Artikel El Latifa Sri Suharto

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ISSN 0126-0472 EISSN 2087-4634
Accredited by DGHE No: 66b/DIKTI/Kep/2011

Media Peternakan, August 2016, 39(2):82-89
DOI: 10.5398/medpet.2016.39.2.82
Available online at http://medpet.journal.ipb.ac.id/

Quality and Antioxidant Activity of Yogurt Supplemented with Roselle during Cold Storage

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(Received 24-11-2015; Reviewed 01-03-2016; Accepted 31-05-2016)

ABSTRACT

The purpose of this study was to determine the quality (physical, chemical, microbiological characteristics), total phenolic content, and antioxidant activity using *1,1-diphenyl-2-picrylhydrazyl radical* **(DPPH) inhibition assay of probiotic yogurt supplemented with roselle flower extract (***Hibiscus sabdariffa L***) during cold storage. The experiment used treatment for types of yogurt as follows: cow's milk probiotic yogurt + roselle, goat's milk probiotic yogurt + roselle, cow's milk yogurt, and goat's milk yogurt. The yogurt was stored in cold storage and evaluated the quality and antioxidant activity variables on days 0, 3, 6, 9, 12, and 15th. The results showed that there were interaction (P<0.05) between types of yogurt and storage time on pH value and total lactic acid bacteria (LAB), but no interaction effect on viscosity. The types of yogurt significantly affected (P<0.05) a_w, total titrable acid (TTA), total phenolic content, and antioxidant activity. Cow's milk probiotic yogurt + roselle and goat's milk probiotic yogurt + roselle were the best yogurt that contributed to a good quality and high antioxidant activity up to 15 d at cold storage.**

Key words: yogurt, roselle, probiotic, antioxidant

ABSTRAK

Tujuan penelitian ini adalah menentukan kualitas (karakteristik fisik, kimia, mikrobiologi), kandungan total fenolat dan aktivitas antioksidan menggunakan uji hambat *1,1-diphenyl-2-picrylhydrazyl* **(DPPH) yogurt probiotik dengan penambahan ekstrak bunga rosella (***Hibiscus sabdariffa L***) selama penyimpanan dingin. Penelitian terdiri atas perlakuan tipe yogurt yaitu yogurt susu kambing, yogurt susu sapi, yogurt susu kambing +** *Lactobacillus acidophilus IIA-2B4* **+ rosella, yogurt susu sapi +** *Lactobacillus acidophilus IIA-2B4* **+ rosella. Yogurt disimpan pada suhu dingin dan dianalisis kualitas dan peubah aktivitas antioksidannya pada hari ke 0, 3, 6, 9, 12, dan 15. Hasil penelitian menunjukkan bahwa terdapat interaksi yang nyata (P<0,05) antara perlakuan jenis yogurt dan lama penyimpanan terhadap nilai pH dan total bakteri asam laktat (BAL), tetapi tidak ada pengaruh interaksi terhadap viskositas. Jenis yogurt berpengaruh nyata (P<0,05) terhadap a_w, total asam tertitrasi (TAT), kandungan total fenolat dan aktivitas antioksidan. Yogurt susu sapi probiotik + rosella dan yogurt susu kambing probiotik + rosella memiliki kualitas fisik, kimia dan mikrobiologi serta nilai aktivitas antioksidan yang paling baik dibandingkan dengan yogurt tanpa pemberian rosella, dan dapat disimpan selama 15 hari pada suhu dingin.**

Kata kunci: yogurt, rosella, probiotik, antioksidan

INTRODUCTION

The development of population welfare that leads to changes in diet has a negative impact on the increase in various kinds of degenerative diseases. Awareness of the enormity of the relationship between food and the possibility of disease incidence has changed the view that food is not just for filling and as a source of nutrients, but also for health. Health has become increasingly important both personally and socially, due to the costs associated with the medication, thus early prevention of health problems is very important. Most of the health complaints are categorized as a disease that can be pre-

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vented by conducting healthy lifestyle. Physical activity and adequate nutrition are essential aspects in influencing a person's health (Altgeld *et al*., 2006).

