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THE CHARACTERISTIC OF COWPEA (*Vigna unguiculata* (L.) Walp.) YOGHURT WITH PROBIOTICS STARTER

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ABSTRACT

Cowpea has high protein (22,9%) and iron (6%) content. Today the utilization of legumes and pulses as plant yoghurt using probiotic microbes are increasing. In this research cowpea was processed using probiotic microbes such as *Lactobacillus acidophillus* (LA), and *Bifidobacterium bifidum* (BB), to yield a yoghurt product. This research utilized Complete Random Design with one factor, five levels and three replications. The variables studied were the types of probiotic starters, which were 1) Combination of *Streptococcus salivarius subsp. Thermophilus* : *Lactobacillus delbrueckii subsp. Bulgaricus* = ST:LB; 2) Combination of *Streptococcus salivarius subsp. thermophilus* : *Lactobacillus delbrueckii subsp. bulgaricus*: *Lactobacillus acidophilus* = ST:LB:LA: 3) Combination of *Streptococcus salivarius subsp. Thermophilus* : *Lactobacillus delbrueckii subsp. Bulgaricus* : *Bifidobacterium bifidum* = LB:ST:BB; 4) LA, and 5) BB. The quality of Cowpea yoghurt were determined by chemical analysis (water content, pH, Total Titratable Acid (TTA), and Total Soluble Solids (TSS)), physical analysis (viscosity), microbiology analysis (total lactic acid bacteria and activity of anti pathogenic bacteria, *E. coli*) and sensory analysis (taste, thickness, color, aroma, general acceptance and rank test). Results showed that the best cowpea yoghurt was the one made by combination of ST:LB:LA with water content 89,56%, pH 3,80, TTA 0,8%, TSS 8,16%, viscosity 380 cp, and total lactic acid bacteria were 4,83 x 10^9 CFU ml^{-1}. In this yoghurt the pathogenic bacteria *E. coli* was totally inhibited. The taste was sour, the texture was moderately viscous, the color was pale white, has beany odor, and moderately liked by the panelist. Every 100 g of cowpea yoghurt contained 5,16 % carbohydrate, 3,87 % protein, 0,0031 % fat, 0,66% ash and 90,31 % water. From overall test, it can be concluded that cowpea yoghurt complied with protein, fat and ash quality requirements of Indonesian National Standard (SNI) number 01-2981-1992.

***Keywords**: cowpea, yoghurt, probiotic micro flora, pathogenic bacteria*

INTRODUCTION

Cowpea is legume that has not been widely cultivated due to its low utilisation. The legumes is currently developed to produce vegetables yoghurt. Yoghurt is a fermented drink that is previously made from milk fermented by lactic acid bacteriaa *Streptococcus salivarius* subsp. *thermophillus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*. These bacteria hydrolyze lactose into lactic acid that lowered pH and coagulate milk protein to form a compact curd. Beside of these, the hydrolysis of lactose and nitrogen metabolism by the bacteria, especially *Lacobacillus delbrueckii* subsp. *bulgaricus*, produce acetaldehyde that give a typical yoghurt aroma (Tamime dan Marshall, 1997; Ono *et al*, 1992; Marshall, 1987 in Suriasih, 2008).

The utilization of legumes has benefit, because it is contain oligosaccharides that can be a suitable substrate for the growth of bacteria both in products and in gastrointestinal tracts. The experiment result from Widowati and Misgiyarta (2003) showed the affectivity of lactic acid bacteria in yielding fermented product from plant protein indicated that the yoghurt produced has level of lactic acid more than 1%, that according to Kosowski (1966), Hargrove (1977) in Harris and Karmas (1989) this showed that the yoghurt has high quality.

To increase the economical value of cowpea yoghurt, therefore prebiotic microbes are used. Because in recent time, customer prefer have a products that can give health benefit more than only have a good taste. Probiotics microbes are alive microbes in food that if consumed in certain amount can give benefit to the health, such as produces antimicrobial substances that killed unwanted microflora in gastrointestinal tracts, and can also inhibits the growth of pathogenic microflora. Probiotic microbes utilized in this research were *Lactobacillus acidophilus* (LA), and *Bifidobacterium bifidum* (BB) as addition of standard microbes used in yoghurt making.

