# Antioxidant Activities and Viability of Lactic Acid Bacteria in Yogurt Made from Buffalo Milk with Addition of Blewah (Cucumis melo L Var. reticulatus Naudin) Juice

*by* Djoko Kisworo

**Submission date:** 16-Mar-2021 09:17PM (UTC-0700)
**Submission ID:** 1535119005
**File name:** ioxidant_Activities_and_Viability_of_Lactic_Acid_Bacteria_in.pdf (207.06K)
**Word count:** 5221
**Character count:** 26408

2
International Journal of Advanced Science and Technology
Vol. 29, No.4, (2020), pp. 4788 – 4796

# Antioxidant Activities and Viability of Lactic Acid Bacteria in Yogurt Made from Buffalo Milk with Addition of Blewah (*Cucumis melo* L Var. *reticulatus* Naudin) Juice

B.R.D. Wulandani[1], Djoko Kisworo[1*], Bulkaini[1], Muhammad Yasin[1], Chusnul Chotimah MR[2], Ahmad Fudholi[3*]
[1]Fakultas Peternakan, Universitas Mataram, Jl. Majapahit 62, Mataram 83125 NTB, Indonesia
[2]House of Education, Library and Publication (HELP), Indonesia
[3]Solar Energy Research Institute, Universiti Kebangsaan Malaysia, 43600 Bangi, Selangor, Malaysia
*Corresponding author: djokokisworo@yahoo.com; a.fudholi@ukm.edu.my*

***Abstract***

*The purpose of this study is to determine the effect of the addition of blewah juice (Cucumis melo L var. reticulatus Naudin) on the viability of lactic acid bacteria and the antioxidant activity of yogurt produced and to obtain the best storage time of yogurt based on the viability of lactic acid bacteria and antioxidant activity produced. The results indicated that the phenolic compounds contained in yogurt added with blewah juice did not contain components that had specific bactericidal activity on the culture starter. It was seen that on the first day of refrigerator storage (4°C), the number of lactic acid bacteria remained at $10^8$ log CFU/g and only showed a decrease in the number of lactic acid bacteria to $10^7$ log CFU/g until the end of the storage process in the refrigerator (4°C). Antioxidant activity of yogurt with the addition of blewah juice occurred at the 7th day of storage in the refrigerator. It was assumed that on the 7th day, lactic acid bacteria were able to metabolize phenolic compounds through hydrolysis of phenolic glycosides to aglycone so as to increase antioxidant activity until the 7th day of storage in the refrigerator.*

***Keywords:*** *Yogurt, blewah (Cucumis melo), lactic acid bacteria, antioxidant activities*

## 1. Introduction

Yogurt is a fermented drink obtained from fresh milk by certain lactic acid bacteria such as *Lactobacillus bulgaricus, Lactobacillus acidophilus, Bifidobacterium longum* and *Streptococcus thermophilus*. Lactic acid bacteria will hydrolyze lactose (milk sugar) into lactic acid which increases the acidity of milk accompanied by a decrease in pH which will cause clumping (coagulation) and form compact (curd) solids [1]. Yogurt is known to have antioxidant activity through the culture starter used. The study by Lin and Yen [2] showed that several strains of lactic acid bacteria namely *Lactobacillus bulgaricus, Lactobacillus acidophilus, Bifidobacterium longum* and *Streptococcus thermophilus* had antioxidant activity that had the ability to chelate metal ions, as scavevenging reactive oxygen species (ROS). On the other hand, blewah (*Cucumis melo*) has been also known to have antioxidant activity. The results of Ismail et al. [3] revealed that the flesh of blewah chose hydroxyl radical trapping activity of 67.19 ± 8.90 (g DMSOE/g extract) with the ability to trap DPPH free radicals at 11.9 ± 1.00 ($IC_{50}$ (mg/ mL)) with a total phenolic compound content of 1.68 ± 0.14 (mg GAE/ g extract). Therefore, in this study we intended to examine how the effect of the addition of blewah juice on yogurt activity was produced, and whether it would affect the viability of lactic acid bacteria during the yogurt fermentation and storage process.

