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The effect of saga milk (*Adenantera pavonina*, L.) and yogurt starter culture concentration on process of yogurt

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Abstract

This study was aimed to obtain the formulation of saga milk (*Adenanthera pavonina*, L), with cow's milk and yogurt starter culture concentration which is optimal in the manufacturing of yogurt-based saga milk. The analysis carried out periodically (0, 2, 4, 6 hrs after incubation time) was on the change of pH value, total acids concentration, and lactic acid bacteria (LAB), as well as the preference test of the product only on the finale product. The material used in this study were saga milk, cow's milk and commercial yogurt starter culture consist of *Lactobacillus acidophilus, Bifidobacterium,* and *Streptococcus thermophilus*. This experiment design was random block design, factorial pattern (3×3). Factor a is the concentration of starter culture consisting of three levels by 3% (a1), 4% (a2), 5% (a3), and formulation of saga milk compared to cow's milk (b) with the ratio of 40:60 (b1), 50:50 (b2), 60:40 (b3), this experiment replicated three times. The result showed that with more cow's milk and yogurt starter culture concentration, the pH of yogurt decreased, which was accompanied by increased acidity. Although LAB in products with a starter culture 3% and 4% were slightly lower than in products with 5% starter but not significant ($p = 0.05$). The addition of the starter culture increased the acceptance of panelists. The addition of saga milk, up to 60%, provides results that can still be accepted with the addition of coco-pandan syrup by panelists. The preferred saga yogurt has a white color like cow's milk, with sufficient viscosity and an acceptable taste

1. Introduction

Yogurt is a processed product obtained from a fermented dairy product that has several health benefits and is usually mixed with a yogurt starter culture consisting of thermophilic lactic acid bacteria *Stretptococcus thermophilus* and *Lactobacillus bulgaricus* ssp. *bulgaricus* (Aswal *et al*., 2012). According to Winarno *et al*. (2003), fresh milk contains various microbes which produce various kinds of acids and flavors. However, commercial yogurt traditionally uses two popular types of lactic acid bacteria, namely *S. thermophilus* and *L. bulgaricus.*

According to the Data Center for Agricultural Information Systems (Pusat Data dan Sistem Informasi Pertanian, 2016), the increase in the availability of local cow's milk is usually accompanied by an increase in imported cow milk commodities. Another source of protein having a protein value that is almost the same as cow's milk is from some types of beans, such as soybeans and saga beans. Soybean protein values per 100 g are 34.9%, while saga bean seeds per 100 g are 30.6%, meaning that the protein content in both types of beans can be an alternative source of vegetable protein.

The import of soybeans in Indonesia from 2015 until 2020 is predicted to reach 3,398008 tons (Aimon and Sutranto, 2014). *Adenanthera pavonina* is a plant that is viable to be developed in tropical countries. It can produce 100-150 kg per tree per year and can be harvested throughout the year (Suita, 2013). Saga bean plant (*Adenanthera pavonina*, L.) is a perennial plant that has the advantages as follows, it grows well in various types of soils including low pH soil, and it is a large tree suitable for tropical wetland areas such as Indonesia and needs easy maintenance (Amar, 2017).

Saga trees have many benefits. *Adenanthera pavonina*, L. bark extract, with various solvents can

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inhibit the growth of Gram-positive and Gram-negative bacteria including *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Enterobacter aerogenes, Staphylococcus epidermidis,* and *Salmonella enterica* serova Typhimurium (Hussein *et al*., 2011). Other researchers used the dry bark of *A. pavonina,* L. extract and have found it very useful. A dried and ground bark of *A. pavonina,* L. extract with methanol is able to have anti-inflammatory activity in animal experiments with mice (Arzumand *et al*., 2018). Another research reported that the *Adenanthera pavonine* seed aqueous extract was able to treat diabetes in mice and associated lipid disorders (Pandhare *et al*., 2012). The use of saga seeds is not only for tempeh (Amar, 2020) but it can also be extracted to produce heteropolysaccharide galactomannan as biopolymers (Melo *et al*., 2018). The raw material of yogurt is always identified with cow's milk. Actually, vegetable milk such as soy milk or saga milk can also be produced into yogurt. The use of saga seeds as milk has been reported by another researcher who also used an extract of sesame seeds to give a brighter colour to saga milk (Yenrina *et al*., 2014). Tempeh of Saga with certain starter cultures has the potential to produce long-chain fatty acids that are good for health (Amar, 2020). Another research reported that saga seeds can reduce cholesterol in quail eggs (Hartono *et al*., 2012).

