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Yoghurt Fortified Formulation of *Lakum* Fruit (*Cayratia trifolia* (L.) Domin) Extract as an Antioxidant

Weni Puspita[1,2], Nurkhasanah[1*], Ika Dyah Kumalasari[1]
[1]Ahmad Dahlan University, Yogyakarta; [2]Akademi Farmasi Yarsi, Pontianak

ABSTRACT

Lakum (*Cayratia trifolia* (L.) Domin) contains a lot of anthocyanin. Anthocyanin in Lakum fruit has a high stability if stored at low temperatures, acidic conditions, and without light. The acidic product such as yogurt can provide an optimum condition for anthocyanin stability and antioxidant activity. The purpose of this study was to optimize yogurt formulation fortified by lakum fruit extract as an antioxidant according to the requirements of the Indonesian National Standard (SNI). Lakum fruit extract was formulated in yogurt with various concentrations at 0%, 5%, 7.5% and 10%. Evaluations were carried out including tests of physical, chemical and microbiological properties and anthocyanin test and antioxidant of yogurt. The results showed that the yogurt formula fortified *lakum* fruit extract at 7.5% concentration resulted highest anthocyanin stability and antioxidant activity that fulfilled SNI requirements and the preferred level of respondents' preference with anthocyanin levels of 53.35±1.04 mg/L, antioxidant activity 69.15±0.24%, t_{90} 7.97 days, total of lactic acid bacteria (LAB) $48.2x10^7$ colony/gram, fat 3.72±0.03%, pH 3.87±0.03, total acid 0.83±0.06%, viscosity 639.07±2.06 cP, protein of 4.90±0.11%, hedonic test, 6.88 (like), and organoleptic purple (5), the distinctive aroma of *lakum* fruit (4.95), sour taste (4.85), fine texture (4.9) and slightly liquid viscosity (4.85).
Key words: Formulation; lakum, *cayratia trifolia* (L.) Domin; antioxidant; yogurt

INTRODUCTION

Lakum contains a considerable amount of anthocyanin pigments, it also contains flavonoid, saponin and alkaloid compounds which have antioxidant activity value IC_{50} about 67.383 ppm (Yeo, and friends. 2012). Panarigas and Idiawati (2015) used *lakum* fruit anthocyanin pigment extract with concentration of 5%, 7.5%, and 10% applied as natural coloring for cold beverages and agar-agar jelly. This information shows other use of *lakum* fruit aside from natural coloring is to give added value to the food product, i.e. as antioxidant.

Anthocyanin in *lakum* fruit extract has enough stability if kept in low temperature, acidic condition, and without light (Neliyanti and Idiawati, 2014). On this research, *lakum* fruit extract is formulated in yoghurt, which is a beverage made of milk fermented by lactic acid bacteria such as *Streptococcus thermophilus* and *Lactobacillus bulgaricus*, where this bacteria could transform lactose into lactic acid. The presence of lactic acid causes low acidity in yoghurt. Thus, this research is expected to improve the anthocyanin stability and antioxidant activity through formulation in *lakum* fruit extract fortified yoghurt products with low acidity. *Lakum* fruit extract fortified yoghurt is formulated with concentration of 0%, 5%, 7.5% and 10% to find out which formula has the best stability of anthocyanin and antioxidant. Based on the background above, this research needs to be done conducted find out the formula on *lakum* fruit fortified yoghurt making method so it has better anthocyanin stability and antioxidant activity.

***Corresponding author : Nurkhasanah**
Email : nurkhasanah@pharm.uad.ac.id

METHODOLOGY

Tools and Ingredients

Tools used on this research are UV-Visible spectrophotometry (Shimadzu®) type 1700, autoclave (Hirayama®), oven (Memmert®), laminar air flow (Mascotte®), and incubator (Memmert®). The ingredients on this research are *lakum* fruits, full cream milk, pure culture of *Streptococcus thermophilus* and *Lactobacillus bulgaricus*, as well as honey. The yoghurt making formula (Table I).

