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OPTIMIZATION OF JAMBLANG *(Syzygium Cumini (L.) Skeels)* FRUIT EXTRACT LEVELS IN YOGHURT FORMULATION AS ANTIOXIDANT

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Abstract

The reactivity of oxidant compounds that exceed the limits of antioxidant protection ability can cause chain reactions which cause interference with the system. Prevention is needed to neutralize the oxidants, so that exogenous antioxidants are needed. One example is the jamblang fruit (Syzygium cumini (L)) which has high antioxidant activity due to its natural anthocyanin. The purpose of this study was to determine the optimal form of yogurt with the addition of jamblang fruit extract with a concentration of 0%, 5%, 7.5% and 10% and the quality of SNI. Tests carried out include physical, chemical and microbiological tests, namely sensory, acid content, total protein, fat content, and a number of bacterial starters, as well as safety tests with storage at 4° C then 0, to 7, 14th, 21st and 28th day. Data analysis using MANOVA. The results of the effect of the product showed that the fruit of yogurt with 10% extract level provided good stability during storage. Decreased antioxidant levels on day 7 to day 28. Antioxidant activity is more stable than antioxidant jamblang fruit extract. The antioxidant activity of yogurt with 5% jamblang fruit extract; 7.5% and 10% on day 0% % inhibition value of 57.48%; 62.06%; and 73.01%.

Keywords: *Antioxidant, Jamblang fruit extract (Syzygium cumini (L.) Skeels), Optimization, Yoghurt*

1. INTRODUCTION

The reactivity of oxidant compounds that exceed the limit of cellular antioxidant protection capability can form a chain reaction that causes disturbances in the work system of organs, causing degenerative diseases (Winarsi, 2007). Hence, prevention is needed to neutralize the formed oxidants, so exogenous antioxidants are needed (Sayuti & Yenrina, 2015). Exogenous antioxidants can be obtained from food or beverages, either processed products or natural ingredients such as vegetables and fruits. Processed products that contain antioxidant compounds are mostly in dosage forms such as tablets, capsules or multivitamin syrup, these are not preferred because they are considered drugs, therefore innovations are made by formulating other preparations such as yogurt drinks.

Yoghurt is a fermented milk product using Lactobacillus Bulgaricus and Streptococcus Thermophilus bacteria, through a pasteurization process, with or without the addition of other food ingredients and permitted food additives (Agarwal & Prasad, 2013). Fortification of natural ingredients in yogurt is an added value in functional foods, which can increase antioxidant activity and increase consumer protection against diseases related to oxidants / free radicals and oxidative stress as well as natural dyes (Pereira et al., 2013). One of the compounds that have antioxidant activity is jamblang fruit (Syzygium cumini, L).

Preclinical studies show that the stems, leaves and fruit of jamblang have activity as an antioxidant, anti-inflammatory, anthelmintic, anticancer, antibacterial, and antidiabetic (Haroon & Arshad, 2015). The results of Tripathy et al (2016) research that the methanolic extract of jamblang fruit can increase cytotoxicity and suppress cell proliferation in H460 lung cancer cells in a concentration-dependent manner, with an IC50 of 35.2 g/mL and research by Zhang & Lin (2009) showed that the acetone extract of jamblang fruit had antioxidant activity (IC50 165 ppm) and in leaves IC50 12.84 ppm (Marliani et al., 2014). The antioxidant activity of jamblang fruit is thought to be from its natural anthocyanin content (Sari et al., 2005). Other content of jamblang fruit is glucose, fructose, citric acid, minerals and vitamins (Ayyanar & Subash-Babu, 2012; Ramya et al., 2012).

Anthocyanins are hydrophilic, so they are often extracted with alcohol or water as a solvent (Seafast Center, 2012). The use of water as a solvent in the extraction process of this research is because it is safe to use in food processing, but anthocyanin pigments have low stability characteristics in processing. In addition, temperature, pH, oxygen, light, and sugar are physical and chemical factors that can affect the stability of anthocyanins (Basuki et al., 2005). It is hoped that the stability of anthocyanins can be overcome by processing food in an acidic environment such as yogurt (Laleh et al., 2006).