The potential of blewah (*Cucumis melo* L var. *reticulatus* Naudin) added to yogurt as a source of antioxidants and its effect on the viability of lactic acid bacteria had



ISSN: 2005-4238 IJAST

never been studied. The following study was to determine the antioxidant activity and viability of lactic acid bacteria for 28 days in refrigerator storage (4°C) in yogurt with the addition of blewah juice (*Cucumis melo* L var. *reticulatus* Naudin). Storage was carried out for 28 days before the maximum shelf life of yogurt at 4°C. This was based on the WFLO Commodity Storage Manual in 2008 that its maximum shelf life was between 35 to 45 days at storage temperatures ranging from 2-4°C.

## 2. Materials and methods

The main material in this study was used blewah obtained around the Yogyakarta city. Bacterial starters used in the yogurt making process were obtained from the Food and Nutrition Culture Collection (FNCC) at the Center for Food and Nutrition Studies, Gajah Mada University, namely: *Lactobacillus bulgaricus* (FNCC 0041) and *Streptococcus thermophilus* (FNCC 0015). The materials used for the calculation of Total Titratable Acid (TTA) during the fermentation process and in yogurt were 0.1N Sodium hydroxide (NaOH), 0.1% phenolphthalein. Material for calculation of Total Phenolic Content (TPC) of yogurt required gallic acid, methanol ($CH_3OH$), Ethanol ($C_2H_5OH$), Reagent Folin-Ciocalteu, sodium carbonate ($Na_2CO_3$) 5%. The ingredients used in the determination of antioxidant activity using 1,1-Diphenyl-2-Picrylhydrazyl (DPPH) radical inhibition assay (1,1-Diphenyl-2-Picrylhydrazyl (DPPH) and Ethanol ($C_2H_5OH$ or $C_2H_6O$).

Equipment for the inoculation and multiplication of starter of lactic acid bacteria, as well as testing of yogurt produced including autoclaves, refrigerators, bottles (dark colored), test tubes, incubators, plastic containers, water baths, centrifuges, freezers, petri dishes, Falcon tubes, goblet, measuring flask, pH meter, and test tube. UV-Vis spectrophotometer was used for testing antioxidant activity and juicer for making blewah juice.

Extraction of blewah juice was based on the work procedure of El-Karmany et al., [4] that was started by washing and peeling the skin part backwards and chopping the fleshy part into small parts and then mixing the fleshy pieces of blewah into the juicer to get the juice that will be added to the yogurt.

Preparation and multiplication of starter cultures of lactic acid bacteria was carried out based on the work procedures contained in the BAL culture starter that the preparation was guided by the Microbiology Laboratory - Center for Food and Nutrition Studies - Gajah Mada University, namely: aseptic by cutting ampoules. Then, were dropped into 3-5 drops of sterile/physiological sodium chloride solution. The suspension formed was then inoculated into MRS broth (50 mL) and incubated at 37°C for 24 hours. Each 12.5 *Streptococcus thermophilus* and *Lactobacillus bulgaricus* cultures from MRS broth were inserted into UHT milk (250 mL) and incubated at 37°C for 24 hours.

The process of making yogurt was based on work procedures according to El-Karmany et al. [4] with several combinations. Other yogurt (yogurt without added blewah juice) and yogurt with the addition of blewah juice are prepared on the same day. Blewah juice (100 mL) was added to previously pasteurized buffalo milk (820 mL) and skim milk (30 mL). Addition of mixed culture starter of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* (50 mL each). The yogurt was then fermented in a water bath (43°C) until the pH reached 4.5 followed by the storage in the refrigerator for storage for 1, 7, 14, 21 and 28 days.

Note: (a) The dose of blewah juice added was based on the results of the research of Wulandani [5] which added extra fruit and leaves of *Ficus glomerata* Roxb in the process of making yogurt. (b) In plain yogurt treatment (control) the addition of 100 mL of blewah juice was replaced with 100 mL of distilled water.

Extraction of yogurt was based on the method [6], each 10 g of yogurt was homogenized with 2.5 ml of sterilized distilled water. The pH of yogurt was determined as 4.0 with HCl (0.1 M). The yogurt was then heated in a water bath (45°C) for 10



ISSN: 2005-4238 IJAST

minutes, followed by centrifugation (5000g, 10 minutes, 4°C), NaOH (0.1M) was added to adjust the supernatant pH, pH 7.0. The supernatant was centrifuged again at (5000g, 10 minutes, 4°C). The supernatant was harvested and stored at -20 °C in the freezer until it was used for further analysis.