Yoghurt

Making Method

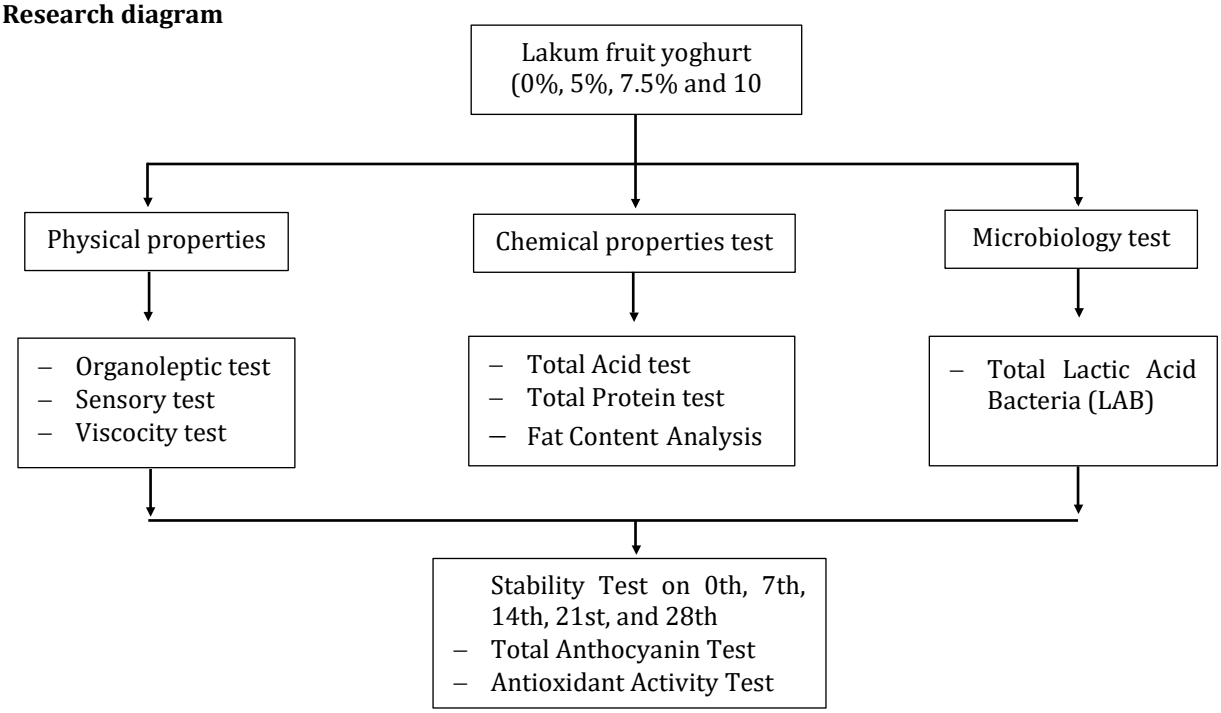
Yoghurt making includes mixing bacteria starter *S. Thermophilus* and *L. Bulgaricus* (1:1) each about 2.5 ml and stirred slowly. Pasteurizing 100 ml full cream milk with temperature of 80°C about 15 minutes, cooling the milk to 45°C, and then adding bacteria culture about 5 ml, followed with

Table I. Yoghurt Making Formula

Materials	Composition			
	FI	FII	FIII	FIV
Full cream milk (13 %)	100 ml	100 ml	100 ml	100 ml
Starter culture (% b/v)	5	5	5	5
Lakum fruit extract (% b/v)	0	5	7.5	10
Honey (% b/v)	8	8	8	8

Ref: Starter culture uses *S. Thermophilus* and *L. Bulgaricus* (1:1 b/v)

Research diagram



fermentation incubation in the incubator (temperature 40°C) for 16 hours. Then *Lakum* fruit extract (*Cayratia trifolia* (L.) Domin) and honey is added.