MATERIALS AND METHODS

Materials used in the production of cowpea yoghurt were cowpea, skimmed dried milk, yoghurt starter (*Streptococcus salivarius subsp. thermophilus* [ST] and *Lactobacillus delbrueckii subsp. Bulgaricus* [LB]), pure culture of probiotic (*Lactobacillus acidophilus* [LA] and *Bifidobacterium bifidum* [BB]) and water.

This research utilized complete randomized design with one factor, five levels and three replications. The factors under studied were probiotic starters.

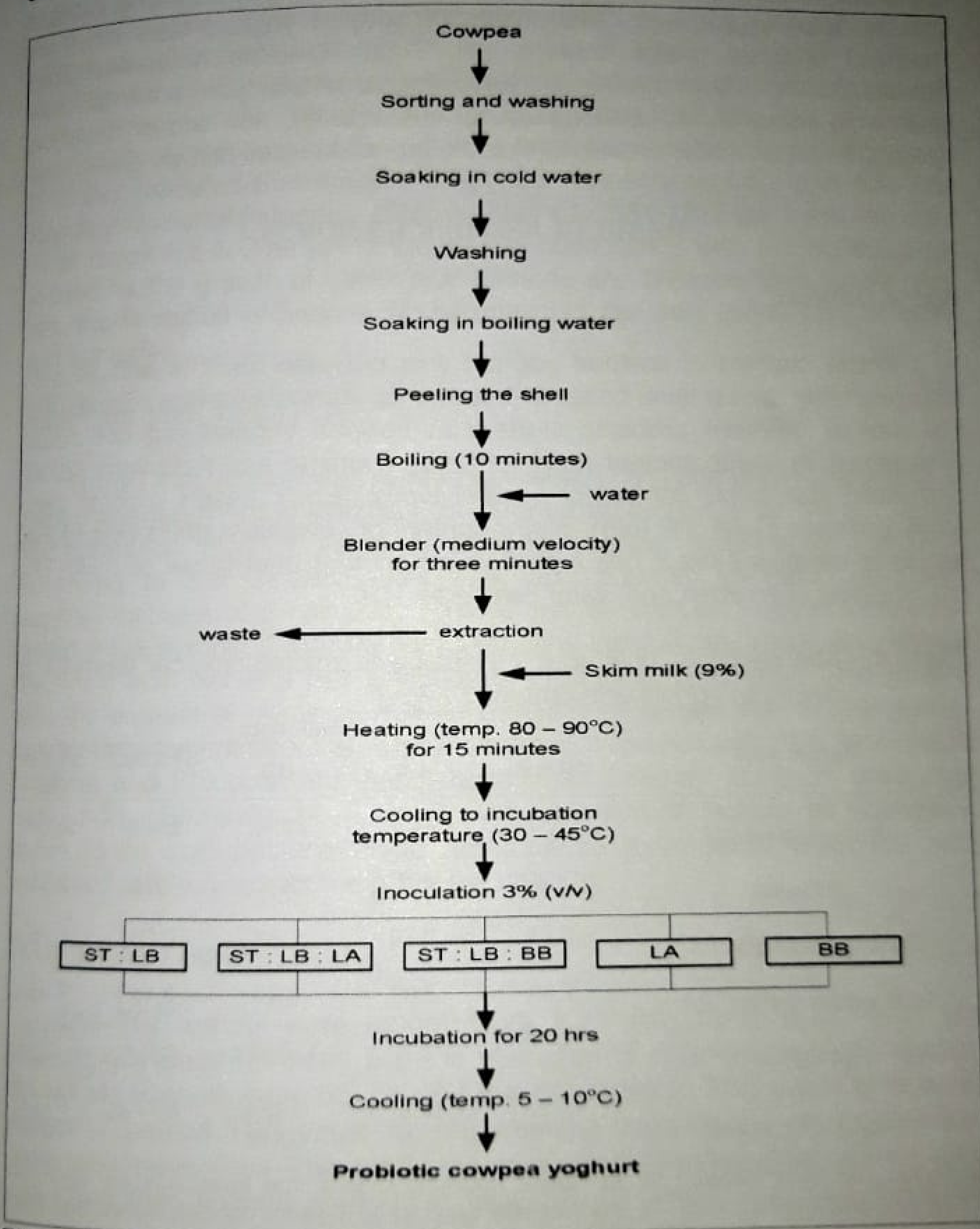

Figure 1. Cowpea yoghurt making process

The starter used was 3% v/v based on volume of cowpea extract with the same composition for each microbes. The combination of microbes were as follows (1) combination of ST:LB; (2) combination of ST:LB:LA: (3) combination of LB:ST:BB; (4) LA; and 5) BB. The yoghurt making process can be seen in figure 1. The quality of cowpea yoghurt determined by chemical analysis (water content, pH, Total Titratable Acid, and Total Soluble Solids, physical analysis (viscosity), microbiological analysis, (total lactic acid bacteria and antibacterial E. coli activity), and sensory analysis (taste, thickness, color, aroma, total acceptance and rank test).

RESULTS AND DISCUSSIONS

Water Content

Water content of cowpea yoghurt was between 89,56% and 90,53% with relatively similar level between treatments. Analysis variant showed that the use of different probiotic starters in cowpea yoghurt did not cause differences in water content level (α=0.05). Tamime and Robinson (2000) explained that water content of several types of milk based yoghurt were approximately 77-84,9% (b/b). Water content of cowpea yoghurt was higher because cowpea extract has higher water content than those of milk. The composition of cowpea and water used was 1:10.

Table 1. The result of chemical, physical and microbiological analysis of cowpea yoghurt with probiotic starter.

Parameters	Treatments				
	ST:LB	LA	BB	ST:LB:LA	ST:LB:BB
Water content (%)	90,46	90,16	90,42	89,56	90,53
pH value	3,99	3,86	5,02	3,80	4,00
Total titratable acid (%)	0,71	0,79	0,68	0,80	0,75
Total soluble solids (° Brix)	7,80	8,00	8,23	8,16	7,33