For viability of lactic acid bacteria during yogurt storage, 1 ml of the yogurt sample was taken and put it in a bottle containing 9 ml of sterile distilled water. Dilution series up to $10^{-7}$ was made. The following steps were to take 1 ml from each dilution and made a pour plate. Incubated for 48 hours at 37°C and continued by calculating the number of visible colonies. The calculated colony was the number of colonies between 30 and 300. The growth curve of LAB during the fermentation process was obtained from calculating the total colonies of lactic acid bacteria (BAL) using the Total Colony Count method using the quebec colony counter tool starting at 0 o'clock with the observation interval once every hour during the fermentation process and every 7 days during the storage process.

Changes in pH and TTA during storage were carried out with observation intervals every 7 days during the storage process. The sample (3 ml) was mixed with 3 ml $dH_2O$ for pH measurement, while for the calculation of TTA during the yogurt storage process was determined by titration using 0.1N NaOH, namely by: 1 ml of yogurt was put into 9 Erlenmeyer tubes filled with 9 ml $dH_2O$. 3-5 drops of 0.1% phenolphthalein were subsequently added as pH indicators. NaOH (0.1 N) titrated into the solution and the solution mixed completely. The process was repeated until the indicator changed to a constant pink color. After 1 ml of 0.1 NaOH was neutralized with 0.009 g of acid, the amount of acid produced during storage could be determined, by:

$$\text{Percentage of lactic acid} = \text{dilution factor (10)} \times V_{NaOH} \times 0.1\ N \times 0.009 \times 100\%$$

Calculation of total phenolic content in blewah and yogurt was based on the method of Shetty et al. [7]. The phenolic content was calculated with observation intervals every 7 days during the storage process. 1 mL of yogurt extract obtained on each was transferred into a test tube that had a lid and mixed with 1 ml of 95% ethanol and 5 ml of $dH_2O$ and added and mixed with 0.5 ml of 50% (v/v) Folin-Ciocalteu reagent (1:1 with water distillation) for 5 minutes at room temperature. 1 ml of 5% $Na_2CO_3$ was added and left for 60 minutes. The absorbance was read at 725 nm. The absorbance value was converted to TPC and shown in the microgram equivalent gallic acid per milliliter sample. The standard curve was determined by using several gallic acid concentrations (5–60 µg/ml) of 95% ethanol.

The method of determining antioxidant activity using 1-Diphenyl-2-Picrylhydrazyl (DPPH) radical inhibition assay involved an antioxidant reaction with DPPH which was stable in 95% ethanol. The sample extract (250 $\mu$l) was added to 3.8 mL 60 mmol/L DPPH (Sigma-Adrich, Germany)/L methanol 95%. The decrease in absorbance was monitored at 517 nm until a constant reading was obtained. Gallic acid used as a control for blewah sample. The results of constant readings for samples) were used to calculate % DPPH oxidation inhibition [8]:

$$\text{DPPH inhibition percentage} = \frac{A.control\ 517 - A.extract\ 517}{A.control\ 517} \times 100$$

where A was the control absorbance (DPPH and ethanol) while B was the sample absorbance (DPPH, ethanol and sample). The correlation between each concentration and the percentage of free radical capture was plotted and $IC_{50}$ values were calculated by interpolation. Antioxidant activity was expressed with $IC_{50}$ as represented the effective concentration of each extract to capture 50% DPPH free radicals.