Yoghurt quality testing

Yoghurt quality testing is performed by analyzing physical-chemical and microbiological characters such as organoleptic, sensory, viscosity, pH, content of lactic acid, total protein, fat content, and lactic acid bacteria total, as well as stability testing by keeping it on 4°C then anthocyanin total testing using UV-Vis Spectrophotometry and activity of antioxidant using dpph method on the 0th, 7th, 14th, 21st and 28th day.

RESULTS AND DISCUSSION

Lakum fruit extract on this research is obtained by extracting using water solvent through heating for 120 minutes on 70°C. This temperature is chosen based on research result by Neliyanti and Idiawati (2014) which shows that the optimal anthocyanin pigment extraction from *lakum* fruit is on temperature of 70°C. Further, *lakum* fruit extract is formulated in the form of yoghurt using lactic acid bacteria *Streptococcus thermophilus* and *Lactobacillus bulgaricus,* where these bacteria can turn lactose into lactic acid. The addition of *lakum* fruit extract to yoghurt aims to use *lakum* fruit extract as natural coloring and adds functional benefit of yoghurt as an antioxidant product due to the anthocyanin compound content. (Yeo, and friends. 2012).

The result of stability of anthocyanin content on *lakum* fruit extract and *lakum* fruit extract fortified yoghurt (Table II) average total value of anthocyanin on *lakum* fruit extract fortified yoghurt on day 28 is higher than *lakum* fruit extract which is around 22.21±2.78 mg/L-42.94±2.36 mg/L, while the average of anthocyanin content on *lakum* fruit extract on day 28 is around 21.54±2.32 mg/L-36.99±0.93 mg/L. Moreover, based on expiration date determination

Table II. Stability of Anthocyanin Content on *Lakum* Fruit Extract and *Lakum* Fruit Extract Fortified Yoghurt

Anthocyanin Content (mg/L)						
Sample	Day 0	Day 7	Day 14	Day 21	Day 28	t_{90} (day)
Lakum 5%	48.57±1.46^{aD}	43.78±0.84^{aD}	38.41±3.27^{aC}	27.50±1.04^{aB}	21.54±2.32^{aA}	3.63
Lakum 7.5%	61.73±1.13^{bD}	56.00±1.60^{bD}	46.90±1.62^{bC}	36.93±0.88^{bB}	28.92±0.63^{bA}	3.89
Lakum 10%	71.17±0.43^{cD}	67.32±0.64^{cD}	60.73±1.36^{cC}	48.57±1.46^{cB}	36.99±0.93^{cA}	4.51
Plain	0	0	0	0	0	0
Yoghurt 5%	38.99±2.79^{aC}	36.37±2.92^{aC}	35.40±2.11^{aC}	23.29±1.11^{aB}	22.21±2.78^{aA}	5.24
Yoghurt 7.5%	53.35±1.04^{bC}	51.32±2.14^{bC}	50.18±1.75^{bC}	40.10±1.06^{bB}	36.82±1.64^{bA}	7.95
Yoghurt 10%	67.02±3.95^{cC}	65.48±2.87^{cC}	62.00±1.72^{cC}	45.67±1.78^{cB}	42.94±2.36^{cA}	6.63

Ref: $^{a-c}$ on the same column with different lowercase shows a significant difference in *lakum* fruit extract concentration level ($p<0,05$); $^{A-D}$ on the same row with different capital letter shows significant difference with storage time ($p<0,05$)

Table III. Stability of Antioxidant Activity on *Lakum* Fruit Extract and *Lakum* Fruit Extract Fortified Yoghurt