The existence of functional foods offers a good effect on public health. Typical functional food products are enriched with substances such as probiotics, prebiotics or omega-3 fatty acids. Various scientific publications have shown that health is an important motivation to consume functional foods (Szakály *et al*., 2012).

Milk contains several physiological functional components, including protein, vitamins such as vitamin E and C as well as carotenoids and flavonoids with antioxidant content. Therefore, milk with high antioxidant capacity can provide potential protection for consumers from the exposure of oxidative stress, that becomes the cause of acute and chronic diseases (Dalle-Donne *et al*., 2006; Valko *et al*., 2007). In recent years, there is a significant increase in the popularity of yogurt as a functional food (Granato *et al*., 2010).

Yogurt is a fermented milk product that rich in nutrients, especially when obtained by fermentation of fresh milk or milk solution with lactic acid bacteria, favored by consumers because of its effect in improving the intestinal environment and boost immunity (Michael *et al*., 2010). Yogurt is produced by lowering the pH of the milk protein on isoelectric point (pH 4.6) through the fermentation of lactose into lactic acid using starter bacteria. Yogurt can be differentiated according to the fat content of milk used in the yogurt production (FAO, 2013).

Previously, two Indonesian probiotics, *Lactobacillus plantarum* IIA-2C12 and *Lactobacillus acidophilus* IIA-2B4, were isolated from beef obtained from Indonesian cattle, Peranakan Ongole (Arief *et al*., 2015a). These bacteria had met the requirements to be classified as probiotic (Arief *et al*., 2014). In addition, these bacteria also had displayed a remarkable ability to prevent EPEC-causing diarrhea (Arief *et al*., 2010) and repair the hematology condition of diarrhea suspected rats (Astawan *et al*., 2011), had functional properties for fermented sausage (Arief *et al*., 2014; Afiyah *et al*., 2015) and *Lactobacillus plantarum* IIA-1A5; another strain; categorized as bacteriocin producer (Arief *et al*., 2013; Arief *et al*., 2015b). In this research, indigenous lactic acid bacteria *Lactobacillus acidophilus* IIA-2B4 was used as probiotic.

The addition of roselle extract can be conducted to improve the quality of the yogurt. Roselle (*Hibiscus sabdariffa* L.) is a herbal plant that belongs to the family Malvaceae (Cisse *et al*., 2011). *H. sabdariffa* contains anthocyanin with high antioxidant activity (El Sherif *et al*., 2011). Therefore, it is necessary to conduct a study in order to evaluate the quality (physical, chemical and microbiological characteristics) and antioxidant activity of the yogurt.

MATERIALS AND METHODS

Materials

The materials used in this study were 6 L of cow's milk and 6 L of goat's milk obtained from the milk processing unit "D-Farm" in Bogor Agricultural University, and roselle flower extract. Yogurt cultures used were *Lactobacillus delbrueckii* subsp *bulgaricus* RRAM-01, *Streptococcus salivarius* subsp *thermophilus* RRAM-01 and *Lactobacillus acidophilus* IIA-2B4, collections of the Laboratory of Animal Product Technology, Faculty of Animal Science, Bogor Agricultural University, Indonesia.

Subculturing of the Starter

This was based on bacteria used in the process, namely: yogurt bacteria, *Lactobacillus delbrueckii* subsp *bulgaricus* RRAM-01 and *Streptococcus salivarius* subsp *thermophillus* RRAM-01. *Lactobacillus acidophilus* IIA-2B4 was used as probiotic. Starter-subculturing was conducted by inoculating 10% of yogurt starter into milk that was sterilized in an autoclave at 115°C for 3 min. Subsequently, it was incubated at 37°C for 18 h to form coagulation in order to obtain yogurt.

Making of Roselle Flower (*Hibiscus sabdariffa L*) Extract

Dried roselle flowers obtained from the grower were finely ground into flour, sieved using a 60-mesh sieve. Roselle flower flour was dissolved in the water at a ratio of 20 g: 100 mL and pasteurized at 63-65°C for 30 min. The liquid (upper solution) was separated carefully and moved to another bottle for next process (Tsai *et al*., 2008).