ISSN: 2005-4238 IJAST

## 3. Results and Discussion

### 3.1. Total Lactic Acid Bacteria during Storage of Plain Yogurt and Yogurt with the Addition of Blewah Juice

In this study, the blewah juice was added before the fermentation process was carried out to protect yogurt from pathogenic bacteria. It was in accordance with Jaziri et al. [10] that the additional of phenolic compounds (such as those found in fruits and plants) into milk before the fermentation process was carried out to protect the end product (yogurt) against unwanted bacterial pathogens and bacteria. The fermentation process lasted for 7 hours and during the fermentation process, an increase in the number of lactic acid bacteria started from the $0^{th}$ hour to the $7^{th}$ hour of fermentation. At the $6^{th}$ and $7^{th}$ hours of fermentation in yogurt with the addition of blewah juice began to show an increase in the population of lactic acid bacteria ($10^8$ log CFU/g) whereas in the plain yogurt treatment showed an increase in population of $10^8$ log CFU / g lactic acid bacteria in the $7^{th}$ hour of fermentation and did not show a significant difference ($P < 0.05$) to yogurt with the addition of blewah juice (Complete data on the viability of lactic acid bacteria during the fermentation process were not included). The number of lactic acid bacteria on the first day of yogurt storage with the addition of blewah juice was still at $10^8$ log CFU/g (plain yogurt) and in plain yogurt at $10^7$ log CFU/g. The number of lactic acid bacteria remained at $10^7$ log CFU/g in plain yogurt and yogurt with the addition of blewah juice, which only showed significant differences on the first day and day 14 of storage, as seen in Table 1.

**Table 1. Total Lactic Acid Bacteria during the Storage Process of Plain Yogurt Compared to Yogurt Added with Blewah Juice**

| Sample | Lactic acid bacteria observed every 7 days (log CFU/g) | | | | |
|---|---|---|---|---|---|
| | 1 | 7 | 14 | 21 | 28 |
| Plain yogurt | 7.18±0.31[a] | 7.78±0.09[a] | 7.89±0.02[a] | 7.91±0.36[a] | 7.86±0.46[a] |
| Yogurt added with blewah juice | 8.32±0.2[b] | 7.85±0.15[a] | 7.76±0.04[b] | 7.86±0.31[a] | 7.93±0.42[a] |

Notes: Different superscript within similar column showed significant difference between treatments ($P \leq 0.05$).

In the treatment of plain yogurt (control) and yogurt with the addition of blewah juice, it was seen that the concentration of lactic acid bacteria *Streptococcus thermophillus* and *Lactobacillus bulgaricus* decreased during the storage process. According to Rotar et al. [11], one of the important properties of lactic acid bacteria was their ability to degrade carbohydrates through fermentation. During the fermentation process, lactic acid bacteria still had enough carbohydrates to synthesize lactic acid and at the time of storage day 1 to day 28, the viability of lactic acid bacteria decreased which affected their ability to metabolize carbohydrates. The activity of probiotic bacteria decreased because in addition to its faster growth, more reducing sugars were used for thwie growth as well as to form lactic acid, so that the reduction in sugar content decreased. With the reduction of reducing sugars, the substrate that was needed for bacterial growth was also reduced so that this substrate would run out. If the substrate in the media ran out, the growth of lactic acid bacteria would decrease because lactic acid bacteria lacked nutrients for their growth.

Based on the results, it could be seen that the presence of blewah juice was thought to be able to increase the metabolic activity of lactic acid bacteria caused by the production of organic acids in yogurt with the addition of blewah juice was higher associated with higher $H^+$ concentrations than plain yogurt. Organic acids (lactic acid, citric acid, formic acid, acetic acid, and butyric acid) were linearly related to the accumulation of TTA in yogurt. According to Vedamuthu [12], the measurement of pH



ISSN: 2005-4238 IJAST

and TTA was important because the acidification was the key mechanism during yogurt fermentation. The value of TTA in yogurt was the equivalent percentage (%) of lactic acid contained in yogurt during fermentation. It was the total hydrogen ion found in fermented milk samples with the exception of being bound to alkaline ions. The determination of TTA was of relevance for evaluating microbial fermentation capacity. With the decreasing pH value during yogurt fermentation process showed increased acid levels in yogurt. Observation of pH at each time of fermentation (in hours) showed a relatively similar initial pH, which was 6.46 ± 0.01 (plain yogurt) and 6.39 ± 0.02 (yogurt added with blewah juice) was superfluous and showed insignificant difference ($P< 0.05$) The pH value of plain yogurt and yogurt with the addition of blewah juice continued to show a decrease in pH until the 7$^{th}$ hour of fermentation even though it did not show a significant difference ($P<0.05$) in plain yogurt and yogurt added with blewah juice, which was 4.42 ± 0.01 and 4.34 ± 0.07, respectively (complete data on the viability of lactic acid bacteria during the fermentation process were not included).