Viscosity (cP)	291,66 ab	358,33 a	170,66 c	380 a	218,33 bc
Total BAL (CFU/ml)	4,15 x 10^9 b	5,70 x 10^9 a	3,90 x 10^9 bc	4,83 x 10^9 b	3,33 x 10^9 c

Remarks: Numbers followed by the same notations means that there was no significant difference at level 5% and numbers followed by different notation means that there was a significant different at level 5%.

pH Value

pH values of cowpea yoghurt were ranged between 3,80 and 5,02. The lowest value was resulted by the combination of bacteria ST:LB:LA, whereas the highest value was found in yoghurt made by using *Bifidobacterium bifidum* starter.

The analysis of variant showed that the utilization of different probiotic starter did not cause the difference in pH values (α=0.05). the pH values were relatively similar, and the result in accordance with was found by Jay, 2000 that revealed that the pH of yoghurt was between 4,2 and 4,6. The *Standard Nasional Indonesia* (SNI) number 01-2981-1992 gave the standard pH at range 3,5-5. The pH range was in accordance with the optimum pH needed for the growth of Lactic acid bacteria and bifidobacteria. Other than that, the limitation of lactose would minimize the acid production of lactic acid bacteria.

Total Titratable Acid (TTA)

The TTA of yoghurt was ranged between 0,68% and 0,80%. Analysis of variant showed that the utilization of different probiotic starter did not cause the difference in TTA value of cowpea yoghurt significantly (α=0.05). According to SNI 01-2981-1992, total acid counted as lactic acid was required between 0,5 and 2,0%. Therefore, all cowpea yoghurt has been tested were complied with the standard. The insignificant result is because the lactic acid bacteria has limitation in producing lactic acid. This related with the amount of skimmed milk as a source of lactose that was added in the same concentration at every treatment. Eventhough the lactic acid bacteria and Bifidobacteria being tested had different ability in producing lactic acid, (Goderska *et al*, 2008), the limitation of lactose as substrate, made lactic acid bacteria could not produce more lactic acid, the acid produced also influenced the pH of the yoghurt.