Making of Probiotic Yogurt with the Addition of Roselle Flower

Goat's milk or cow's milk was heated at 85-90°C for 35 minutes, then cooled until the temperature reached 40-45°C. Yogurt starter (*Lactobacillus delbrueckii* subsp *bulgaricus* RRAM-01 and *Streptococcus salivarius* subsp *thermophilus* RRAM-01 and *Lactobacillus acidophilus* IIA-2B4 as probiotic were added to goat's milk or cow's milk. The population used was more than 10^7 CFU/mL, incubated at 37°C for 16 h to form coagulation (plain yogurt). Then, it was added with 1% of roselle extract. Yogurt was stored at cold temperatures (±4°C) with a different storage duration (Donkor *et al*., 2006).

Analysis of Yogurt Quality

TTA Value (Total Titratable Acid). Measurement of Total Titratable Acid (TTA) in samples was conducted to measure the amount of organic acids contained in samples. Yogurt sample as much as 10 mL was added with 3 drops of Phenolphthalein (PP) as an indicator, then the mixture was titrated using NaOH solution (0.1 N) to form a pink color that did not disappear when homogenized. The total value of titratable acid was calculated by converting it to lactic acid percentage (AOAC, 2005).

pH Value. pH measurement was conducted using a pH meter (Schoot Instrument, SI Analytics GmbH, Mainz Deutschland). PH meter was calibrated with buffer solution at pH 4 and pH 7. The electrode was dipped into a 10 mL sample, then this number was recorded as a specific pH value (AOAC, 2005).

Viscosity. The measurement of viscosity was conducted using a rotational viscotester (RION, Tokyo Japan). 100 ml of sample was loaded into the test cell. The rotor was dipped into the sample and allowed to spin until the needle scale pointer stopped at a certain scale. This scale indicates the viscosity of the sample with dPa.s as the viscosity unit (AOAC, 2005).

Water activity (a_w). a_w measurement was conducted using calibrated a_w meter (Novasina AG, Lachen Switzerland) . a_w meter calibration was conducted using saturated NaCl that has an a_w value of 0.75 and $BaCl_2$ at a_w of 0.90. The sample was introduced into the container/chamber on a_w meter, then closed and waited for a few minutes until the a_w value of the sample was analyzed (AOAC, 2005).

Total lactic acid bacteria. Amount of 5 mL of sample was added to the Erlenmeyer flask containing 45 mL of BPW (Buffer pepton water) solution in order to obtain one-tenth dilution (10^{-1}). Furthermore, from 10^{-1} dilution, 1 mL was pippeted to be dissolved into a 9 mL BPW dilution solution to obtain 10^{-2}, and continued until 10^{-8}. 1 mL of sample from 10^{-6} to 10^{-8} dilutions were inoculated into petri dishes and poured with MRS agar media, shaken thoroughly and then incubated at 37°C for 48 h (AOAC, 2005).

Yogurt extraction. Yogurt with- or without the addition of roselle extract was homogenized with 2.5 mL of sterile distilled water. Yogurt was then heated with a water bath (45°C) for 10 min and centrifuged (10,000 rpm, 10 min at 4°C). The supernatant was obtained and NaOH (0.5 M) was added until the pH reached 7.0. The supernatant was centrifuged again (10,000 rpm, 10 min at 4°C), then the precipitate formed was separated and the supernatant obtained was stored at refrigerator temperature until needed for analysis (Shori *et al.*, 2014).

Total phenolic content. Total plenolic content analysis was conducted using Shetty *et al.* (2005) method. 1 mL of yogurt extract was transferred into a tube and mixed with 1 mL of 95% ethanol and 5 mL of dH_2O. Folin-Ciocalteu reagent (50% v/v; 0.5 mL) was added to each sample, then homogenized with a vortex. After 5 min, 1 mL of 5% Na_2CO_3 was added and allowed to stand for 60 min at room temperature. Absorbance was measured at 725 nm. Absorbance values were converted to total phenol and expressed in micrograms of gallic acid equivalent per mililitre (mL) of sample.

