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Viability of lactic acid bacteria, fatty acid profile and quality of cocoghurt made using local and commercial starters during fermentation

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Abstract Cocoghurt is a novel fermentation product with coconut milk as the main raw ingredient. In this study, the starter concentration and fermentation time on the viability of lactic acid bacteria (LAB) and the fatty acid profile and quality of the cocoghurt were examined. *Lactobacillus casei* sub sp. *casei* R-68 and *Streptococcus thermophilus* were used as starter cultures. The results showed that 3.0% of the *L. casei* subsp. *casei* R-68 and *S. thermophilus* starters resulted in the optimal growth of LAB. Fermentation time significantly affected pH, total lactic acid, total LAB, and protein content but did not significantly influence ash, moisture, fat, and total solid content. The duration of fermentation also did not significantly affect the fatty acid profile. The probiotic cocoghurt fatty acid profiles consisted mainly of medium-chain saturated fatty acids followed by long-chain saturated fatty acids and finally unsaturated fatty acids. Cocoghurt produced using skim milk 3.0% starter and fermentation time for 10 hours had the characteristic of being slightly white, tasting sour and sweet, with an aroma of coconut milk; the texture was relatively thick and preferred by the panelists.

Keywords: Viability, Cocoghurt, Fermentation time, *Lactobacillus casei*, Coconut milk

Introduction

Indonesia is one of the largest coconut producers globally, with production estimated to be 18.3 million tons per year in various regions. Riau Province is the largest coconut-producing area in Indonesia, with a planting area of 520,260 Ha from 3,654,520 Ha. Coconut production was 427,080 tons in 2016 and 418,250 tons in 2017 (Indonesian Central Bureau of Statistics,

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2019). Coconut milk is one of the top products from raw coconut processing. However, the incorporation of coconut milk into local snacks is still limited. Cocoghurt is one type of fermented product made from coconut milk that has the potential to be developed. The importance of fermentation in modern life is characterized by the emergence of various types of food marketed to almost all countries, both developing and developed. This fermentation is intended not only for food preservation and safety but also for producing sensory attributes that are highly valued. Fermented foods should be considered one of the primary nutritional sources in developing countries contributing to food security. This fact is due to the fermentation process improving nutritional quality and the digestibility of food and increasing food safety. It is traditionally acceptable and accessible at affordable prices (Holzapfel, 2002; Rolle and Satin, 2002). At present, there is an increasing interest in developing various milk or non-milk fermented products that have health benefits. There is also an interest in preventing toxins from food-borne pathogens and spoilage bacteria that enter the human body (Shah, 2007; Ali, 2010).

Fermented foods and beverages may vary based on the nature of the food, the fermentation time, the number of initial starters, and the intentional application of microbes utilized (Anal, 2019). *S. thermophilus* lowers the pH by metabolizing the lactose in milk into lactate and lactic acid in yogurt production. This triggers the growth of *L. bulgaricus* (Tamime and Robinson, 2007). Based on the role that *S. thermophilus* plays in yogurt fermentation, it was hypothesized that the addition of *S. thermophilus* to coconut milk fermentation triggers the growth of *Lactobacillus casei* subsp. *casei* R-68 (LCR-68) to produce cocoghurt. LCR-68 was isolated from *dadih*, a fermented food produced from local buffalo milk originating from West Sumatra, Indonesia (Venema and Surono, 2018). Fermentedcocoghurtis produced using the commercial starter *S. thermophilus* and the local probiotic LCR-68. The two starter cultures were expected to optimize the production and quality of the cocoghurt.

Several factors affect the final quality of the fermentation products, namely the length of fermentation and the number of initial starters. Fermentation time affects bacterial activity because the longer the fermentation time, the more active the bacteria and the higher the total LAB. As a result, increased amounts of substrate in the media are metabolized (Nor-Khairuza *et al*., 2019). The duration of fermentation is one of the most critical factors affecting the physical, chemical, and organoleptic properties of fermented processed products (Kunaepah, 2008). Previous findings show that 6 hours of fermentation using *Enterococcus faecalis* UP-11 resulted in cocoghurt (Imam *et al*., 2015) that both met the Indonesian standard of fermented milk (Indonesia