New other research about the use of saga seeds has been reported that saga milk has the potential to improve neurological diseases. This is evidenced by a decrease in the activity of Cholinesterase in the liver, heart and kidneys of mice as experimental animals. It is assumed that saga milk can facilitate the transfer of ions so they can penetrate the cell membrane (Afolabi *et al*., 2018). Saga milk can also be made into fresh cheese, like fresh cheese made from cow's milk (Amar *et al*., 2017).

The objective of this study was to obtain the optimal formula of saga milk and concentration of yogurt starter culture to produce yogurt which can be accepted organoleptically by the panellists. *Adenanthera pavonina*, L. as one of the tropical country's biodiversity assets must be optimized for the welfare of humanity.

2. Materials and methods

2.1 Procedures

The ingredients used were the saga bean seeds (*Adhenantera pavonina*, L.), originating from the campus of the Institut Teknologi Indonesia, Serpong, fresh plain pasteurized milk, skim milk powder, plain yogurt starter culture (Yummy fresh product), that was purchased at PT. Yummy Food Utama, Jakarta. Other ingredients were neutral aquadest, alcohol 70%, PP indicator 1%, NaOH 0.1N (Merck), MRS-Agar (CM0361-Oxoid) and sodium bicarbonate ($NaHCO_3$), Merck. The research was conducted at the Fermentation Laboratory and Microbiology Laboratory, Agro-Industrial Technology Study Program, Institut Teknologi Indonesia, Serpong, Tangerang Selatan, Banten. The research was conducted in a block randomized design consisting of two factors, namely factor A was the concentration of commercial yogurt culture starter, factor B was milk formulation, factor A consisted of concentrations as follows: a1 = 3%, a2 = 4%, and a3 = 5%. Factor B consisted of a ratio between saga milk to cow's milk as follows: b1 = (40:60), b2 = (50:50), b3= (60:40), and the parameters measured were pH of the product (using Hanna Instrument, and before using, the instrument was calibrated with buffer solution), total acid calculated as lactic acid (SNI 01-2981-2006 yoghurt in Badan Standar Nasional, 2009) and total Lactic acid bacteria (LAB) by Jannah *et al.* (2014), during the fermentation process. The Preference Test was carried out using the Fliedner and Wilhelmi (1993) method.

2.2 Saga milk process

Saga bean seeds were soaked in water for 24 hrs and then boiled for 60 mins. Then, peeled to separate the skin and washed thoroughly. After that, the endosperm of the saga bean seeds was washed and followed by boiling for 15 mins. Then, milled with 80°C hot water for 2 mins followed by filtering and separating the pulp. The separated pulp was milled again for 30 s. After that, the filtration results were added with 0.5% $NaHCO_3$ and pasteurized at 80°C for 10 mins. Saga milk was then ready for use.

2.3 Saga yogurt process, pH value, total titrated acids, and total lactic acid bacteria analysis

The yogurt process was conducted as follows 1800 mL prepared saga milk added to sodium hydrogen carbonate as much as 0.5% and then pasteurized at 80°C for 10 mins then cooled to a temperature of 40°C, parallel with that, also pasteurized 1800 mL of cow's milk which has been added with skim milk powder as much as 3% at a temperature of 95°C and then cooled to a temperature of 40°C. The formulations were made as followed: 400mL saga milk + 800mL cow's milk mixed homogeneously (b1) then divided into 12 sterilized glass bottles and inoculated each 4 bottles consecutively with 3% (a1), 4% (a2), and 5% (a3) commercial yoghurt starter cultures, 600mL saga milk + 600 mL cow's milk mixed homogeneously (b2) then divided into 12 glass bottles and inoculated each 4 bottles consecutively with 3%, 4%, and 5% commercial yoghurt starter culture, 800 mL saga milk + 400 mL cow's milk mixed homogeneously (b3) then divided into 12 glass bottles and inoculated 4 bottles respectively with 3%, 4% and