2. RESEARCH METHOD

2.1. Tools and materials

The tools in this research are UV-Visible spectrophotometry (Shimadzu®) type 1700, autoclave (Hirayama®), oven (Memmert®), laminar air flow (Mascotte®), and incubator (Memmert®). The material in this study was jamblang fruit which was obtained from Mulekan Village 2 RT 002, Kirobayan Village, Kec. Kretek, Kab. Bantul - Yogyakarta, full cream Dancow® milk powder, Streptococcus thermophilus culture, Lactobacillus bulgaricus, honey.

2.2. Method

Parameters tested are test sensory, pH, acid content, total protein, fat content, number of starter bacteria, and Yogurt stability test with storage at 4°C then tested for antioxidant activity on the 0, 7th, 14th, 21st and 28th days

2.3. Jamblang Extract Making

Samples of ripe jamblang fruit were weighed as much as 100 grams, then added 50 mL of distilled water. The jamblang fruit was then mashed using a blender for 3 minutes, then heated at 54°C for 50 minutes. The crude extract obtained was then filtered to obtain jamblang fruit extract (Maran et al., 2014).

2.4. Yogurt Making

2.4.1. Yogurt Formula

Yogurt is made with 3 types of formulas. Each formula with a volume of 100 mL formula can be seen in Table I. The process of making yogurt is full cream milk powder which has been pasteurized at a temperature of 85ºC for 10 minutes and lowered the temperature to 40ºC. Then inoculated milk with a starter that has been made as much as 5%.

After that, it was incubated at 37ºC for 24 hours. Then add the Jamblang fruit extract and honey until homogeneous, store in the refrigerator at 5ºC.

Table 1 Yoghurt Formulas

Ingredients	Composition			
	F 0	F II	F III	F IV
Full cream milk (mL)	100	100	100	100
Starter culture (% b/v)	5	5	5	5
Jamblang fruit extract (% b/v)	0	5	7,5	10
Honey (% b/v)	8	8	8	8

2.4.2. Sensory Test

The test uses the senses of taste, smell, sight and touch, including tests of liking, taste, odor, color, texture, and viscosity. This test involved 20 panelists. The preference scale is divided into 7 levels: 1 (dislike very much), 2 (dislike), 3 (dislike somewhat), 4 (neutral), 5 (like somewhat), 6 (like), and 7 (like very much). The scoring test was carried out to determine the response to more specific product characteristics, namely color (white to purple), aroma (typical of yogurt to very typical of Jamblang fruit), taste (very not sour to very sour), texture (very rough to very smooth).), and viscosity (liquid to very thick), the scoring test scale is 1-5 (Sunarlim et al., 2007).

2.4.3. pH analysis

The pH test was carried out using a pH meter. Before measuring the pH meter, it must be calibrated before use with a buffer solution of pH 7 and 10. The electrode tip of the pH meter is dipped in the yogurt sample, the pH meter screen will show the pH value of the yogurt sample.

2.4.4. Acidity Analysis

Determination of acidity based on pH measurements and titrated acid levels (AOAC, 2000). In the titrated acid test, the amount of acid is calculated as lactic acid. The test used 0.1 N NAOH as the titrant with the end point of the titration marked by a change in the color of the solution to pink. Based on the standard lactic acid 0.5–2.0 (SNI, 2009).

$$Amount\ of\ acid\ (\%) = \frac{V\ x\ N\ x\ 90}{W} x100\%$$

Note:
W = Sample Weight
V = Volume of NaOH Solution
N = Normality of NaOH Solution
90 = BM of lactic acid Orgenoleptic test.