National Standard SNI 2981, 2009). Since coconut milk is the main ingredient in cocoghurt, the food product contains medium fatty acids, which may benefit health. The major MCFA (medium-chain fatty acid) present in coconut milk is lauric acid, which has many health benefits such as increasing immunity as well as being an antimicrobial agent (Lieberman *et al*., 2006; Nakatsuji *et al*., 2009; Shilling *et al*., 2013; Dayrit, 2014; Anzaku *et al*., 2017). Until now, there have not been found the report on the fate of the MFCA during fermentation. Therefore, the objective of the present study was to evaluate the effect of the starter concentration and fermentation time on the viability of the lactic acid bacteria, the fatty acid profile, and the quality of the cocoghurt.

Materials and methods

Study area

This study was conducted at the Laboratory of Agricultural Product Processing, Faculty of Agriculture, Universitas Riau, and the Integrated Laboratory of Food Science and Technology, IPB University, Bogor, Indonesia. The study was conducted from October 2018 to March 2019.

Experimental design

The study was carried out experimentally using a completely randomized design for determining the effects of starter concentration and fermentation time on cocoghurt quality. For the first experiment, the starter concentration was varied as follows: C1 (starter concentration 1.5%), C2 (starter concentration 3.0%), C3 (starter concentration 4.5%), C4 (starter concentration 6.0%) and C5 (starter concentration 7.5%). In the second experiment, the fermentation time was varied as follows: T1 (fermentation time for 6 h), T2 (fermentation time for 10 h), T3 (fermentation time for 14 h), T4 (fermentation time for 18 h), and T4 (fermentation time for 22 h). Each treatment was repeated three times.

Bacterial starters

Lactobacillus casei subsp. *casei* R-68 (LCR-68) was isolated from dadih (Venema and Surono, 2018), and commercial *Streptococcus thermophilus* were used as the bacterial starters in this study.

Preparation of coconut milk

Coconut milk was prepared according to Pato *et al*. (2019). Grated coconut was squeezed using a coconut milk press until the coconut milk was wholly extracted from the grated coconut. The coconut milk was then filtered using a filter cloth, and pure coconut milk was obtained.

Preparation of cocoghurt starters

The used starter was prepared in two stages; the first stage involved preparing the medium containing 5% skimmed milk and 2% sucrose. This medium was stirred before transferred into a glass jar and sterilized at 121 ℃ for 10 min. Following cooling the mixture down to 30 - 40 ℃, skimmed milk medium was inoculated with LCR-68 and *S. thermophilus* separately (2% v/v) and incubated at 37 ℃ for 13 h. Next, a second medium was consisted of equal volumes of skimmed milk and coconut milk. The mix was stirred, transferred into glass jars, and sterilized at 121 ℃ for 10 min. After cooling the mixture down to 30 - 40 ℃, the skimmed milk/coconut milk mix was inoculated with bacteria from the first skimmed milk medium. The second medium was used as the active starter for making the cocoghurt.

***Making process of the probiotic* cocoghurt**

The cocoghurt was prepared following the formulation previously described by Pato *et al*., 2019). Coconut milk (400 mL) was mixed with sucrose 2% (v/w), skimmed milk 5% (v/w) and carboxymethyl cellulose 0.05%. The mixture was then homogenized using a blender for 5 min. The homogenized media was heated to 85 ℃ for 15 min and then cooled to a temperature of 37 ℃. The media was then inoculated with starter LCR-68 and *S. thermophiles* at concentrations ranging between 1.5, 3.0, 4.5, 6.0, and 7.5%. The mixture was then incubated at 37 ℃ for 10 h. The best concentration was then used to determine the optimal fermentation time of 6,10,14,18, and 22 hours to produce the best quality cocoghurt as described.

Parameters measured in the probiotic cocoghurt

The measured cocoghurt parameters were pH, total lactic acid, total LAB, protein, fat, total solids, moisture, and ash content. The pH was determined using a pH meter, the total lactic acid was determined by alkalimetric titration using 0.1N NaOH. Protein, ash, total solids, moisture, and fat contents were

analyzed according to the method described by AOAC (2012). Total LAB was determined according to the methods described by Indonesian National Standard (2009). The fatty acid profile was analyzed using gas chromatography (Seppanen-Laakso and Laakso, 2002). Cocoghurt sensory testing refers to Yuceer and Drake (2007). Sensory tests conducted were descriptive tests by 30 semi-trained panelists and hedonic tests by 80 panelists.