	% of Inhibition				
Formula	Day 0	Day 7	Day 14	Day 21	Day 28
Lakum 5%	50.91±0.52^{aD}	43.20±1.29^{aD}	37.48±0.38^{aC}	30.14±0.07^{aB}	25.54±1.01^{aA}
Lakum 7.5%	59.08±1.20^{bD}	57.26±1.01^{bD}	49.79±0.47^{bC}	39.47±0.28^{bB}	30.14±0.07^{bA}
Lakum 10%	64.64±0.68^{cD}	61.11±0.47^{cD}	52.53±1.06^{cC}	40.88±1.80^{cB}	32.88±0.64^{cA}
Plain	45.48±1.88^{aC}	39.97±0.62^{aC}	35.95±0.69^{aC}	27.36±0.89^{aB}	19.82±0.80^{aA}
Yoghurt 5%	60.07±0.57^{bC}	55.01±0.68^{bC}	49.79±0.47^{bC}	39.47±0.28^{bB}	30.63±0.73^{bA}
Yoghurt 7.5%	69.15±0.24^{cC}	64.64±0.68^{cC}	60.07±0.57^{cC}	52.53±1.06^{cB}	44.61±0.56^{cA}
Yoghurt 10%	75.79±0.72^{dC}	70.85±0.51^{dC}	64.64±0.68^{dC}	57.26±1.01^{dB}	49.79±0.58^{dA}

Ref: $^{a-d}$ on the same column with different lowercase shows a significant difference in *lakum* fruit extract concentration level ($p<0,05$); $^{A-D}$ on the same row with different capital letter shows significant difference with storage time ($p<0,05$)

(t_{90}) of *lakum* fruit extract and *lakum* fruit extract fortified yoghurt formula on reaction order one shows that t_{90} of yoghurt is around 5.24-7.95 days. Meanwhile, on *lakum* fruit extract shows a shorter t_{90} which is around 3.63-4.51 days. The degradation of quality during storage is influenced by reaction such as oxidation, flavor compound degradation, texture changes due to water contribution and color changes (Irsyad and friends. 2017). This shows that *lakum* fruit extract formulated on the form of yoghurt with low acidity can improve its anthocyanin content stability. It is in accordance to the research by Neliyanti and Idiawati (2014) which stated that anthocyanin in *lakum* fruit has a high stability if kept on low temperature, acidic condition, and without exposure to light.

A reaction can be determined as order reaction one if the reaction rate depends on the single reactant concentration. On this type, a single substance directly degrade into one product or more. The reaction is directly proportional with substance concentration that reacts. It is in accordance to research result by Hayati, and friends. (2012), that the total reduction degradation rate of anthocyanin on rosella flower petals toward heating temperature timing follows the first order of reaction. Besides, the same result is also found by Ulfiah (2014) that the changes of anthocyanin content on maltodextrin coated purple sweet potato on capsule form follows the 1st order of reaction. Thus, based on storage stability in 28 days, *lakum* fruit extract fortified yoghurt with the best stability is 7.5% concentration, so it can be used as foodstuff for 7.95 days if kept on temperature 4°C or refrigerated.

Stability of Anthocyanin Activity on *Lakum* Fruit Extract and *Lakum* Fruit Extract Fortified Yoghurt

The stability result of antioxidant activity on *lakum* fruit extract and *lakum* fruit extract fortified yoghurt (Table III) 10% *lakum* fruit extract yoghurt formula has the highest antioxidant activity (% of inhibition), where the higher

Table IV. Evaluation Result of *Lakum* Extract Fortified Yoghurt

Evaluation	Result				
	F I Plain	F II *Lakum* 5%	F III *Lakum* 7.5%	F IV *Lakum* 10%	Standard Indonesia National
Total BAL	$42,10^7$±2,0[a]	$47,10^7$±1,0[b]	$48.2,10^7$±0.52[bc]	$49.5,10^7$±0.55[c]	Min. 10^7
Fat Content	4.13±0.09[c]	3.95±0.04[bc]	3.73±0.03[b]	3.52±0.04[a]	Min. 3.0%
pH	4.25±0.13[c]	4.06±0.06[bc]	3.87±0.03[b]	3.62±0.03[a]	Max. 4.5
Total Acid	0.74±0.2[a]	0.78±0.12[b]	0.83±0.06[bc]	0.90±0.15[c]	0.5-2.0%
Viscosity (cP)	825.20±4.18[c]	762.78±3.87[bc]	639.07±2.06[b]	510.88±3.15[a]	-
Protein Content	2.80±0.12[a]	3.26±0.4[ab]	4.90±0.11[b]	5.04±0.28[c]	Min. 2.7%