Antioxidant activity using radical *1,1-diphenyl-2-picryl hydrazyl* (DPPH) inhibition test. Yogurt extract (250 µL) was added to 3 mL of 60 µM DPPH in ethanol. The decrease in absorbance was observed at 517 nm until constant readings. The readings were compared to control containing 250 µL of dH_2O as the extract replacement (Apostolidis *et al.*, 2006).

Statistical Analysis

The experiment was carried out in three different batches of yogurt (n= 3). Data were expressed as mean ± deviation standard). Randomized block design with factorial 4x6 was used in the research. The first factor was type of yogurt (YSSPR= cow's milk probiotic yogurt + roselle, YSKPR= goat's milk probiotic yogurt + roselle, YSS= cow's milk yogurt, YSK= goat's milk yogurt). The second factor was duration of cold storage (0, 3, 6, 9, 12, and 15 d). The statistical analysis was performed using one way analysis of variance (ANOVA), followed by Tukey test (Steel & Torrie, 1995).

RESULTS

Viscosity

The type of yogurt and storage duration significantly (P<0.05) affected the viscosity of yogurt, but did not interact each other. The observation of viscosity is presented in Table 1. Viscosity describes the consistency of a foodstuff. Table 1 shows the average viscosity of goat's milk probiotic yogurt+roselle stored at refrigerator temperature (4°C) (Table 1) that demonstrated the highest score at 3.51±1.60 dPa.s, while the lowest average viscosity was showed by cow's milk yogurt at 1.96±0.65 dPa.s. Goat milk had higher total solid and higher fat content than cow's milk, so that the viscosity of goat's milk yogurt was higher than cow's milk.

Water Activity (a_w)

The type of yogurt significantly (P<0.05) affected the a_w, but the storage duration and the interaction between them did not significantly affect the a_w. a_w values in yogurt ranged from 0.85-0.86 (Table 2).

In the cow's milk probiotic yogurt with the addition of roselle extract, a_w value decreased on day-6 of storage and increased on day-9 of storage, then a_w value decreased again on the day-12 of storage and increased on day-15 of storage, whereas in the cow's milk yogurt a_w values on day-0 to day-9 of storages were relatively similar, then decreased on day-12 of storage. In goat's milk probiotic yogurt with the addition of roselle extract, a_w value decreased on the day-6 of storage and increased again on the day-12 of storage, then dropped on the day-15 of storage. In goat's milk yogurt, a_w value decreased on the day-3 of storage and the a_w values were relatively similar until the day-9 of storage and finally decreased again on the day-15 of storage. Water activities were affected complex chemical reaction by many factors such as pH value, viscosity and texture of the product.

pH Value

The pH value is affected by many factors such as products from lactic acid bacteria metabolism, addition of flavor, colorings and other food additives. Table 3 shows the decrease in the pH value of the four types of yogurt.

Table 1. Viscosity of yogurt during cold storage temperatures (dPa.s)

Storage duration (Days on-)	Type of yogurt				Average
	YSSPR	YSS	YSKPR	YSK	
0	1.90±0.35	1.27±0.55	2.43±1.50	2.37±1.03	1.99±0.96[d]
3	2.20±0.50	1.70±0.53	2.93±1.96	2.67±1.03	2.38±1.11[cd]
6	2.33±0.50	1.90±0.62	3.30±1.85	3.07±1.12	2.65±1.15[bcd]
9	2.57±0.42	2.07±0.57	3.77±1.66	3.40±1.01	2.95±1.13[abc]
12	2.77±0.42	2.27±0.57	4.13±1.72	3.83±0.85	3.25±1.18[ab]
15	3.07±0.45	2.53±0.67	4.47±1.48	4.27±0.74	3.58±1.15[a]
Average	2.47±0.54[b]	1.96±0.65[b]	3.51±1.60[a]	3.27±1.06[a]	

Note: Mean in the same row or column with different superscripts differ significantly (P<0.05); YSSPR= cow's milk probiotic yogurt + roselle; YSKPR= goat's milk probiotic yogurt + roselle; YSS= cow's milk yogurt; YSK= goat's milk yogurt.

