. b^* value in 5% bitter melon juice incorporated yogurt also was increased from 4.07 to 5.40 after 24 h fermentation. The treated results showed that the yogurts supplemented with bitter melon juice in to different concentrations were darker and more yellow color than the control, which could be attributed by the color of bitter melon juice. Bitter melon juice is a yellowish and may contributed the color of yogurt and decreasing L^* and increasing a^* and b^* values.

Table 3. *Changes in Hunter's color values of yogurt incorporated with various amount of bitter melon juice during incubation.*

	Incubation time (hr)	Bitter melon juice (%)			
		0	1	3	5
L	0	52.47±0.20^{dA}	51.73±0.23^{cA}	50.70±0.24^{bA}	49.35±0.30^{aA}
	3	52.65±0.12^{dA}	51.99±0.24^{cA}	51.15±0.18^{bA}	49.72±0.30^{aA}
	6	55.94±0.34^{cB}	51.48 ±0.36^{bA}	50.32±0.34^{aA}	50.81±0.29^{aA}
	12	56.95±0.51^{cB}	54.82±0.47^{bB}	52.36±0.29^{aB}	51.54±0.49^{aB}
	24	58.53±0.57^{cC}	58.35±0.64^{cC}	56.40±1.90^{bC}	53.84±1.90^{aC}
a	0	-2.18±0.03^{aA}	-1.77±0.02^{bA}	-1.67±0.04^{cA}	-1.38±0.01^{dA}
	3	-1.92±0.03^{aB}	-1.76±0.03^{bA}	-1.58±0.02^{cB}	-1.38±0.01^{dA}
	6	-1.89±0.01^{aB}	-1.71±0.07^{bAB}	-1.58±0.10^{cB}	-1.39±0.01^{dA}
	12	-1.74±0.02^{aC}	-1.69±0.03^{bB}	-1.48±0.03^{cC}	-1.40±0.03^{dA}
	24	-1.73±0.05^{aC}	-1.59±0.02^{bC}	-1.28±0.03^{cD}	-1.30±0.05^{cB}

	Incubation time (hr)	Bitter melon juice (%)			
		0	1	3	5
b	0	3.50±0.11^{aA}	3.87±0.05^{bA}	3.98±0.08^{bA}	4.07±0.08^{cA}
	3	3.65±0.10^{aA}	3.93±0.07^{bA}	3.92±0.05^{bA}	4.24±0.06^{cB}
	6	3.94±0.02^{aA}	4.21±0.09^{bB}	4.26±0.07^{bB}	4.72±0.09^{cB}
	12	4.00±0.03^{aAB}	4.53±0.15^{bB}	4.64±0.14^{bB}	5.31±0.05^{cC}
	24	4.53±0.06^{aB}	5.14±0.10^{bC}	5.23±0.02^{bcC}	5.40±0.20^{cC}

Data were the mean ± SD of three separate experiments.
$^{a-d}$Values with the different superscript within the same row are significantly different at $p<0.05$.
$^{A-D}$Values with different superscript letters within the same column are significantly different at $p < 0.05$.

4.3. LAB Counting

Table 4 shows the LAB counts in yogurts incorporated with various amounts of bitter melon juice. The log number of LAB was detected from 1.08~1.18 CFU/mL before starting the incubation in control and bitter melon juice added samples and the initial number of LAB was not different among samples. After 3 h incubation, the number of LAB was increased rapidly in both control and bitter melon juice treated samples. The number of LAB was detected after 3 hr incubation from 5.93~6.65 log CFU/mL and 1~5% bitter melon juice added samples showed similar number of LAB with control. The number of LAB was increased over 24 h incubation in bitter melon juice added samples. The number of LAB in control and 1% bitter melon juice containing yogurt showing that 11.99 and 12.07 log CFU/mL, respectively. The number of LAB in 3 and 5% bitter melon juice containing yogurt showing that 12.06 and 12.01 log CFU/mL, respectively. There were no significantly differences among the control and three different samples in LAB numbers after 24 h incubation of yogurt. The standard number of LAB in semi-liquid yogurt is 1.0×10^8 CFU/mL [22] and 24 h incubation of yogurt was satisfied this criteria. LAB have very important role in yogurt flavor during fermentation. They convert carbohydrate into lactic acid or other metabolites, hydrolyzed caseins into peptides and free amino acids, and break milk fat into free fatty acids [23, 24]. These reactions produce various flavors in yogurt [25]. Bitter melon juice at 1~5% does not inhibit the growth of LAB compared with the control.