Total Soluble Solids (TSS)

The TSS values were ranged from 7.33° Brix up to 8,23° Brix. The analysis of variant revealed that the utilization of different probiotic starters did not significantly (α=0.05) caused the difference in TSS value of cowpea yoghurt. The low TSS value related with the composition of cowpea and water which was 1:10. This made the water content was higher but the TSS was lower than yoghurt made from milk. SNI 01-2981-1992 did not explain the requirement of TSS value, however according to Tamime dan Robinson (2000) the TSS value of milk yoghurt was between 12 and 13%.

Viscosity

The viscosity of cowpea yoghurts were from 170,66 cP to 380 cP. The average viscosity was varied among treatments (Figure 2). The lowest viscosity can be found in yoghurt with BB starter (170.66 cP) whereas the highest in ST:LB:LA starter (380 cP). Analysis of variant showed that different probiotic starter resulted in different viscosity at α=0,01. Duncan post test revealed that the viscosities of cowpea yoghurt produced using starter LB:ST:LA, LA and ST:LB were different with yoghurt made by using LB:ST:BB and BB starters. However, the viscosity of yoghurt made by ST:LB starter was not different from yoghurt made by LB:ST:BB starter.

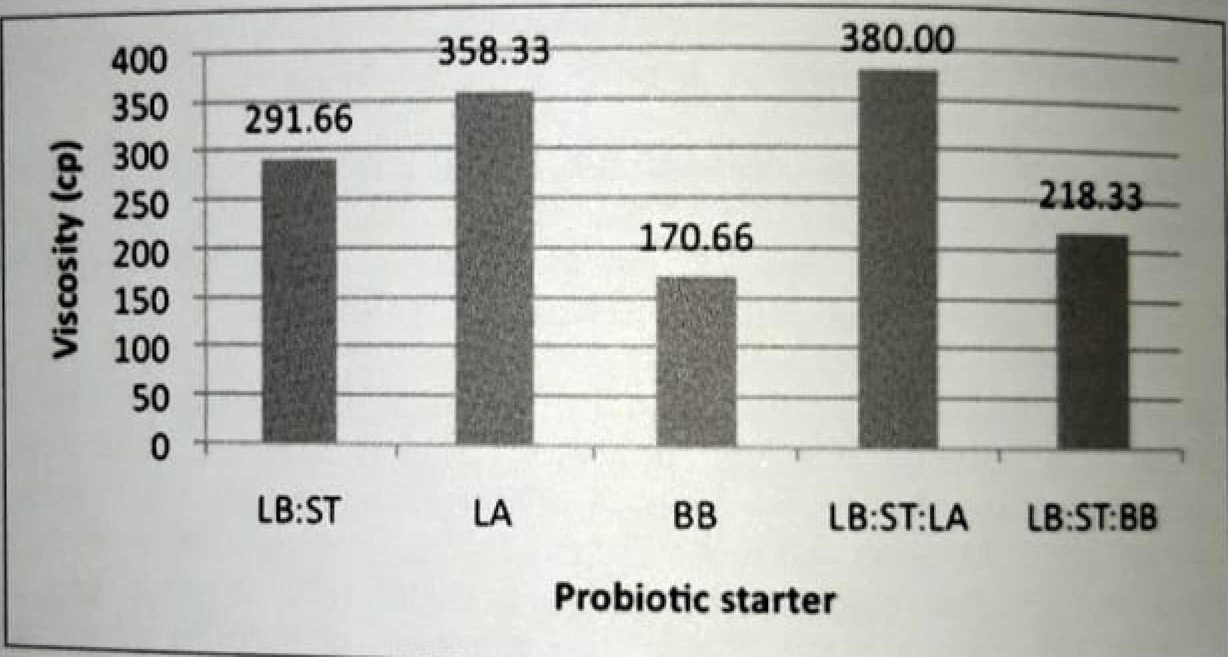

Figure 2. Viscosity of cowpea yoghurt with probiotic microbes

According to Muchtadi dan Sugiyono (1992) in Hartoto (2003), the viscosity of yoghurt were between 1507 cP and 1708 cP. Cowpea yoghurt had low viscosity. The factors determined the property were (1) high water content and low TSS; 2) low protein content of cowpea yoghurt; Protein was a substance important in forming yoghurt coagulum (Tamime and Robison, 2000) that determined the viscosity. (3) the ability of microbes in producing different types of acid. *Lactobacillus acidophilus* produced higher lactic acid than *Bifidobacterium bifidum* (Goderska *et al*, 2008). Therefore pH values of cowpea made by using ST:LB:LA and LA starters were lower than those made by using ST:LB:BB and BB starters. *Bifidobacterium bifidum* produced little amount of lactic acid because this bacteria was pure anaerob that need more complete nutrition for the growth (Kearney *et al*, 2008), and the nutrition could not be provided by cowpea as substrate, due to low protein and fat contents.

The decrease in pH value influenced by lactic acid production. The lower the pH value, the more compact the coagulum, so the yoghurt became more viscous. Cowpea yoghurt made by using *Lactobacillus acidophilus* was more viscous than those made by using *Bifidobacterium bifidum*. Eventhough the value between treatment was not significantly different, the small different was enough to make a significant changing in viscosity of the yoghurt.

Total Lactic Acid Bacteria (LAB)

The total number of LAB in cowpea yoghurt was varied from $1{,}3 \times 10^9$ CFU/mL to $6{,}3 \times 10^9$ CFU/ml (Figure 3). The total LAB was higher in yoghurt with LA starter than those with BB starter. According to Kearney *et al.* (2008), food products was categorized as probiotic if it contained at least 10^7 CFU/ml probiotic starter. Base on this fact, it can be concluded that all cowpea yoghurt in this research fulfilled the requirements.