Table 2. A_w value of yogurt during cold storage temperatures

Storage duration (Days on-)	Type of yogurt				Average
	YSSPR	YSS	YSKPR	YSK	
0	0.86±0.01	0.86±0.00	0.85±0.01	0.86±0.01	0.85±0.01
3	0.86±0.01	0.86±0.01	0.85±0.01	0.85±0.02	0.86±0.01
6	0.85±0.01	0.86±0.00	0.84±0.01	0.85±0.03	0.85±0.02
9	0.87±0.00	0.86±0.01	0.84±0.01	0.85±0.02	0.85±0.02
12	0.85±0.00	0.85±0.02	0.85±0.02	0.87±0.01	0.85±0.01
15	0.86±0.01	0.85±0.01	0.84±0.01	0.84±0.01	0.85±0.01
Average	0.86±0.01[a]	0.86±0.01[a]	0.85±0.01[b]	0.85±0.02[ab]	

Note: Mean in the same row with different superscripts differ significantly (P<0.05); YSSPR= cow's milk probiotic yogurt + roselle; YSKPR= goat's milk probiotic yogurt + roselle; YSS= cow's milk yogurt; YSK= goat's milk yogurt.

Table 3. pH value of yogurt during cold storage temperatures

Storage duration (Days on-)	Type of yogurt			
	YSSPR	YSS	YSKPR	YSK
0	3.72±0.03[f]	4.64±0.07[a]	3.51±0.01[f]	4.50±0.11[ab]
3	3.71±0.09[f]	4.46±0.10[ab]	3.52±0.03[f]	4.28±0.07[bc]
6	3.73±0.05[f]	4.39±0.12[abc]	3.52±0.01[f]	4.21±0.09[cd]
9	3.71±0.08[f]	4.32±0.15[bc]	3.52±0.05[f]	4.19±0.13[cd]
12	3.74±0.07[ef]	4.29±0.16[bc]	3.53±0.04[f]	4.17±0.13[cd]
15	3.69±0.04[f]	4.17±0.21[cd]	3.52±0.03[f]	3.98±0.09[de]

Note: Mean in the same row or column with different superscripts differ significantly (P<0.05); YSSPR= cow's milk probiotic yogurt + roselle; YSKPR= goat's milk probiotic yogurt + roselle; YSS= cow's milk yogurt; YSK= goat's milk yogurt.

The pH value of yogurts added by roselle was lower than yogurt without roselle. The type of yogurt, storage duration, and the interaction between them significantly (P<0.05) affected the pH value of yogurt. The pH values obtained were in the range of 3.51-4.64. This result was relevant with the elevation of the titratable acidity value of yogurt that was counted as total lactic acid, and acids from roselle addition. This reduction of pH value enhanced the sour and unique flavor of yogurt during storage (Paseephol *et al.*, 2009; Astawan *et al.*, 2012).

Total Titratable Acid (TTA)

The type of yogurt was significantly (P<0.05) affected total titratable acid (TTA) in yogurt (Table 4), but the storage duration and the interaction between them were not significantly (P>0.05) affecting total titratable acid (TTA) in yogurt. The total titratable acid obtained ranged from 0.92%-2.08%. TTA values of 4 types of yogurt during storage still met the Indonesian National Standard (INS) of Yogurt Quality (BSN, 2009).

Total Lactic Acid Bacteria (LAB)

The total population of lactic acid bacteria in a yogurt product becomes an indicator of microbiological quality of the product. The type of yogurt, storage duration and the interaction between them significantly (P<0.05) affected the total LAB (Table 5).

Total Phenolic Content

The result of this study showed that total phenolic content of four types of yogurt with different storage duration ranged between 28.17-64.37 µg GAE/mL. Type of yogurt significantly (P<0.05) affected total phenolic content (Table 6), but the storage duration and the interaction between them did not significantly (P>0.05) affect total phenolic content.