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5% commercial yoghurt starter culture. All preparations were fermented in an incubator at 44.1°C, and periodically at 0 hr (c1); 2 hrs (c2), 4 hrs (c3) and 6 hrs (c4) after incubation was analysed for pH and total acid, as well as total lactic acid bacteria. These experiments were repeated three times. Data were analysed using Variance analysis and further test was conducted with Duncan's New Multiple Range Test (DMRT) at the level of 5%.

The measurement of the pH value of the product, 50 mL of the sample was prepared and filled in a beaker glass and the tip of the electrode was inserted into the sample as deep as 5 cm. The measurement result was read as the pH value. The determination of the total titrated acids (is calculated as the total lactic acids) in the sample was done by titration using 0.1 N NaOH according to the Standard Nasional Indonesia (SNI 01-2981-2006 Yoghurt, 2009). Meanwhile to determine the total growth of LAB in the product using the Pour Plate method (Jannah *et al*., 2014) means that the sample was diluted until the determined concentration and then was cultured in the MRS-Agar media and incubated at 40°C. Then the sample was calculated for the total LAB.

2.4 Preference test

The test used the Fliedner and Wilhelmi method (1993). The category of panellists used was not properly trained panelist, with ages ranging between 18-27 years. Panelists were asked to give an evaluation of the yogurt product, which was served by adding coco-pandan syrup, on a scale of 1 to 9 for the performance, texture, aroma and taste attributes. The range of scores given was 1-9, starting from like exceedingly (9), like very much (8), like (7), rather like (6), neutral (5), rather dislike (4), dislike (3), dislike very much (2) and dislike exceedingly (1). [Whether or not a product was accepted was subject to the following conditions: The score the panellists must give is 6 or above. When the score was 6 or above, then they are included in the calculation for acceptance. However, if the assessment was below 6 then it is not counted in the calculation for acceptance. If the percentage of panellists that give the above score is between 0-65 %, it means not accepted; 66-72% it means far from accepted or a very little accepted (weighty complaints, require more improvements on some sensory parameters); 73-79% it means almost accepted (complaints given, require more improvements on one or two sensory parameters); 80-86% it means accepted (not free of complaints, require improvements one of sensory parameters); 87-93% it means more than accepted (almost free of complaints, no improvement necessary); 94-100% it means exceptionally accepted (free from complaints, no improvement necessary)

3. Results and discussion

The pasteurization of cow's milk was carried out at a temperature of 95°C, which is higher than the pasteurization temperature of saga milk. This must be done so that the lactoglobulin and lactoalbumin present in cow's milk are completely denaturized. The denaturized condition causes the lactoglobulin and lactoalbumin to have no capability anymore to disturb the stability of yogurt gel. Meanwhile, the pasteurization saga milk only aims to kill pathogenic microorganisms. The preliminary research that has been done (data unpublished) using the saga milk formula for yogurt shows that if the concentration of saga milk is more than 60%, the flavour of the resulting yogurt has a very sharp beany flavour and the gel formed was unstable. Therefore, only three milk formulas were chosen in this main research. Previous studies showed that soymilk fermented with *Lactobacillus plantarum* bacteria in the process of producing yogurt was able to improve the dysregulation of cholesterol metabolism in rats fed with a high cholesterol diet (Kim *et al*., 2014). The concentration of starter culture used in saga yogurt was only 3%, 4% and 5%. This was determined because the culture used was commercial plain yogurt. It is therefore believed that with 3 to 5% commercial yogurt culture starters are able to form normal yogurt. Even the 4th starter culture (F4) generation stored in the refrigerator for 25 days still has a 7-8 Log CFU/mL of LAB viability and is still suitable for use as a starter culture (Fitrianingrum *et al*., 2016). Many researchers state that the culture can actually start at 1%. This is possible if the culture is freshly developed. This research used commercial yogurt starter culture, previous study used *L. acidophilus* strain SBT 202 culture still has 100% viability for 7 days stored at 4°C (Ng *et al*., 2011). However, in the same type of culture with a different strain of *L. acidophilus* strain NCFM at the same storage condition the viability is only 10% (Ng *et al*., 2011). The yogurt culture starter used in our research was a mixed culture containing *S. thermophilus*, *L. acidophilus* and *Bifidobacterium bifidum*. The last two species include probiotic bacteria. The probiotic microorganism is able to produce more acetaldehyde than the usual yogurt culture, thereby reducing the aroma of beany flavour in soy yogurt (Donkor *et al*., 2007). According to Chang *et al*., 2010 the mixed culture for soy yogurt produced good quality characteristics. It is expected that the saga milk-based yogurt also produces a reduced beany flavour.