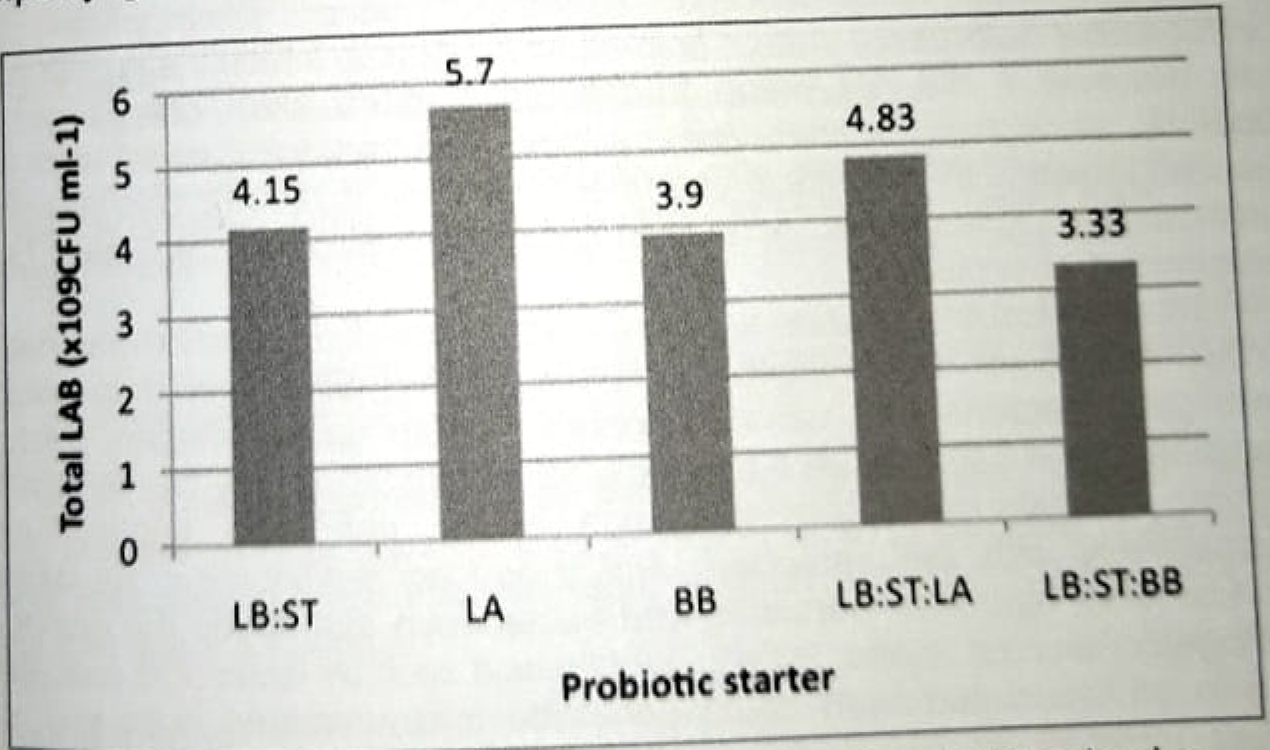

Figure 3. Total LAB in cowpea yoghurt with different probiotik microbe

Analysis of covariant showed that the different probiotic starter resulted in significantly different number of LAB at $\alpha = 0{,}01$. Duncan post test at $\alpha = 0{,}05$ revealed that the use of *Lactobacillus acidophilus* starter both single or in combination, produced yoghurt with the highest number of LAB among other starter. The lowest number of total LAB can be found in yoghurt produced by using *Bifidobacterium bifidum* both single or in combination although the number of bacteria had fulfilled the requirement. *Bifidobacterium bifidum* was pure anaerobik, oxygen sensitive and needed complete nutrition for the growth. Because the nutrition provided by cowpea as substrate was limited (with low fat and protein), therefore it was not adequate for optimal growth of the bacteria (Kearney *et al*, 2008).

Anti Pathogenic Bacteria Activity

Antibacteria activity was an ability of a chemical substances against unwanted microbes. The substances can destroy the cell surface by inhibiting the formation or caused lysist of formed cell walls (Pelczer *et al.*, 1993 in Farida, 2006). The using of probiotic starter produced cowpea yoghurt with no *E. coli* growth. This meant that bacteria in the yoghurt produced anti pathogenic bacteria substances against *E. coli*.