Ref: [a-c] on the same line with different lowercase shows a significant difference in *lakum* fruit extract concentration level ($p<0,05$)

anthocyanin compound content on the extract, the higher the antioxidant activity. It is due to antioxidant substances on the *lakum* extract pigment, such as anthocyanin pigment (Widhiana, *et al.,* 2012). Moreover, the average value of yoghurt antioxidant activities of *lakum* extract on day 0 is higher than *lakum* extract approximately between 60.07±0.57%-75.79±0.72%, meanwhile the average % of inhibition of *lakum* extract is around 50.91±0.52%-64.64±0.68%, due to plain yoghurt formula has antioxidant activity (45.48±1.88%) obtained from components in yoghurt, so % of inhibition in yoghurt is higher with antochyanin substances from *lakum* extract and components in yoghurt. This is in line with the research of Mohamed *et al.,* (2014) stating that yoghurt as functional foodstuff due to the bioactive compound components such as peptide active and amino acid acting as antioxidants, thus the stability and product quality of *lakum* extract in yoghurt formula will increase.

Evaluation Result of *Lakum* Extract Fortified Yoghurt

Total BAL (Table IV) indicates that yoghurt produced on each treatments are ranged from 42.10^7±2,0 colony /gram-$49.5,10^7$±0.55 colony/ gram. Produced yoghurt formula still meet the standard amount of BAL corpuscle, at least 10^7 colony/gram (SNI, 2009). The addition of *lakum* extract may improve growth rate of BAL because *lakum* extract has carbohydrate substance used by BAL to produce lactic acid as primary product (Kumar *et al.,* 2012). The combination of *Lactobacillus bulgaricus* with *Streptococcus thermophillus* give a better growth, since during incubation period yoghurt starter provides nutrients as stimulator for second growth of bacteria (Muhsinin *et al.,* 2016).

The evaluation result of yoghurt (Table IV) indicates that the highest fat content is in plain yoghurt (4.13±0.09%), the lowest fat content is in 10% *lakum* extract-fortified yoghurt (3.52±0.04%). Fat content obtained in this research is in accordance with SNI (2009) that is minimum 3.0%. Fat content in yoghurt is determined by its base material, full-cream milk which contain high fat. According to the fat content value obtained, it can be observed that increased concentration of *lakum* extract is able to lower fat content of yoghurt because of water content of *lakum* extract is high, in accordance with research result of Mulyani *et al.,* (2016) stating that the higher water content of extract, the lower the fat content of *Soyghurt* from plaintain rinds.

Evaluation result of yoghurt (Table IV) indicates that the lowest pH is in 10% *lakum* extract yoghurt (3.62±0.03), while the highest pH is in plain yoghurt (4.25±0.13%). The pH value of each yoghurt formulas obtained indicate normal pH range value for yoghurt product, as stated by Pereira, Barros, and Ferreira (2013) formation of lactic acid induces sour taste in yoghurt and maximum pH 4.5. The low pH value of yoghurt with the addition of *lakum* extract may be influenced by the acidity of *lakum* pigment extract and the symbiosis between lactic acid bacteria with antioxidant substances. Moreover, low pH in product has a role in suppressing the growth rate of other unwanted microorganisms (Widagdha and Nisa, 2015).