2.4.5. Fat Content Analysis

The fat in the sample was hydrolyzed with ammonia and alcohol then extracted with ether which was obtained and then evaporated to dryness in an aluminum container and the fat content was calculated gravimetrically (National Standardization Agencies, 2009).

$$Fat\ (\%) = \frac{(W1 - W0)}{W} \times 100\%$$

Note :
W = sample weight (g)
W0 = weight of empty container (g)
W1 = weight of empty container and fat (g)

2.4.6. Number of Starter Bacteria

Prepare and homogenize, make the dilution level as needed using the Butterfield's Phosphate -Buffered Dilution Water (BPB) dilution solution, pipette 1 mL each from the dilution level 10-3 to 10-5 into sterile petri dishes in duplicate, pour 12 mL to 15 mL of MRS medium which is still liquid at a temperature of (45 ± 1) °C into each petri dish, shake the petri dish carefully (turn and shake it forward, backward, right and left) so that the sample and media are evenly mixed and solidified, put all the petri dishes upside down into the incubator cabinet at a temperature of 35°C for 3 days or a temperature of 30°C for 5 days. If possible, incubation is carried out in air enriched with CO2 in an anaerobic jar, and record colony growth on each petri dish containing 25 to 250 colonies after 3 or 5 days (National Standardization Agencies, 2009).

2.4.7. Antioxidant Activity Analysis

1) Preparation of Sample Supernatant for Testing (Kartikasari & Nisa, 2014). Weighing 10 grams of yogurt, centrifuged for 40 minutes at a speed of 8000 rpm, then separate the supernatant and the sample supernatant is ready to be tested.
2) Antioxidant Activity of DPPH Method (Sulandi, 2013).

The antioxidant activity test of the water extract radical scavenger was carried out using the DPPH method with slight modifications. A total of 1 mL of extract with a concentration of 10 g/mL, 30 g/mL, 50 g/mL, 70 g/mL and 90 g/mL were added to 2 mL of 0.1 mM DPPH. The mixture was then shaken and incubated at room temperature for 30 minutes in the dark. The absorbance of this solution was then measured at max 517 nm. The same treatment was also carried out for the blank solution (DPPH solution that did not contain the test material) and the positive control vitamin C with concentrations of 2 g/mL, 4 g/mL, 6 g/mL, 8 g/mL, and 10 g/mL. The max used for vitamin C is 515 nm. The blank solution consisted of 2 mL of 0.1 mM DPPH and 1 mL of methanol pa. The absorbance measurement results were analyzed for the percentage of antioxidant activity using the following equation.

$$\%\ Inhibition = \frac{A\ blank - A\ sample}{A\ blank} \text{ x } 100\%$$

Note :
A = Absorbance value

3. RESULT AND DISCUSSION

3.1. Sensory

The average hedonic and descriptive test results on the yogurt formulations, the assessment of preference for color, aroma, taste, texture and viscosity in the yogurt formulation with the addition of jamblang fruit extract tended to be somewhat favored (5.27) for yogurt with 5% jamblang fruit extract (criteria disliked to like) and preferred (criteria dislike to like very much) for yogurt with 7.5% jamblang fruit extract (6.12) and 10% (6.22), while for plain yogurt as a control / comparison, the panelist's assessment was somewhat dislikes (3.74).

Table 2 Sensory Test Results of Plain Yoghurt and Fortified Yoghurt Jamblang Fruit Extract

Yoghurt formula	Average Score					Total Score
	Color	Odor	Flavor	Texture	Viscosity	
Plain	3,55	3,05	3,75	4,15	4,2	3,74
Jamblang Extract 5 %	6,05	4,1	4,6	5,45	6,15	5,27
Jamblang Extract 7,5%	6,5	5,4	6,2	6,35	6,15	6,12
Jamblang Extract 10 %	6,35	5,9	6,3	6,45	6,1	6,22

Directions:

*Hedonik: 1 = Very Dislike, 2 = Dislike, 3 = Rather dislike, 4 = Neutral, 5 = Rather like, 6 = Like, 7 = Very like.