The pH value on storage day 1 was 4.82 ± 0.07 (plain yogurt) and 4.84 ± 0.03 (yogurt added with blewah juice) and each showed a decrease in pH value on the 28$^{th}$ day of storage in the refrigerator (4$^{o}$C), which was each 4.78 ± 0.04 and 4.55 ± 0.01 which did not show significant differences ($P \geq 0.05$), as seen in Table 2.

**Table 2. pH During the Storage Process of Plain Yogurt Compared to Yogurt Added with Blewah Juice (4°C).**

| Sample | pH observed every 7 days | | | | |
|---|---|---|---|---|---|
| | 1 | 7 | 14 | 21 | 28 |
| Plain yogurt | 4.82±0.07$^{a}$ | 5.23±0.10$^{a}$ | 5.09±0.08$^{a}$ | 5.22±0.01$^{a}$ | 4.78±0.04$^{a}$ |
| Yogurt added with blewah juice | 4.84±0.03$^{a}$ | 4.77±0.21$^{a}$ | 4.79±0.14$^{a}$ | 4.84±0.01$^{b}$ | 4.55±0.01$^{a}$ |

Notes: Different superscript within similar column showed significant difference between treatments ($P \leq 0.05$).

According to Helferich and Westhoff [13], *Lactobacillus bulgaricus* could reduce pH or increased acidity as well as synthesized pyruvic acid which could stimulate the growth of *Streptococcus thermophillus* bacteria so that the acidity value would also increase rapidly. The pH value was inversely proportional to the total titrated acid value so that the higher the total acid titration value, the lower the pH value. pH is a measurement of the $H^{+}$ ion while TTA is a measure of the total organic acid contained in yogurt. With the decrease in pH, the value of TTA continued to increase until the 28$^{th}$ day of storage showed that the extension of yogurt storage time resulted in a decrease in pH and increased TTA values in both yogurt and resulted in a number of lactic acid bacteria ranging from 7.86 ± 0.46 log CFU/g (plain yogurt) and 7.93 ± 0.42 log CFU/g (yogurt with 10% *Ficus glomerata* Roxb fruit extract) which did not show a significant difference ($P> 0.05$) between plain yogurt and yogurt added with blewah juice. The TTA value of plain yogurt and yogurt added with blewah juice on the first day was 0.029 ± 0.00% and 0.033 ± 0.00% respectively, which did not show a significant difference ($P> 0.05$). Further, it continued to show an increase with the decreasing pH value of plain yogurt and yogurt added with blewah juice with TTA values of 0.060 ± 0.00 and 0.085 ± 0.00 respectively which showed significant differences in each treatment ($P <0.05$), as seen in Table 3.



ISSN: 2005-4238 IJAST

**Table 3. Total Titratable Acid (TTA) during Storage Process of Plain Yogurt Compared to Yogurt Added with Blewah Juice**

| Sample | TTA observed every 7 days (%) | | | | |
|---|---|---|---|---|---|
| | 1 | 7 | 14 | 21 | 28 |
| Plain yogurt | 0.029±0.00[a] | 0.033±0.00[a] | 0.045±0.00[a] | 0.049±0.00[a] | 0.060±0.00[a] |
| Yogurt added with blewah juice | 0.033±0.00[a] | 0.065±0.00[b] | 0.068±0.00[b] | 0.075±0.00[b] | 0.085±0.00[b] |

Notes: Different superscript within similar column showed significant difference between treatments (P ≤0.05).

Decreasing the number of acidic bacteria during the final fermentation process until the last day of storing yogurt in the refrigerator due to the accumulation of organic acids. As explained by Joung et al. [14], supplementation of plant extracts was able to support the production of lactic acid bacteria by culture starter, but with the extension of the storage time of yogurt resulted in accumulation of diacetic acid, acetaldehyde, formic acid and lactic acid.

### 3.2. Antioxidant Activity of Yogurt during the Fermentation and Storage Process

In fermented milk products, the presence of culture strains of lactic acid bacteria could degrade the main components of milk such as proteins, carbohydrates and lipids into various forms such as amino acids, peptides, organic acids, free fatty acids and wrong sequence of peptides one function was to increase antioxidant activity [15].

The parameters used to measure antioxidant activity in control yogurt (plain) and yogurt added with blewah juice were $IC_{50}$, a number that indicated the concentration of yogurt which could inhibit a radical activity of 50% [16].