Table 4. Total titratable acid (TAT) value of yogurt during cold storage temperatures (%)

Storage duration (Days on-)	Type of yogurt				Average
	YSSPR	YSS	YSKPR	YSK	
0	1.59±0.13	0.81±0.12	2.09±0.28	1.09±0.15	1.39±0.54
3	1.58±0.15	0.87±0.10	2.09±0.37	1.16±0.09	1.43±0.51
6	1.60±0.13	0.90±0.10	2.09±0.31	1.21±0.07	1.45±0.49
9	1.59±0.13	0.91±0.10	2.06±0.31	1.24±0.11	1.45±0.47
12	1.59±0.13	0.92±0.09	2.06±0.36	1.28±0.02	1.46±0.47
15	1.58±0.14	1.11±0.32	2.10±0.36	1.56±0.33	1.59±0.45
Average	1.59±0.12^{b}	0.92±0.17^{d}	2.08±0.28^{a}	1.26±0.21^{c}	

Note: Mean in the same row with different superscripts differ significantly (P<0.05); YSSPR= cow's milk probiotic yogurt + roselle; YSKPR= goat's milk probiotic yogurt + roselle; YSS= cow's milk yogurt; YSK= goat's milk yogurt.

Table 5. Total lactic acid bacteria (LAB) of yogurt during cold storage temperatures (log cfu/mL)

Storage duration (Days on-)	Type of yogurt			
	YSSPR	YSS	YSKPR	YSK
0	9.02±0.19^{a}	9.43±0.13^{a}	8.67±0.29^{a}	9.49±0.01^{a}
3	8.77±0.19^{a}	9.43±0.08^{a}	8.32±0.45^{a}	9.44±0.09^{a}
6	8.72±0.45^{a}	9.41±0.14^{a}	7.86±0.61^{ab}	9.38±0.08^{a}
9	8.55±0.39^{a}	9.39±0.06^{a}	6.43±1.28^{bc}	9.33±0.22^{a}
12	8.53±0.06^{a}	9.33±0.05^{a}	6.40±1.43^{bc}	9.33±0.12^{a}
15	8.33±0.44^{a}	9.20±0.55^{a}	6.02±1.37^{c}	9.25±0.34^{a}

Note: Mean in the same row or column with different superscripts differ significantly (P<0.05); YSSPR= cow's milk probiotic yogurt + roselle; YSKPR= goat's milk probiotic yogurt + roselle; YSS= cow's milk yogurt; YSK= goat's milk yogurt.

Antioxidant Activity Value (DPPH)

The results showed that the lowest antioxidant activity was shown by goat's milk yogurt, while the highest antioxidant activity produced by goat's milk probiotic yogurt with the addition of roselle extract. Results of analysis of variance showed that the type of yogurt significantly (P<0.05) affected the antioxidant activity (Table 7), but the storage duration and the interaction between them did not significantly (P>0.05) affect the antioxidant activity. According to the IC_{50} calculation, four types of yogurt have the IC_{50} between 9.63–15.06 ppm and IC_{50} value of vitamin C of 15.07 ppm (Table 8).

DISCUSSION

The effect of storage duration treatment in the refrigerator temperature at 4°C on the viscosity of yogurt was progressively increasing. This is due to the influence of the low temperature in the refrigerator, causing clots in the yogurt. According to Astawan *et al.* (2012), the cooling and the storage process after fermentation increased viscosity caused by protein hydration and compaction of yogurt gel structure. The changes of milk acidity affected protein isoelectric point and changed the protein solubility. In this research, beside affected by the metabolism yield acidity of the starter bacteria that convert lactose into lactic acid, pH value was also affected by roselle addition. The activity of microorganisms converted milk lactose into lactic acid followed by lowering pH. The decrease in pH depends on the activity and amount of LAB in producing lactic acid. Not only lactic acid bacteria, but also roselle contributed to pH value of yogurt. Roselle extract has low pH value as 2.00 with total titratable acid 0.27%.