Table 3 Descriptive Test Results of Plain Yoghurt and Fortified Yoghurt Jamblang Fruit Extract

Yoghurt formula	Average Score					Total Score
	Color	Odor	Flav	Texture	Viscosity	
Plain	1	1,5	3,85	3,9	2,6	2,57
Jamblang Extract 5 %	4,05	3,15	4,05	4,4	3	3,73
Jamblang Extract 7,5%	4,15	3,85	4,25	4,2	3,05	3,9
Jamblang Extract 10 %	4,33	3,85	4,45	4,4	3,15	4,036

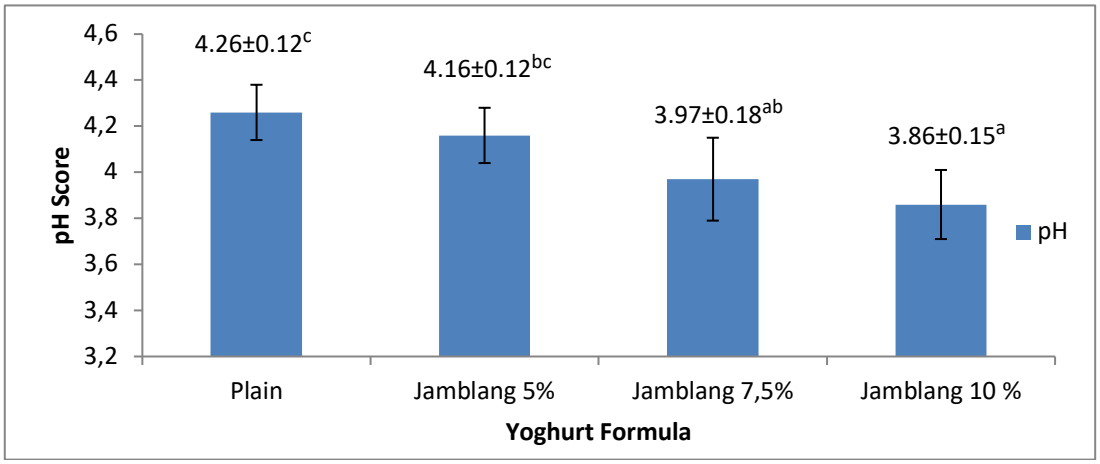
*Descriptions:

1 = Identic of yoghurt, 2 = Very Identic of yoghurt, 3 = Rather Identic of yoghurt,

4 = Identic of jamblang fruit, 5 = Very identic of jamblang fruit

3.2. pH

The pH value of the yogurt formulation is inversely proportional to the total titrated acid. The pH value of yogurt with jamblang fruit extract decreased compared to plain. The highest pH value was in plain yogurt (4.26%) while the lowest pH value was in yogurt with the addition of 10% jamblang fruit extract (3.86). Prastyaharasti and Zubaidah (2014) which states that the decrease in pH is one of the consequences of the fermentation process that occurs due to the accumulation of lactic acid as the main product of the activity of lactic acid bacteria. Another factor of the decrease in the pH value is the monosaccharide sugar content in the jamblang fruit extract sample. The more sources of sugar that can be metabolized, the more organic acids are produced so that automatically the pH will also be lower. This is because of the activity of LAB in breaking down lactose into lactic acid, the result of sugar metabolism causing a decrease in the pH of yogurt (Jannah et al., 2014).

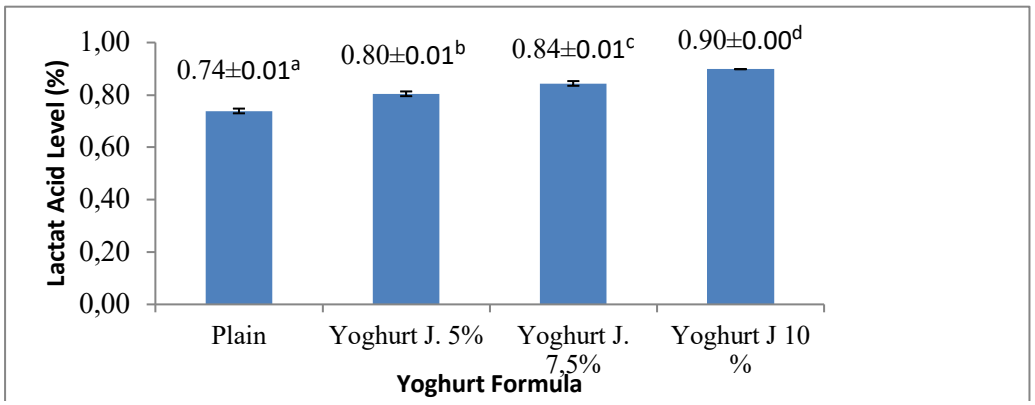


Note : [a - d] with different lowercase letters showed a significant difference with the concentration level of jamblang fruit extract ($p<0.05$)