3.1 pH Value

Fermentation occurs as indicated by the change in pH value of the yogurt, its acidity level. It can be seen in Table 1 that the initial milk formulation has an average pH value of 6.97 but after incubation for 6 hrs the final

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Table 1. Effect of incubation time on total bacteria lactic acid pH value and total titrated acid of saga yogurt

Incubation time (hours)	Average of total lactic acid bacteria (Log CFU/mL)	Average of pH value of saga yoghurt	Average of total titrated acid (%)
6	5.48±0.24[a]	4.69±0.05[c]	0.21±0.03[a]
4	4.39±0.41[b]	5.00±0.16[c]	0.15±0.03[b]
2	4.05±0.70[bc]	6.03±0.32[b]	0.08±0.01[c]
0	3.23±1.05[c]	6.94±0.28[a]	0.04±0.01[d]

Values are presented as mean±standard deviation (n = 9). Values with different superscript within the column are significantly different ($p \leq 0.05$).

pH value dropped to 4.69, which was an ideal pH value for yoghurt. The research was set up until the measured pH reached 4.6-4.8, and on observation 6 hrs after incubation, the pH value had reached the pH which was usually owned by commercial yogurt. Therefore, the observation was stopped and the product was immediately stored in the refrigerator at 8-10°C to develop its flavour and the next day the panellist's preference test was carried out. Incubation time significantly affects the pH value of yogurt, this is due to the increasing growth of lactic acid bacteria. It was also observed that the longer the incubation time up to 6 hrs, the pH value is significantly reduced, and the average total titrated acids reached up to 0.21%. This condition indicates that an increase in the length of the incubation time, the number and the activity of microbes will increase the lactose which is converted into lactic acid, causing a decrease in pH. This is in line with Wardhani's research (2015) which states that the longer duration of fermentation and the more the starter concentration will be accompanied by a decrease in pH value. However, it was reported by Wardhani *et al*. (2015) that the incubation time reached 15 hrs pH value of Corn yoghurt only has a pH of 4.0.

However, based on our observations in this study, the increase in the concentration of yoghurt starter cultures, the data is shown in Table 2, and differences in saga milk formulations (Table 3) did not significantly affect the pH value of yogurt. The addition of saga milk was able to slow down the pH decrease even though it was not significant. This was because saga milk has a lactose content which is much less compared to cow's milk. Another previous study showed that the addition of date palm extract can accelerate the decrease in pH of yogurt drinks (Hartati *et al*., 2012).

3.2 Total titrated acid value as lactic acid

Decreasing pH value and increasing concentration of lactic acid in yogurt saga during fermentation is caused by the lactose in cow's milk. Lactose is hydrolysed by LAB in yogurt culture (*L. acidophilus*, *Bifidobacterium* and *S. thermophilus*). The capability of LAB to use lactose in this fermentation process can be explained as follows: lactose will be hydrolysed by the permease enzyme, which is then converted by lactase to galactose and glucose, and the glucose formed will then be metabolized to lactic acid.