The results obtained during the fermentation process showed a lower antioxidant activity of $IC_{50}$ in the treatment of yogurt added with blewah juice when compared to plain yogurt, even though at the 4$^{th}$ and 6$^{th}$ hours of fermentation there was a higher antioxidant activity of $IC_{50}$ in the yogurt added with blewah juice which showed a significant difference ($P<0.05$) for each observation hour ($IC_{50}$ antioxidant activity data during the fermentation process were not included). This might indicate a change in the structure of phenolic compounds during the fermentation process by lactic acid bacteria which could increase antioxidant activity. The mechanism for the effect of fermentation on oxidative activity in plain yogurt was likely that peptide peptides with antioxidant properties or amino acids during fermentation by *Lactobacillus bulgaricus* and *Streptococcus thermophillus* which continued to the maximum took place on the 1$^{st}$ day of storage in the refrigerator with $IC_{50}$ antioxidant activity values was 876.94 ± 1.83 ppm which was not significantly different ($P> 0.05$) on yogurt added with blewah juice, which was 661.30 ± 1.51 ppm. The antioxidant activity of yogurt added with blewah juice occurred on the 7$^{th}$ day of storage in the refrigerator which was equal to 509.413 ± 1.67 ppm compared to plain yogurt, which was 971.87 ± 0.90 ppm and showed significant differences ($P <0.05$), as seen in Table 4. It was assumed that on the 7$^{th}$ day, lactic acid bacteria were able to metabolize phenolic compounds through hydrolysis of phenolic glycosides into their aglycones so as to increase antioxidant activity until the 7$^{th}$ day of storage in the refrigerator.



ISSN: 2005-4238 IJAST

**Table 4. DPPH Antioxidant Activity $IC_{50}$ during Storage Process of Plain Yogurt Compared to Yogurt Added With Blewah Juice (4°C)**

| Sample | Antioxidant activity observed every 7 days (ppm) | | | | |
|---|---|---|---|---|---|
| | 1 | 7 | 14 | 21 | 28 |
| Plain yogurt | 876.94±1.83[a] | 971.87±0.90[c] | 929.90±1.88[d] | 1105.777±0.77[b] | 1725.581 ±1.91[a] |
| Yogurt added with blewah juice | 661.30±1.51[e] | 509.413±1.679[e] | 751.27±1.47[c] | 862.828±0.84[b] | 2241.439 ±1.71[a] |

Notes: Different superscript within similar column showed significant difference between treatments (P ≤0.05).

At the beginning of the fermentation process, the highest TPC value was in the treatment of yogurt added with blewah juice which was 7.10±0.64 μgGAE/g, with $IC_{50}$ antioxidant activity values which were lower than plain yogurt, which was 415.607 ± 0.99 and the number of lactic acid bacteria was 7.00 ± 0.48 log CFU/g which did not show a significant difference (P> 0.05) to plain yogurt with the number of lactic acid bacteria 7.13±0.29 log CFU/g (data on $IC_{50}$ antioxidant activity during fermentation process were not included). According to Amirdivani and Baba [9], yogurt fermentation affected the bioactive components contained in herbs. It could be assumed that the increase in TPC value contributed to the further breakdown of phenolic compounds during fermentation as a result of microbial metabolic activity.

On storage day 1 at 4°C, the total phenolic content of each yogurt treatment ranged from 7.790 ± 0.53 μg/ g (plain yogurt) and 6.869 ± 0.84 μgGAE/ g (yogurt added with blewah juice). The test results for total phenolic content on storage day 7 began to show a decrease in plain yogurt, but showed an increase in total phenolic content in the yogurt added with blewah juice, which was 9.900 ± 0.33 μgGAE/ g and showed significant differences for each treatment (P <0.05). On storage day 14 in a refrigerator (4°C), yogurt added with blewah juice began to show a decrease in total phenolic content, i.e. to 7.890 ± 0.89 μgGAE/ g.

**Table 5. Total Phenolic Content (μggae/G) during Storage Process of Plain Yogurt Compared to Yogurt Added with Blewah Juice (4°C)**

| Sample | *Total phenolic content* every 7 days (μg GAE/g) | | | | |
|---|---|---|---|---|---|
| | 1 | 7 | 14 | 21 | 28 |
| Plain yogurt | 6.869±0.84[a] | 6.652±0.65[a] | 5.365±0.44[a] | 4.950±0.29[a] | 5.027±0.05[a] |
| Yogurt added with blewah juice | 7.790±0.53[b] | 9.900±0.33[b] | 7.890±0.89[b] | 6.717±0.00[b] | 8.127±0.34[b] |

Notes: Different superscript within similar column showed significant difference between treatments (P ≤0.05).