Figure 1 Diagram of pH Value in Fortified Yoghurt Formulation of Jamblang Fruit Extract

3.3 Total acidity

The average value of yogurt lactic acid levels, the measurement results show that yogurt with the addition of jamblang fruit extract increased, the results obtained were between 0.74 - 0.90%. The highest lactic acid value was in yogurt with 10% jamblang fruit extract (0.90±0.00%), while the lowest was in plain yogurt (0.74±0.01%). The value of lactic acid yogurt produced is still in accordance with the provisions of National Standardization Agencies (2009), namely 0.5-2.0%. Acidity is inversely proportional to pH, the acidity value increases as the yogurt pH decreases. This is due to the use of a mixed starter Lactobacillus bulgaricus which will release the amino acids valine, glycine and histidine needed by Streptococcus thermophilus, on the other hand Streptococcus thermophilus helps lower pH and produces a number of formic acid which stimulates the growth of Lactobacillus bulgaricus (Machmud et al., 2013).

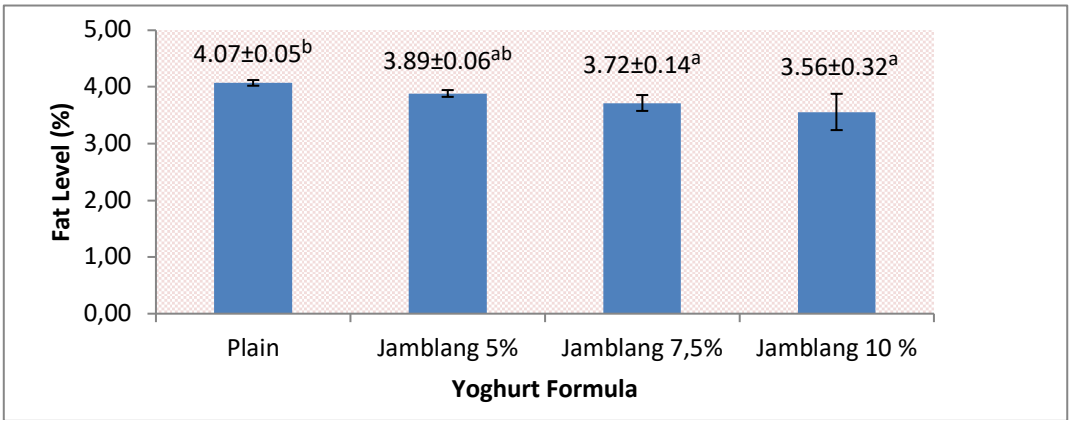


Note : [a - d] with different lowercase letters showed a significant difference with the concentration level of jamblang fruit extract ($p<0.05$)

Figure 2 Diagram of Acid Levels in Jamblang Fruit Extract Yoghurt Formulation

3.4. Fat level

Increasing the concentration of jamblang fruit extract can reduce fat content in yogurt because of the high water content of jamblang fruit extract. In line with research Mulyani, et al. (2016), namely the higher the water content of the extract, the lower the fat content in the banana peel Soyghurt, so that the resulting yogurt texture will be more liquid. This decrease in fat content was related to the growth of L. bulgaricus, where at 44°C it was known to be the optimum temperature for its growth, while the fermentation product of S. thermopilus stimulated the growth of L. bulgaricus to produce more lactic acid. These lactic acid bacteria will produce lipase enzymes that will break down fat into fatty acids, then these fatty acids will be broken down into compounds that have a distinctive aroma of yogurt.



Note : [a - d] with different lowercase letters showed a significant difference with the concentration level of Jamblang fruit extract ($p<0.05$)

Figure 3 Fat Content Diagram Fortified Yoghurt Jamblang Fruit Extract

3.5. Number of Starter Bacteria

The average number of starter bacteria L. bulgaricus (80.107 colonies/g) and S. Thermophillus (3.1.107 colonies/g) met the standard for the number of live cells of lactic acid bacteria (LAB), which was at least 107 colonies/gram (National Standardization Agencies, 2009). This is in accordance with the research of Chandan and Shahani (1993), that the number of active microbes is at least 107 colonies/gram in the yogurt preparation. Microbiological qualitative analysis of foodstuffs is important to determine the quality of foodstuffs. The combination of L. bulgaricus with S. Thermophillus gave better growth, this was because during the incubation period the yogurt starter provided nutrients that functioned as stimulators for the growth of the two bacteria (Muhsinin et al., 2016).