Table 2 was shown that the addition of yogurt culture starter concentration tends to increase the concentration of lactic acid, although not significant between 3% and 4% yogurt starter culture. However, when compared between 3% and 5%, there is a significant difference, and between 4 and 5% was also not significant, therefore it is chosen for the use of 4% yogurt starter culture in manufacturing of saga yogurt. However, it all depends also on the panellists' preference test. Total titrated acid as lactic acid in the saga yogurt product reached up to 0.21±0.03%, relatively smaller compared to other yogurts in several studies. But it is still comparable to soy yogurt, including saga yogurt derived from saga milk mixed with 40-60% cow's milk. Previous soy yogurt studies have total lactic acid ranging from 0.13-0.40 (without the addition of cow's milk) while cow's milk yogurt has higher lactic acid content with a range between 0.08-0.43% (Horáčková *et al*., 2015). In another study, yogurt derived from cow's milk added with tomato juice extract has higher lactic acid than the control sample in the range of 0.48-0.61%. This is relatively high due to the high sugar content in tomato juice, which can stimulate the growth of LAB. (Savitry *et al*., 2017). The addition of the incubation time was

Table 2. Effect of addition of starter on lactic acid bacteria, pH value and total titrated acids of saga yogurt

Concentration of yogurt starter culture (%)	Average of total lactic acid bacteria (Log CFU/mL)	Average of pH value of saga yoghurt	Average of total titrated acid (%)
5	4.80±0.55[a]	5.78±0.95[a]	0.13±0.07[a]
4	4.24±1.03[a]	5.59±0.95[a]	0.13±0.07[a]
3	3.82±1.25[a]	5.62±0.95[a]	0.11±0.07[b]

Values are presented as mean±standard deviation (n = 9). Values with different superscript within the column are significantly different ($p \leq 0.05$).

Table 3. Effect of formulation of saga milk on lactic acid bacteria, pH value, and total titrated acid of saga yogurt

Formulation of saga milk: cow milk	Average of total LAB (Log CFU/mL)	Average of pH value of saga yoghurt	Average of total titrated acid (%)
40:60	4.36±0.56[a]	5.66±0.94[a]	0.13±0.08[a]
50:50	4.23±0.41[a]	5.66±0.94[a]	0.12±0.07[ab]
60:40	4.25±0.39[a]	5.84±1.04[a]	0.11±0.07[ab]

Values are presented as mean±standard deviation (n = 9). Values with different superscript within the column are significantly different ($p \leq 0.05$).

able to increase the total titrated acid as shown in Table 1, and the addition of saga milk up to 50% was able to increase the total titrated acid significantly, the data is shown in Table 3.

3.3 Total lactic acid bacteria

In Table 1, it is shown that the total LAB in saga yogurt increases with increasing incubation time. It is easy to understand that because LAB grows and develops with incubation time. However, the number of LAB cells produced was relatively low 6 hours after fermentation, only reaching 5.48±0.24 log CFU/mL. It might be due to saga milk media being a new media for yoghurt starter culture, so that adaptation is needed for cell proliferation. However, the presence of lactose from cow's milk provides a relatively fast adaptation for the active metabolic processes of microorganisms. Thus, within 6 hrs it reaches the pH that is usually owned by commercial yogurt derived from cow's milk.

In another study using soy milk in the yogurt process, at the age of 16 hrs, the *Lactobacillus bulgaricus* culture was able to reach 6.38 Log CFU/mL (Horáčková *et al.*, 2015). Furthermore, another study with yogurt based on cow's milk and added with tomato juice at the age of 4 hrs of fermentation reached 6.8-8.0 Log CFU/mL (Safitry *et al*., 2015). The low number of cells of LAB in saga yogurt is in accordance with the total acid in Table 2 also in Table 3. The question arises whether there is indeed a compound in saga milk that inhibits the growth of LAB. A previous study by Radziah *et al*. (2011) proved that saga seed oil has antibacterial properties. The increase of the concentration of yogurt starter culture also seems not to be significant to increase the number of LAB cells.