Table 4. *Changes in lactic acid bacteria number (log CFU/mL) of yogurt incorporated with various amount of bitter melon juice during incubation time.*

Incubation time (hr)	Bitter melon juice (%)			
	0	1	3	5
0	1.11±0.00^{aA}	1.08±0.00^{aA}	1.18±0.00^{aA}	1.18±0.00^{aA}
3	6.46±0.04^{abB}	5.93±0.05^{aB}	6.41±0.03^{abB}	6.65±0.06^{bB}
6	9.29±0.25^{aC}	9.22±0.32^{aC}	9.31±0.47^{aC}	9.27±0.26^{aC}
12	11.30±0.52^{aD}	11.40±0.41^{aD}	11.38±0.19^{aD}	11.38±0.26^{aD}
24	11.99±0.48^{aD}	12.07±0.36^{aE}	12.06±0.41^{aE}	12.01±0.77^{aE}

Data were the mean ± SD of three separate experiments.
$^{a-b}$Values with the different superscript within the same row are significantly different at $p<0.05$.
$^{A-E}$Values with different superscript letters within the same column are significantly different at $p < 0.05$.

4.4. Total Phenolic and Flavonoid Contents of Yogurt

Table 5 shows the total polyphenol and flavonoid contents of the yogurt incorporated with different amount of bitter melon juice. The total polyphenol content was detected from 17.47~35.79 mg GAE before starting the incubation in control and bitter melon juice added samples and the initial total polyphenol content was significantly higher in higher amount of bitter melon containing yogurt. During the incubation, the total polyphenol concentration was increased as proportional to the concentration of bitter melon juice and incubation time. The highest total polyphenol content (43.59 mg GAE/g dry weight) was found in the yogurt incorporated 5% of bitter melon juice after 24 h incubation. In contrast, the lowest polyphenol content (20.90 mg GAE/g dry weight) was found in the control. The total polyphenol contents were increased upto 1.5- and 1.2-fold in 1 and 5% bitter melon juice added yogurt, respectively.

Total flavonoid content was ranged from 68.97 to 86.86 in control and three bitter melon juice added yogurt at 0 incubation time. Control sample showed the lowest flavonoid content and the flavonoid content was increased in more bitter melon juice added yogurt and 5% bitter melon juice added yogurt showed the highest content of 86.86 mg CE among the samples. By fermentation, flavonoid content was increased than initial content in control sample was increased from 68.97 to 129.76 after 24 h fermentation. Total flavonoid content in 3 and 5% bitter melon juice incorporated yogurt was increased from 79.70 to 153.94 and 86.86 to 158.22, respectively after 24 h fermentation. After 24 h incubation, the total flavonoid content in the control yogurt was 129.76 mg CE/g. While, the yogurt incorporated 1~5% of bitter melon juice contained higher flavonoid contents than control with ranged from 134.94 to 158.22 mg CE/g. By 24 h fermentation, the total flavonoid content was increased from 1.7~1.9-fold compared to 0 h.

Bitter melon juice added into the yogurt contributed higher

amount of total polyphenols and flavonoids in yogurt. Bitter melon contains relative high amounts of polyphenols, particularly quinic, syringic, 4-quimaric, caffeic and ferulic acids [26, 27] and the average of phenolic contents in bitter melon were 283.58~361.93 ng/mg dry weight [27].

Table 5. *Total polyphenol and flavonoid contents of yogurt incorporated with different amount of bitter melon juice.*