The total titratable acid in yogurt was inversely proportional to the pH value. This is due to the higher amount of acid produced that caused the higher decrease in pH. This statement can be proven in Table 4, in which the average total titratable acid in goat's milk

Table 6. Total phenolic content of yogurt during cold storage temperatures (μg GAE/mL)

Storage duration (Days on-)	Type of yogurt				Average
	YSSPR	YSS	YSKPR	YSK	
0	51.70± 4.98	31.72± 0.72	63.51± 8.72	25.25±3.17	43.05±16.62
3	50.72± 5.72	34.21± 4.20	65.62± 5.29	27.99±6.27	44.63±16.02
6	55.54± 1.33	43.33±10.56	61.79± 3.65	27.61±5.16	47.07±14.62
9	55.93± 3.97	37.79± 7.00	63.92±12.18	28.40±5.29	44.68±16.21
12	51.49± 9.18	34.90± 4.41	58.40±12.82	29.55±3.65	45.42±14.31
15	54.72±10.08	35.94± 3.43	72.96± 9.17	30.21±3.35	48.46±18.60
Average	53.35± 5.95^{b}	36.31± 6.22^{c}	64.37±9.02^{a}	28.17±4.21^{d}	

Note: Mean in the same row with different superscripts differ significantly (P<0.05); YSSPR= cow's milk probiotic yogurt + roselle; YSKPR= goat's milk probiotic yogurt + roselle; YSS= cow's milk yogurt; YSK= goat's milk yogurt.

Table 7. Antioxidant activity value of yogurt during cold storage temperatures (%)

Storage duration (Days on-)	Type of yogurt				Average
	YSSPR	YSS	YSKPR	YSK	
0	79.31±12.31	56.83±21.51	70.65±30.12	42.04±16.70	62.21±23.36
3	86.57± 4.61	42.54± 5.32	86.62± 3.32	53.91±13.16	67.41±21.48
6	83.20± 3.93	57.08±32.85	86.81± 2.87	45.36±14.30	68.11±23.87
9	90.14± 1.54	66.71±26.02	78.00±16.67	44.89± 2.24	63.09±21.00
12	83.84± 8.00	45.62±12.04	77.31± 8.87	52.65± 8.76	71.70±18.95
15	85.97± 3.68	60.98±16.91	78.58±15.39	38.87± 8.95	66.10±21.71
Average	84.84± 6.58[a]	54.96±19.75[b]	79.66±14.57[a]	46.29±11.27[b]	

Note: Mean in the same row with different superscripts differ significantly (P<0.05); YSSPR= cow's milk probiotic yogurt + roselle; YSKPR= goat's milk probiotic yogurt + roselle; YSS= cow's milk yogurt; YSK= goat's milk yogurt.

Tabel 8. The concentration of antioxidant and IC_{50} value from type of yogurt and Vitamin C

Sample	Type of yogurt/ concentration	Average of antioxidant activity (%)	Antioxidant concentration (ppm)	IC50 (ppm)
Type of yogurt	YSSPR	84.84±6.58	25.98±2.06	9.63±0.03[a]
	YSS	54.96±19.75	16.62±6.19	15.06±0.18[b]
	YSKPR	79.66±14.57	24.36±4.57	10.27±0.12[a]
	YSK	46.29±11.27	13.91±3.53	17.99±0.21[b]
Vitamin C	0 ppm	0.00± 0.01	0.00±0.00	
	5 ppm	19.23± 0.01	5.43±0.01	15.07±0.20
	10 ppm	35.59± 0.01	10.55±0.01	
	20 ppm	64.52± 0.00	19.62±0.00	

Note: Mean in the same column with different superscripts differ significantly (P<0.05); YSSPR= cow's milk probiotic yogurt + roselle; YSKPR= goat's milk probiotic yogurt + roselle; YSS= cow's milk yogurt; YSK= goat's milk yogurt.

probiotic yogurt with roselle has the highest with the lowest pH value. Nutrient composition of goat milk that was used in this research were 87.34% moisture, 0.8% ash, 5.22% fat, 3.9% protein, and 0.19% crude fiber based on a wet basis. While, cow's milk had 2.8% fat and 2.7% protein based on wet basis. Higher nutrient composition of goat milk than cow's milk caused yield of the fermentation process from lactic acid bacteria on goat's milk produced higher acidity than cow's milk. Beside that, roselle liquid extract also contributed to total titratable acid of yogurt. Roselle liquid extract has high total titratable acid 0.27%.