3.6. Antioxidant Activity

The research data showed that the highest % inhibition value on day 28 was obtained at a concentration of 10% jamblang fruit extract (44.32 ± 1.57%) while the lowest % inhibition was obtained from jamblang fruit extract with a concentration of 5% (33.46 ± 2.99 %). Based on the results of the study, it was shown that the higher the anthocyanin content of an extract, the higher the antioxidant activity. This is caused by the pigment from jamblang fruit extract which has antioxidant substances, one of which is anthocyanin pigment (Widhiana et al., 2012).

Effect of storage time on antioxidant stability of yogurt with jamblang fruit extract on the storage process day 0 - day 28 decreased antioxidant levels. This is directly proportional to the decreasing anthocyanin levels in the jamblang fruit extract, the antioxidant activity also decreases. This is due to the degradation of anthocyanin properties which are highly dependent on oxygen, enzymes, light, environment, temperature and pH, presence of metal ions, sugar content, and the effect of dioxide tendrils (Seafast Center, 2012)

Table 5 MANOVA Analysis Result Data Effect of Storage Time on Antioxidant Stability of Jamblang Fruit Extract

Antioxidant Level (% inhibition)					
Sample	**Day 0**	**Day 7**	**Day 14**	**Day 21**	**Day 28**
Jamblang 5%	49.21 ± 3.24^{aE}	47.97 ± 2.39^{aD}	45.61 ± 1.87^{aC}	38.39 ± 0.92^{aB}	33.46 ± 2.99^{aA}
Jamblang 7,5%	59.83 ± 1.06^{bE}	57.17 ± 1.07^{bD}	50.00 ± 2.17^{bC}	45.98 ± 0.81^{bB}	39.10 ± 1.77^{bA}
Jamblang 10%	67.74 ± 2.30^{cE}	62.56 ± 2.06^{cD}	57.96 ± 1.86^{cC}	52.57 ± 1.74^{cB}	44.32 ± 1.57^{cA}

Note : *a-c in the same column with different lowercase letters showed a significant difference with the concentration level of jamblang fruit extract ($p<0.05$)
*A-D in the same row with different capital letters showed a significant difference with storage time ($p<0.05$)

Table 6 MANOVA Analysis Result Data Effect of Storage Time on Antioxidant Stability of Fortified Yoghurt Jamblang Fruit Extract

Antioxidant Level (% inhibition)

Yoghurt	Day 0	Day 7	Day 14	Day 21	Day 28
Plain	45.48±1.89^{aD}	39.97±0.63^{aD}	35.95±0.69^{aC}	27.36±0.90^{Ab}	24.75±0.78^{aA}
Jamblang 5%	59.41±4.44^{bD}	56.67±2.92^{bD}	53.98±1.29^{bC}	46.10±5.67^{bB}	43.03±8.13^{bA}
Jamblang 7,5%	62.06±2.06^{cD}	60.41±13.61^{cD}	55.47±9.81^{cC}	48.22±11.07^{cB}	42.95±12.70^{cA}
Jamblang 10%	73.26±1.95^{dD}	68.49±1.62^{dD}	66.79±0.42^{dC}	59.00±3.38^{dB}	50.21±5.20^{dA}

Note : *a-c in the same column with different lowercase letters showed a significant difference with the concentration level of jamblang fruit extract ($p<0.05$)
*A-D in the same row with different capital letters showed a significant difference with storage time ($p<0.05$)

4. CONCLUSION

Yogurt fortified jamblang fruit extract (Syzygium cumini, L). has better stability of antioxidant activity, meets the requirements of the Indonesian National Standard (SNI), and at a concentration of 10% gives the respondent's most preferred level of preference.

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