The decrease in total phenolic content in plain yogurt and yogurt added with blewah juice continued to show a decline until the 28th day of storage in the refrigerator, which was 5.027 ± 0.05 μgGAE/g (plain yogurt) and 8.127 ± 0.34 μgGAE/g compared to 7th day of yogurt storage, as shown in Table 5.



ISSN: 2005-4238 IJAST

Based on a for ementioned explanations, it was assumed that the flavonoid compounds contained in blewah during yogurt fermentation until the 7th day of storage contributed to the hydroxyl group in each of the phenolic compounds that form hydrogen bonds to optimally took place on the 7th day of storage yogurt which was reflected in the $IC_{50}$ antioxidant activity produced. The activity of $IC_{50}$ antioxidant on the 7th day of yogurt storage with the addition of blewah juice was also thought to be due to metabolic activity of lactic acid bacteria, as Papadimitriou et al. [17] suggested that high antioxidant activity in yogurt was due to the metabolism of lactic acid bacteria that were still active even at low temperatures, which mighta be able to change phenolic compounds so it would affect the antioxidant activity produced. There was a decrease in $IC_{50}$ antioxidant activity on the 21st day to 28th day of storage in the refrigerator and it continued to elevate from a high $IC_{50}$ value of 1725.581 ± 1.91 (plain yogurt) and 2241.439 ± 1.71 (yogurt added with blewah juice) and did not show significant differences ($P > 0.05$).

## 4. Conclusion

Based on the results of this study, the phenolic compounds contained in yogurt with the addition of blewah juice did not contain components that had specific bactericidal activity on the culture starter. It was clearly seen that on the first day of refrigerator storage (4°C), the number of lactic acid bacteria remained at $10^8$ log CFU/g and only showed a decrease in the number of lactic acid bacteria to $10^7$ log CFU/g until the end of the storage process in the refrigerator (4°C). Antioxidant activity of yogurt with the addition of blewah juice was occured at the 7th day of storage in the refrigerator. It was assumed that on the 7th day lactic acid bacteria were able to metabolize phenolic compounds through hydrolysis of phenolic glycosides to aglycone so as to increase antioxidant activity until the 7th day of storage in the refrigerator.

## Conflict of Interest

The authors declare that they have no conflict of interest.

## References

[1] Tamime, A.Y., Robinson, R.K., 1999. Yogurt: science and technology. (pp 1-9)2nd Ed. Combridgde : Woodhead Publishing Ltd.
[2] Lin, M., Yen, C. 1999. Antioxidative Ability of Lactic Acid Bacteria. Journal of Agricultural and Food Chemistry, 47: 1460-1466.
[3] Ismail, H.I., Chan, K.W., Mariod, A.A., Ismail, M., 2010. Phenolic content and antioxidant activity of cantaloupe (Cucumis melo) methanolic extract. Food Chemistry, 119 (2): 643-647.
[4] El-Karmany, A.M.M., Abol-Ela, M.F., Hassanein, A.M., 2013. Production of A New Yoghurt- Like Products Fortified With Some Legumes and Sweet, Cereal Crops and Its Evaluation of Fungal Load. Journal of Food and Dairy Sciences, 4(4): 133-148. Mansoura University.
[5] Wulandani, B.R.D., Rahayu, E.S., Marsono, Y., Utami, T., 2017. Aktivitas Antioksidan dan Angiotensin Coverting Enzyme Inhibitor oleh Yoghurt dengan Ekstrak Daun Ficus glomerata Roxb. Agritech Jurnal Teknologi Pertanian, 37(3): 246-255.
[6] Baba, A.S. Najarian, A., Shori, A.B., Lit, K.W., Keng, G.A., 2014. Viability of Lactic Acid Bacteria, Antioxidant Activity and In Vitro Inhibition of Angiotensin-I-Converting Enzyme of Lycium barbarum Yogurt. Arabian Journal for Science and Engineering, 3 (7): 5355-5362.
[7] Shetty, K., Curtis, O.F., Levin, R.E., Witkowsky, R., Ang, W., 1995. Prevention of verification associated with in vitro shoot culture of oregano (Origanum vulgare) by Pseudomonas spp. J. Plant Physiol., 147: 447-451.
[8] Apostolidis, E., Kwon, Y.I., Shetty, K., 2007. Inhibitory potential of herb, fruit, and fungal-enriched cheese against key enzymes linked to type 2 diabetes and hypertension. Innov. Food Sci. Emerg. Technol. 8:46-54.
[9] Amirdivani, S., A.S, Baba. 2011. Changes in yogurt fermentation characteristics, and antioxidant potential and in vitro inhibition of angiotensin-1 converting enzyme upon the inclusion of peppermint, dill and basil. Jounal of LWT - Food Science and Technology, 44: 1458 -1464.