The evaluation result of yoghurt (Table IV) shows that the lowest acid amount in plain yoghurt (0.74±0.2%), while the highest acid amount is in 10% *lakum* extract yoghurt (0.90±0.15). Acid amount obtained in this research is in accordance with SNI (2009) that is between 0.5-2.0%. Level enhancement of *lakum* extract addition linearly affect lactic acid enhancement rate associated with *β-gal* become more effective with active compounds such as flavonoid and anthocyanin derivatives that are high within *lakum* extract. It has a high lactic acid level, as the application of mixed starter *Lactobacillus bulgaricus* might

release valine, glycine, and histidine amino acid required by *Streptococcus thermophilus*, otherwise *Streptococcus thermophilus* helps reduce pH and produce formic acid to stimulates *Lactobacillus bulgaricus* growth (Muhsinin *et al*., 2016).

The evaluation result of yoghurt (Table IV) shows that the highest viscosity is found in plain yoghurt (825.20±4.18 cP) and the lowest viscosity is in 10% *lakum* extract yoghurt (510.88±3.15 cP). Viscosity impairment in *lakum* extract fortified yoghurt may be influenced by the addition of *lakum* extract into yoghurt. The higher concentration of *lakum* extract added, the greater the amount of free water content available so that the viscosity decreases. This is in line with the research result by Febrihantana *et al.,* (2015) stating that with the increased concentration of carrot juice with high water content, the viscosity of yoghurt produced decreases. Furthermore, the inoculation of lactic acid bacteria starter *Lactobacillus bulgaricus* and *Streptococcus thermophillus* in the amount of 5% with 1:1 ratio may produce better yoghurt viscosity.

The evaluation result of yoghurt (Table IV) shows that the highest protein content is in 10% *lakum* extract yoghurt (5.04±0.28%), and the lowest protein content is in plain yoghurt (2.80±0.12%). Protein content obtained in this research is in accordance with SNI (2009) that is minimum 2.7%. High protein content in yoghurt with the addition of *lakum* extract is due to the protein, carbohydrate, water, vitamin, and mineral contents in *lakum* fruits (Kumar *et al.,* 2012). Mulyani *et al.,* (2016) stating that the protein content of yoghurt is determined by the quantity of additional ingredients, the higher the protein content in additional ingredients, then the higher the protein content of yoghurt produced.

Based on organoleptic and sensory test, the plain yoghurt used as comparison is rather disliked by panelists with score of 3.74. Meanwhile the *lakum* extract fortified yoghurt is liked by panelists with score of (6.28-6.88). The best result of hedonic test for yoghurt produced with score of 6.88 (liked) and organoleptic purple (5), distinctive aroma of *lakum* (4.95), sour taste (4.85), fine texture (4.9), and rather liquid viscosity (4.85) is *lakum* extract yoghurt with 7.5% concentration. It is in line with the research by Panarigas and Idiawati (2015) applying purple *lakum* extract as natural coloring that has high anthocyanin pigment in cold beverages and agar jelly, where according to organoleptic test the addition of *lakum* extract is most preferred at a concentration of 7.5%.

Anthocyanin has antioxidant benefits by acting as electron donor or hydrogen atom transfer on free radicals (Widhiana *et al.,* 2012). The research of Neliyanti and Idiawati (2014) explained that anthocyanin in *lakum* extract has high stability if stored in low temperature, acidic state, and with no exposure to light. Where the influence of long duration of sunlight and light on *lakum* extract may cause the degradation of absorbance, since water when exposed directly to sunlight can stimulate the formation of hydrogen peroxide (H_2O_2), that is able to decomposed color-producing compound that may cause the color to fade, resulting in a decline of antioxidant activity. Moreover, the addition of pH may cause an increase in absorbance value which affects the color change in anthocyanin extract, so the lower the pH added, the brighter the color and the antioxidant activity will increase.

CONCLUSION

Lakum extract fortified yoghurt (*Cayratia trifolia* (L.) Domin has better anthocyanin stability and antioxidant activity, meeting the requirements of Indonesian National Standard (SNI/Standar Nasional Indonesia), and at concentration of 7.5% provides the most likability from respondents.

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