In gastrointestinal tract, one function of LAB was to inhibit pathogenic bacteria, therefore LAB especially *Lactobacillus acidophilus dan Bifidobacterium bifidum* were categorized as probiotik. From the results it was seen that *Streptococcus salivarius subsp. thermophilus* and *Lactobacillus delbrueckii subsp. bulgaricus* could also inhibit the growth of *E. coli*, however it did not mean that these bacteria were categorized as probiotik, since these bacteria could not sustain in gastrointestinal tract, and reached intestine in insignificant number (Senok *et al.*, 2005). This differ with *Lactobacillus acidophilus* and *Bifidobacterium bifidum* that had been categorized as probiotic.

Streptococcus salivarius subsp. thermophilus, Lactobacillus delbrueckii subsp. bulgaricus, Lactobacillus acidophilus, and *Bifidobacterium bifidum were* good bacteria that naturally inhabit human gastrointestinal tract. In digestion, these bacteria were beneficial for health since they can reduce the number of pathogenic bacteria such as *E. coli*. The bacteria lived competitively with the pathogen, and produced antibacterial substances such as acid, hydrogen peroxide, and bacteriocyn that inhibit the growth of pathogenic bacteria (Saito, 2004). Lipofilic acid such as lactic acid and acetic acid in undissociated form could penetrate into microbes' cells and at a higher intracellular pH it dissociated and produced hydrogen ion that disturbed metabolism of pathogenic bacteria (Ouwenhand, 1998 in Farida, 2006).