	Incubation time (hr)	Bitter melon juice (%)			
		0	1	3	5
Total polyphenol (mg GAE[1)]/g)	0	17.47±0.79^{aA}	19.85 ±0.26^{bA}	28.93±0.64^{cA}	35.79±1.36^{dA}
	3	18.3 ±1.25^{aA}	20.80 ±0.64^{bA}	29.3 ±0.50^{cA}	37.79±0.76^{dB}
	6	20.7 ±0.95^{aB}	21.71±1.68^{aB}	33.52±0.71^{bB}	38.40 ±0.86^{cB}
	12	20.71 ±0.56^{aB}	26.57±0.65^{bC}	34.65±1.23^{cB}	40.80 ±0.77^{dC}
	24	20.90 ±1.51^{aB}	29.75±2.08^{bD}	33.48±0.41^{cB}	43.59±0.41^{dD}
Total flavonoids (mg CE[2)]/g)	0	68.97±2.48^{aA}	80.85 ±0.47^{bA}	79.7 ±0.75^{bA}	86.86 ±1.74^{cA}
	3	72.89±1.75^{aB}	83.93 ±2.03^{bB}	89.18±0.98^{cB}	88.15 ±2.09^{cA}
	6	71.75±1.04^{aB}	97.53±0.85^{bC}	103.76±1.78^{cC}	99.89 ±5.86^{aB}
	12	105.95±1.82^{aC}	111.36±1.33^{bC}	138.95±1.21^{cD}	115.78±2.86^{bC}
	24	129.76±1.66^{aD}	134.94±0.82^{bD}	153.94±0.27^{cD}	158.22±1.38^{cD}

[1)]GAE = gallic acid equivalent, [2)]CE = catechin equivalent
Data were the mean ± SD of three separate experiments.
[a-d]Values with the different superscript within the same row are significantly different at $p<0.05$.
[A-D]Values with different superscript letters within the same column are significantly different at $p < 0.05$.

4.5. The Antioxidant Activities of Yogurt

The antioxidant activities of the yogurt adding various amount of bitter melon juice is shown in Table 6. The DPPH radical scavenging activity of plain and bitter melon juice containing yogurt was ranged from 30.86 to 33.70 at 0 incubation time. Control sample showed the lowest DPPH radical scavenging activity and 5% bitter melon juice added yogurt showed the highest DPPH radical scavenging activities than other samples. By fermentation, DPPH radical scavenging activities were increased than initial activities. After 24 h incubation, the DPPH radical scavenging activity of the plain yogurt was 58.65% and 1~3% bitter melon containing yogurt had similar antioxidant activity than plain yogurt. The average inhibition of DPPH radical formation in 1 and 5% bitter melon juice added yogurt was 58.53% and 61.73%, respectively at 24 h incubation.

The ABTS radical scavenging activity of plain and bitter melon juice containing yogurt was ranged from 38.30 to 41.07 at 0 incubation time. Control sample showed the lowest ABTS radical scavenging activity and the ABTS radical scavenging activity was increased in more bitter melon juice added yogurt and 5% bitter melon juice added yogurt showed the highest ABTS radical scavenging activities among the samples. By fermentation, ABTS radical scavenging activity was increased than initial ABTS radical scavenging activity in plain and bitter melon containing yogurt. The average inhibition of ABTS radical formation in plain yogurt was 62.92%, whereas it was increased up to 66.37% in 1% bitter melon juice incorporated yogurt after 24 h incubation. The average ABTS radical scavenging activities in 3 and 5% bitter melon juice added yogurt was 65.76% and 71.64%, respectively at 24 h incubation. The higher antioxidant activities in bitter melon yogurt might relate to individual phytochemical contents of the juice and as a result of microbial metabolic activities.

Table 6. *DPPH & ABTS radical scavenging activity (%) of yogurt incorporated with different amount of bitter melon juice.*

	Incubation time (hr)	Bitter melon juice (%)			
		0	1	3	5
DPPH	0	30.86 ±3.25^{aA}	31.19±2.94^{aB}	33.41±4.42^{bA}	33.70 ±2.02^{bA}
	3	37.83 ±5.02^{aB}	37.87±2.74^{aC}	36.1 ±7.28^{aA}	41.94±2.38^{bB}
	6	46.18 ±1.84^{aC}	45.15±0.92^{aA}	46.24±3.54^{aB}	52.52±2.43^{bB}
	12	57.64 ±1.46^{aD}	58.21±3.21^{aD}	58.60 ±1.20^{aB}	59.18±2.25^{aC}
	24	58.65 ±2.67^{aD}	58.53±2.40^{aD}	58.03±2.89^{aB}	61.73±4.29^{bC}
ABTS	0	38.30±1.56^{aA}	42.1 ±0.52^{aA}	41.44±1.03^{aA}	41.07±0.72^{aA}
	3	46.34±1.06^{aB}	44.52±0.57^{aB}	49.86±1.58^{bB}	51.03±1.63^{bB}
	6	54.00±0.95^{aC}	58.92±0.67^{bC}	60.49±0.71^{cC}	69.41±1.27^{dC}
	12	64.02±0.56^{aD}	63.81±0.39^{bD}	65.48±1.29^{cD}	69.72±1.56^{dC}
	24	62.92±0.34^{aD}	66.37±1.13^{bD}	65.76±1.98^{bD}	71.64 ±2.3^{cD}

Data were the mean ± SD of three separate experiments.
[a-d]Values with the different superscript within the same row are significantly different at $p<0.05$.
[A-D]Values with different superscript letters within the same column are significantly different at $p < 0.05$.