Data analysis

The obtained data were analyzed using analysis of variance (ANOVA). For the test results with an F count greater than or equal to the F table, further testing was performed using the Duncan New Multiple Range Test (DMRT) at 5% to determine the differences between each treatment.

Results

The concentration of starter LCR-68 and *S. thermophilus* significantly affected the pH, total lactic acid, and total LAB in cocoghurt ($p<0.05$), as shown in Figure 1. The use of various starter concentrations resulted in a relatively similar total LAB ranging from 8.89 log CFU/g at a concentration of 1.5% to 9.29 log CFU/g at a concentration of 4.5%.

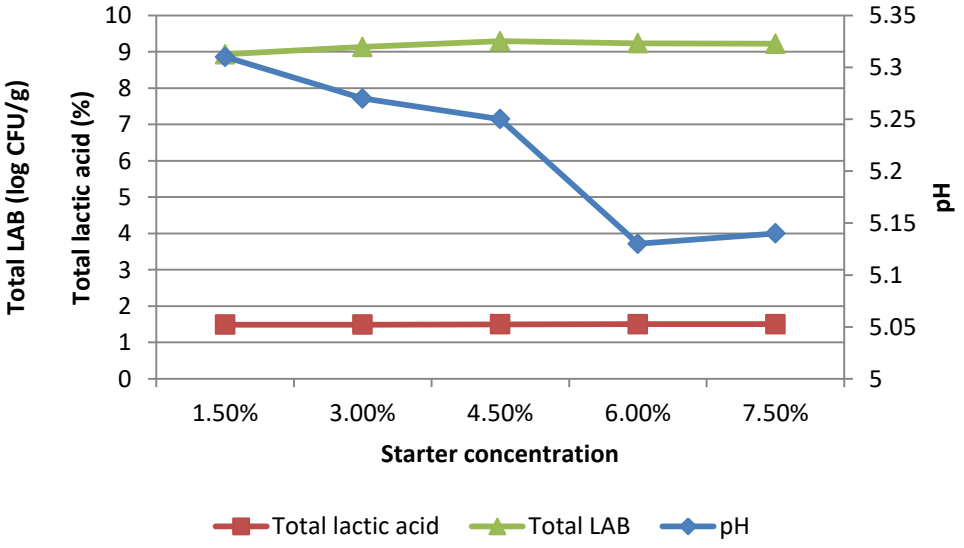

Figure 1. pH, total lactic acid, and total LAB during fermentation using various concentrations of starter *Streptococcus thermophilus* and *Lactobacillus casei* subsp. *casei* R-68

The results indicated that 3.0% starter results in the optimal growth of LAB (Figure 1). The growth of LCR-68 and *S. thermophilus* in the coconut milk and skimmed milk mix during the fermentation process, the pH, total lactic acid, and total LAB were measured against time. The fermentation time significantly influenced the pH, total lactic acid, and total LAB in the cocoghurt ($p<0.05$). The results are presented in Figure 2. The longer the fermentation, the higher the total BAL with optimal time at 14 hours resulted in the amount of LAB as much as 9.64 log CFU/g. Fermentation time led to increase in the total amount of lactic acid from 1.17 - 2.45% and decreased pH value from 5.48 - 4.67 at fermentation time of 6 and 22 hours.