Bacteriocyn was one of antimicrobial produced by *Bacillus* bacteria (such as *B. cereus, B. subtilis,* and *B. stearothermophilus*), *Lactobacillus* and

Bifidobacteria. Bacteriocyn could destroy the cell's surface of pathogenic bacteria (Khalil *et al*., 2009). Hydrogen peroxide as antimicrobials can cause oxidative stress in *E. coli* that lowered the virulency of pathogenic bacteria (Hedge *et al*., 2008).

Sensory Evaluation

Sensory evaluation on cowpea yoghurt included acidity, thickness, color, aroma and general acceptance. Rank test was also conducted to appraise the most liked product. The average value of sensory evaluation on the quality of cowpea yoghurt can be seen in Table 2.

Table 2. The average value of sensory evaluation on the quality of cowpea with probiotic starters

Treatments	Average scores				
	Acidity	Thickness	Color	Aroma	General acceptance
ST:LB	3,9 a	3,2 a	3,4 b	3,5	2,9 ab
LA	3,6 a	3,2 a	3,7 b	3,4	3,0 a
BB	2,8 b	2,0 b	3,4 b	3,3	2,5 b
ST:LB:LA	4,2 a	3,4 a	4,5 a	3,4	3,3 a
ST:LB:BB	3,8 a	3,0 a	3,2 b	3,5	3,1 a

Score remarks :
- Acidity: 1 (not sour); 2 (less sour); 3 (rather sour); 4 (sour); 5 (very sour)
- Thickness: 1 (very thin); 2 (thin); 3 (rather thick); 4 (thick); 5 (very thick)
- Color: 1 (very brown); 2 (rather brown); 3 (cream); 4 (rather white); 5 (white)
- Aroma: 1 (very beany); 2 (beany); 3 (rather beany); 4 (not beany); 5 (not beany al all)
- General acceptance: 1 (very dislike); 2 (dislike); 3 (rather like); 4 (like); 5 (very like)

Remarks: Numbers followed by the same notations means that there was no significant difference at level 5% or 1 % and numbers followed by different notation means that there was a significant different at level 5% or 1%.

Sensory test was conducted by adding 5% sugar solution (sugar : water = 1:2) into the product. According to Kroeger (1976) in Suriasih (2008) most of the consumer did not like very sour yoghurt and like yoghurt with fruit flavor and sugar addition.

Sensory acidity

Cowpea yoghurt had a ranged sour taste from rather sour until sour (score 2,8-4,2). The highest acidity found in yoghurt with ST:LB:LA starter, and the lowest found in yoghurt with *Bifidobacterium bifidum* starter.

The analysis of variant revealed that the utilization of different probiotic resulted in significant different value of sour taste at α = 0,01. Duncan post test showed that cowpea yoghurt with *Bifidobacterium bifidum* starter had significantly different level of acidity compared to another cowpea yoghurts. Eventhough from pH value there was no significant different, the panelist could detect the difference in acidity.

Thickness

The thickness of cowpea yoghurt were varied from thin until rather thick (score 2.0-3.4). The analysis of variant showed that the utilization of different probiotic starter influenced the thickness of the yoghurt significantly (α 0,01). The thickness of cowpea yoghurt related with physical test-viscosity. The highest and lowest value gained from physical test was relatively the same with the result of sensory analysis. However in sensory test, the notation was not much varied. This because the yoghurts had thin texture that made semi trained panelists could not detect the differences.

Color

The color of cowpea yoghurt were ranged from cream to rather white (score 3.2-4.5). The highest score was found in yoghurt with ST:LB:LA starter (score 4,50=rather white), and the lowest with ST:LB:BB starter (score 3,20 =cream).

The analysis of variant proved that the utilization of different probiotic starter caused a significant different in color. (α 0,01). Duncan post test showed that yoghurt with ST:LB:LA starter had a distinct color compared with others. This phenomena was also found in previous study conducted by Schaffner and Beuchat (1986) that studied the fermentation process of aqueous extract of legumes (included cowpea) using LAB. In that study, the conclusion gained is that the lowest pH could brighter the color of legumes extract (Schaffner dan Beuchat, 1986).