Increasing the concentration of starter cultures did not significantly increase the total LAB (Table 2). Another study by Jumah *et al.* (2001), reported that starter culture level had a definite effect on yogurt viscosity during the gelation process. Likewise, the addition of saga milk in the formula up to 60% does not affect the total LAB (Table 3)

3.4 Preference test

The addition of saga milk in milk formula for manufacturing yogurt has an influence on the preference test. With increasing concentrations of saga milk, the panellist assessment of the colour, texture, aroma and taste of yogurt decreases. The taste of saga yogurt is similar to soy yogurt. Research with soy yogurt fermented with *Bifidobacterium bifidum* shows it can reduce the total level of plasma cholesterol in the rat (Champagne *et al.*, 2009). Other researchers in their experiment on rats suggest that soy yogurt can prevent hepatic lipid accumulation (Kitawaki *et al.*, 2009). Therefore, health function must be highlighted in comparison to the taste of the product. Because the taste can be accustomed.

Table 4 illustrates the overall panellist acceptance of saga yogurt. Variations in acceptance for yogurt colour ranged from low, only 37% of panelist who liked the saga yogurt, to 100% of panellists who liked this product colour. The addition of fruit flavour gives a positive effect on the Preference Test, whereby strawberry is the most favoured by panellists compared to other fruit flavours (Osundahunsi *et al.*, 2007). Therefore, the Preference Test in this research used Cocopandan syrup to increase the panellist's acceptability. Other research about soy yogurt shows that the use of orange fruit flavour or banana flavour also gives a positive effect on acceptance by panellists (Raeisi *et al*., 2017). All saga yogurt inoculated with 5% yogurt starter culture were accepted by the panellists. This is indicated by 85% of the panellists choosing "likes" of the hedonic scale. On the contrary, for saga yogurt with either 3% or 4% yogurt starter culture, only one formula was almost accepted by the panellists.

All panellists noted that the yogurt with the addition of 5% starter culture had a white colour, like cow's milk, with moderate viscosity, and was less acidic and even had a slight beany flavour. Most of the panellists like that the texture of the yogurt saga is slightly viscous than usual, is this why the panellists like the texture still need to be further explored. The appearance of moderate viscosity of saga yogurt texture may also be caused by galactomannan biopolymer compounds that have saga seeds. It might be assumed, the presence of polysaccharides in Saga milk stimulates the formation of a thicker gel. This is in line with the research by Jumah *et al.* (2001) which showed that polysaccharides and

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Table 4. Acceptance percentage of saga yogurt

Treatment		Acceptance of panelist (%)					
Starter culture concentration	Milk formula Saga milk: cow's milk	Appearance/ colour	Texture	Aroma	Taste	Overall Acceptance	Average acceptance
3%	40:60	67	80	50	50	NA	
	50:50	73	73	67	70	283	70.8[e]
	60:40	67	67	67	57	NA	
4%	40:60	73	77	83	77	310	77.5[d]
	50:50	37	67	50	57	NA	
	60:40	40	47	60	33	NA	
5%	40:60	90	90	73	87	340	85[c]
	50:50	100	87	97	97	381	95[a]
	60:40	83	87	90	83	343	86[b]

NA: Not accepted.
Level of acceptance are categorized with the following:
[a]free from complaints, no improvement necessary;
[b]almost free of complaints, no improvement necessary;
[c]not free of complaints, require improvements one of sensory parameters;
[d]complaints given, require more improvements one or two sensory parameters;
[e]weighty complaints, require more improvements on some sensory parameters.

proteins collaborate in forming a solid gel. Heteropolysaccharide in saga seeds have been isolated by Melo *et al*. (2018) and it is nontoxic. Thus, yoghurt saga is also expected to be non-toxic or safe for consumption and even has good functional properties, for example, saga milk fat which contains lignoceric acid.

Preference test plays an important role in product acceptance which includes appearance/color, texture, aroma and taste. The use of a 5% yogurt starter culture in this process of saga yogurt was favored by panellists. Saga yogurt with a formulation of 50:50 (saga milk: cow's milk) and inoculated yogurt starter culture of 5% tended to be favoured by panellists because of the white colour like cow's milk, with sufficient viscosity in texture and an acceptable taste, and flavour.

4. Conclusion

The addition of the starter culture up to 5% increased the acceptance of panellists. The addition of saga milk, up to 60%, provides results that can still be accepted by panellists. The preferred saga yogurt has a white colour like cow's milk, with sufficient viscosity and an acceptable taste.

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