ISSN: 2005-4238 IJAST

[10] Jaziri, I., Slama, M.B., Mhadhbi, H., Urdaci, M.C., Hamdi, M., 2009. Effect of green and black teas (Camellia sinensis L.) on the characteristic microflora of yogurt during fermentation and refrigerated storage. Food Chemistry, 112 (3): 614–620.
[11] Rotar, A.M., Vodnar, D.C., Bunghez, F., Catunescu, G.M., Popo, C.R., Jimborean, M., Semeniuc, C.A., 2015. Effect of goji berries and honey on lactic acid bacteria viability and shelf life stability of yoghurt, Not Bot Horti Agrobo, 43 (1): 1842-4309.
[12] Vedamuthu, E. R. 1982. Fermented milks. In A. H. Rose (Ed.), Economic microbiology: Fermented foods (pp. 199-225). London: Academic Press
[13] Helferich, W., Westhoff, D.C., 1980. All about Yoghurt. New Jersey: Prentice – Hall : 76-81.
[14] Joung, J.Y., Lee, J.Y., Ha, Y.S., Shin, Y.K., Kim, Y., Kim, S.H., Oh, N.S., 2016. Enhanced microbial, functional and sensory properties of herbal yogurt fermented with korean traditional plant extracts, Korean J Food Sci Anim Resour., 36 (1):90-99.
[15] Gjorgievski, N., Tomovska, J., Dimitrovska, G., Makarijojoski, B., Shariati, M.A., 2014. Determination of the antioxidant activity in yogurt. Journal of Hygienic Engineering and Design: 88-92.
[16] Molyneux, P., 2004, The use of the stable free radical diphenylpicryl-hydrazyl (DPPH) for estimating antioxidant activity, Songklanakarin Journal Science and Technology (SJST), 26(2), 211-21.
[17] Papadimitriou, C.G., Vafopoulou-Mastrojiannaki A, Silva, S.V., Gomes, A.M., Malcata, F.X., Alichanidis, E. 2007. Identification of peptides in traditional and probiotic sheep milk yoghurt with angiotensin I-converting enzyme (ACE)-inhibitory activity. Food Chem., 105: 647–656.



ISSN: 2005-4238 IJAST

# Antioxidant Activities and Viability of Lactic Acid Bacteria in Yogurt Made from Buffalo Milk with Addition of Blewah (Cucumis melo L Var. reticulatus Naudin) Juice

ORIGINALITY REPORT

| 7% | 7% | 5% | 3% |
|---|---|---|---|
| SIMILARITY INDEX | INTERNET SOURCES | PUBLICATIONS | STUDENT PAPERS |

PRIMARY SOURCES

| | | |
|---|---|---|
| 1 | repository.um.edu.my<br>Internet Source | 4% |
| 2 | Submitted to Universitas Brawijaya<br>Student Paper | 3% |

| | | | |
|---|---|---|---|
| Exclude quotes | Off | Exclude matches | Off |
| Exclude bibliography | Off | | |

# Antioxidant Activities and Viability of Lactic Acid Bacteria in Yogurt Made from Buffalo Milk with Addition of Blewah (Cucumis melo L Var. reticulatus Naudin) Juice

GRADEMARK REPORT

FINAL GRADE

/0

GENERAL COMMENTS

**Instructor**

PAGE 1

PAGE 2

PAGE 3

PAGE 4

PAGE 5

PAGE 6

PAGE 7

PAGE 8

PAGE 9