The brightness of cowpea yoghurt might be related with pigment in the legumes. Cowpea had several color variations; black, brown until white. In eastern Africa, the color was a combination of white and brown, in western Africa and a part of Latin America the color of the legumes was usually brownish-red. In Indonesia, Cuba and Caribbean the color was black. There were two pigments responsible for the color of legumes. They are anthocyanin that gave purple, red and black color and melanin-like structured pigment that gave pale yellow color. (Mustapha, 2007). There were high possibility that melanin-like structured pigment was the pigment responsible for cowpea available in Indonesia.

Melanin was known as dark brown pigment with acid properties that also influenced human skin (Irianto, 2008). Melanin can also be found in fruits and vegetables, but the occurrence more likely viewed as disadvantages because the dark color it brought. (Gao *et al.*, 2007). Melanin was very sensitive to oxidation, with oxidation, melanin could change to be more bright, this is used for hair coloring technique with the aid of hydrogen peroxide (Anonim, 2008). Hydrogen peroxide was a substances produced by lactic acid bacteria (LAB) that function as antibacterial agent.

If it was correlated between color change and LAB activities, it can be predicted that the LAB activity in cowpea yoghurt with ST:LB:LA starter were higher that those other treatments. With high activity of LAB, the hydrogen peroxide produced was high so that could brighter the melanin. This could be related with chemical and physical analysis such as pH, TTA and viscosity. The cowpea yoghurt made by ST:LB:LA starter could produce a higher lactic acid, so it was predicted that the hydrogen peroxide produced was also high, therefore the color was brighter.

Aroma

Cowpea yoghurt had a rather beany flavor (score 3.3-3.5). This score was relatively similar among all treatments. Boiling for 20 minutes did not remove all beany aroma in cowpea. The beany arome would be a disadvantages for the product since panelist did not like the aroma. The analysis of variant showed no difference in aroma of cowpea yoghurt.

General Acceptance

Generally, panelist gave score 2.5 to 3.1 to the product. It meant that the products were disliked until rather liked by the panelist. Panelis did not like yoghurt made by using BB starter because the product less sour, too thin and had dark color compared with others.

Rank Test

The evaluation to the preference rank of the yoghurt showed that yoghurt with ST:LB:LA starter was at first rank, followed by yoghurt with LA, ST:LB:BB, ST:LB and BB starter respectively. The rank test determined the best yoghurt made.

Supporting Test

Table 3. The nutrition content per serving size of cowpea yoghurt

Serving size 100 gr		% RDI*	SNI
Charbohydrate	5,16 g	1,72 %	-
Protein	3,87 g	9 %	Min. 3,5
Fat	0,0031 g	0 %	Maks. 3,8
Total LAB (CFU)	$4,5 \times 10^{11}$	-	-
Ash	0,66	-	Maks. 1,0
Moisture	90,31	-	-

* RDI based on energi 2000 calory.

From the overall supporting test it can be concluded that cowpea yoghurt complied with quality requirements of SNI 01-2981-1992.

CONCLUSION

1. Cowpea yoghurt had the following characteristics: creamy to white color, rather thick to thin texture, compact no separation, and had strong beany flavor.
2. Based on chemical, physical, sensory and microbiology analysis, the utilization of *Lactobacillus acidophilus* bacteria resulted a better quality of yoghurt than the utilization of *Bifidobacterium bifidum*. However, not all chemical, physical and microbiology variables showed a significant value.
3. The using of probiotic starter influenced the viscosity, total LAB, sensory acidity, thickness, color and general acceptance of cowpea yoghurt. The best cowpea yoghurt was the one made by using ST:LB:LA starter.
4. Cowpea yoghurt complied with quality standard of oleh SNI 01-2981-1992.

Further exploration is needed to reduce the strong beany flavor exist in cowpea yoghurt. In vivo experiment is also needed to evaluate viability and ability in reducing the number of pathogenic in gastrointestinal tracts.

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