4.6. Sensory Evaluation of Yogurt

Table 7 shows the sensory scores of the yogurts incorporated with various amount of bitter melon juice. The color score of control and 1% bitter melon juice containing yogurt showed relatively higher values than other treatments. The control showed the value of 6.55 and 1% bitter melon juice containing yogurt showed the similar score of 6.70 while decreased scores up to 5.25 to 5.30 by adding 3% and 5% of bitter melon juice. The flavor score of yogurt with 1% bitter melon juice showed the highest score of 6.25 and this was similar value with the control. While the flavor scores of yogurt with 3% and 5% bitter melon juice showed lower values of 4.20 and 4.50, respectively. The taste score of control and 1% bitter melon juice containing yogurt showed relatively higher values than other treatments. The taste values of control and 1% bitter melon juice containing yogurt were 6.55 and 6.85, respectively. While, 3% and 5% bitter melon containing yogurt showed lower taste values of 4.85 and 3.90, respectively. In mouth feel and thickness, again, control and 1% bitter melon containing yogurt showed the higher score than 3% and 5% of bitter melon containing yogurt. In overall acceptance, the values of control and 1% bitter melon juice containing yogurt were 6.65 and 7.20, respectively and there were no statistically differences (p<0.05.). While, 3% and 5% bitter melon containing yogurt showed lower values of 4.20 and 3.90, respectively.

There are no statistically differences in color, taste, flavor, mouth feel, thickness and overall acceptance of yogurts in control and 1% bitter melon juice containing yogurts. Usually, bitter melon itself has bitter taste but if we incorporated into the yogurt, the 1% of bitter melon juice in the yogurt did not any adverse effects on the color, taste, flavor, mouth feel, thickness and overall acceptance of yogurt.

Table 7. *Consumer preference of yogurt incorporated with different amount of bitter melon juice.*

Sensory properties	Bitter melon juice (%)			
	0	1	3	5
Color	6.55±1.57^{b}	6.70±1.34^{b}	5.30±1.81^{a}	5.25±1.37^{a}
Flavor	6.10 ± 1.68^{b}	6.25 ± 1.41^{b}	4.20 ± 1.96^{a}	4.50 ± 1.19^{a}
Taste	6.25 ± 1.29^{b}	6.85 ± 1.46^{b}	4.85 ± 1.69^{a}	3.90 ± 1.92^{a}
Mouth feel	6.45 ± 1.39^{b}	7.00 ± 1.21^{b}	4.25 ± 1.55^{a}	3.55 ± 1.97^{a}
Thickness	6.35 ± 1.50^{b}	6.70 ± 1.34^{b}	5.20 ± 1.06^{a}	4.75 ± 1.71^{a}
Overall acceptance	6.65 ± 1.87^{b}	7.20 ± 1.24^{b}	4.20 ± 1.40^{a}	3.90 ± 1.71^{a}

Data were the mean ± SD of three separate experiments.

$^{a-b}$Values with the different superscript within the same column are significantly different at p<0.05.

5. Conclusions

The purpose of this study was to investigate the quality characteristics, LAB number, physiologically active substance, antioxidative activity and sensory characteristics of yogurt by adding 0, 1, 3, 5% of bitter melon juice. As the content of added bitter melon juice increased from 0% to 5%, the pH of yogurt decreased from 4.32 to 4.39. The total acidity of the control was 0.95%, and the acidity increased with increasing incubation time, and the acidity was found to be 00% when 5% of bitter melon was added after 24 h of incubation. As cultivation progressed, the brightness of yogurt decreased in proportion to the amount of bitter melon, but a and b values increased. The number of lactic acid bacteria in yogurt increased with increasing incubation time regardless of the amount of bitter melon added. Total polyphenol and flavonoid contents in yogurt were significantly increased in proportion to the content of bitter melon juice, and the antioxidant activity was significantly higher than that of the control. The sensory characteristics of the yogurt prepared by adding 1% of bitter melon juice and control were superior to those of the other groups.

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