According to Prayitno (2006), the difference in lactic acid levels is due to the different of lactose contents in different dairy ingredients, thus affecting the lactose breakdown rate and lactic acid synthesis. During 15 days of storage, lactic acid levels tended to decrease. Changes in lactic acid levels during storage were also proportional to the changes in the number of microbes in yogurt. The decreased of the lactic acid level was related to the reduction of lactose as a main source of carbon for the bacteria. This is due to a decrease in the number of LAB cells closely related to a decrease in the pH of the product due to accumulation of organic acids as metabolites result of fermentation process (Shah, 2009).

The addition of rosella extract also affect the total LAB due to phenolic compounds in roselle extracts. Yogurt with roselle supplementation had lower total lactic acid bacteria than yogurt without roselle supplementation. Phenolic compounds of roselle are flavonoids (anthocyanin). Phenol compounds has antibacterial activity by interacting with the bacterial cells through the absorption process involving hydrogen bonding, and disrupted the cytoplasmic membrane so that the metabolism becomes inactive and bacterial growth will be inhibited (Kao *et al*., 2009). The phytochemical rosella extract also contains saponins and flavonoids. Saponins can act as an antibacterial, by penetrating the cell membranes of the microorganisms, disrupting cell wall and causing cell lysis (Soetan *et al.*, 2006).

The highest total phenolic content was showed by goat's milk probiotic yogurt product supplemented with roselle, and the lowest total phenolic content was showed by goat's milk yogurt product without roselle supplementation. Roselle could increase the total phenolic content in the product. The total phenolic of roselle extract was 66.20 μg GAE/mL, with antioxidant activity was 86.86%.

Roselle has antioxidant substances; one of them is anthocyanin pigment. Anthocyanin is a pigment as flavonoid from polyphenol compounds with carbon structure C6C3C6 (Sari *et. al.*, 2015), so that the total phenol of roselle included anthocyanin compounds. Anthocyanins have antioxidant benefits by acting as an electron donor or transferring hydrogen atom to free radicals (Widagdha *et. al.*, 2015). Oxidation reaction can produce free radicals that have unpaired electrons which in turn can start chain reactions. Antioxidants prevent chain reactions by removing free radical intermediates and inhibit other oxidation reactions. These unpaired electrons in pairs with the presence of an antioxidant (hydrogen donor / electron) (Valko *et al.*, 2007).

Roselle also contributed to the antioxidant activities of yogurt. The antioxidant mechanism of antioxidant compounds were inhibition and oxidation preventing of fat, so it could protect cells from oxidative damage by free radicals such as singlet oxygen, superoxide, peroxyl radicals, hydroxyl radicals and peroxynitrite. In this research, yoghurt without roselle supplementation had antioxidant activities, although lower than yoghurt with roselle addition. Lactic acid bacteria have high antioxidant activity, which could increase the antioxidant activity in yoghurt and preventing lipid peroxidation. The ability of lactic acid bacteria to break down protein (proteolytic) into small peptides (bioactive peptides) and secondary metabolites from bacterial metabolism (Zhang, 2011).

IC_{50} is a number that indicates the product concentration (micrograms/milliliter) that is able to inhibit the oxidation process by 50%. The smaller the IC_{50} value means higher antioxidant activity (Molyneux, 2004). According to the IC_{50} calculation, four types of yogurt have the IC_{50} between 9.63–15.06 ppm and IC_{50} value of vitamin C of 15.07 ppm (Table 8). IC_{50} of yogurt with roselle addition were lower than yogurt without roselle supplementation.

CONCLUSION

Roselle extract contributed to quality of goat's milk and cow's milk probiotics yogurt. Cow's milk probiotic yogurt with the supplementation of roselle extract had the highest antioxidant activities. The four types of yogurt were still good to be consumed up to 15 d at 4°C storage.

ACKNOWLEDGEMENT

This research was partially supported by the Grant for Research and Community Services from DIKTI of the Indonesian Ministry of Education and Culture. The article processing cost also supported by the IBIKK program from DIKTI of the Indonesian Ministry of Education and Culture 2016.

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