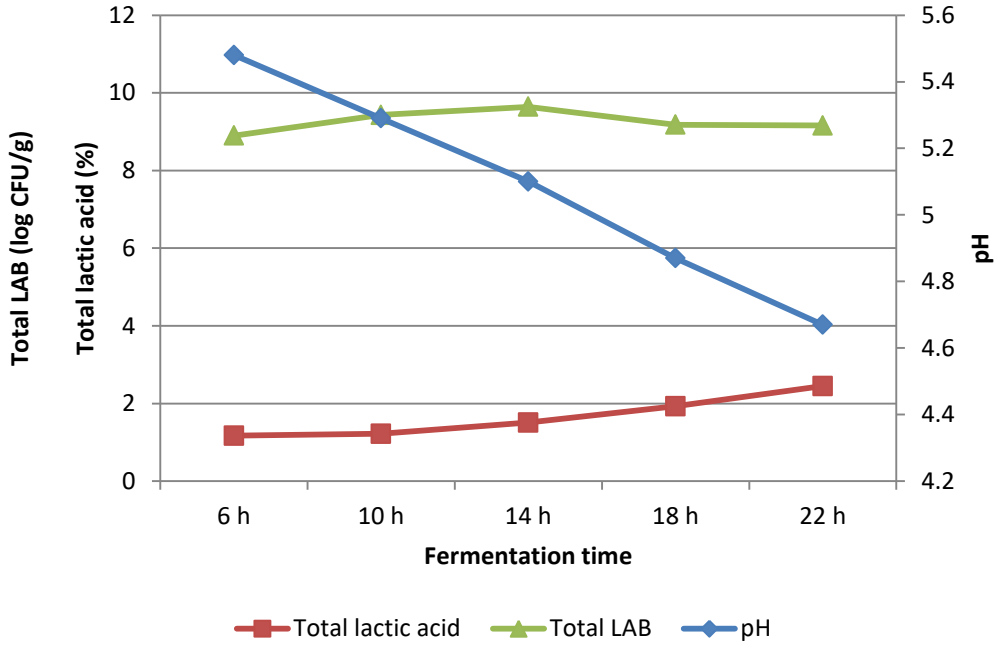

Figure 2. pH, total lactic acid, and total LAB during fermentation using the starter *Streptococcus thermophilus* and *Lactobacillus casei* subsp. *casei* R-68

Likewise, a slightly decreased in pH from 5.31 to 5.13 and a slightly increased in total lactic acid from 1,489 to 1,502 at concentrations of 1.5 and 6.0%. The longer the fermentation time, the more lactic acid is produced and accumulated in the fermentation medium. During fermentation, carbohydrate in the form of lactose and sucrose is metabolized by *L. casei* subsp. *casei* R-68 and *S. thermophilus* to form lactic acid. Residual lactose and sucrose are not metabolized into lactic acid in the medium, as shown in Figure 3. The longer the fermentation, the lower the amount of sucrose from 5.48 - 4.67% and lactose from 0.37 - 0.06% during fermentation 6 and 22 hours, respectively.

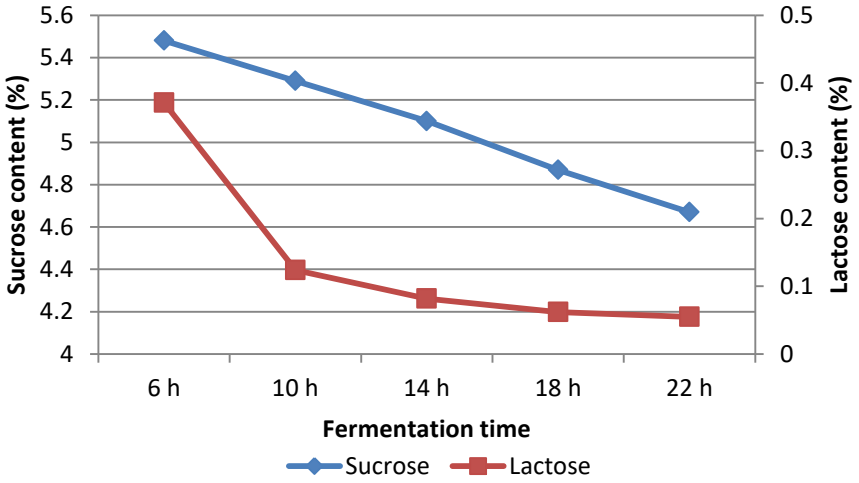

Figure 3. Sucrose and lactose leftover during fermentation using the starter *Streptococcus thermophilus* and *L. casei* subsp. *casei* R-68

LCR-68 and *S. thermophilus* used these two types of disaccharides as energy sources to produce lactic acid was the final product of lactate fermentation. This fact was indicated by a decreased in the amount of both types of sugar during fermentation.

During growth, LAB required the nutritional compounds which contained in the fermentation medium (Table 1). Fermentation time did not significantly affect moisture, ash, and total solid contents but significantly influenced the protein content of cocoghurt (p<0.05). Protein level significantly increased from 5.07 to 6.30% at fermentation time 6 and 22 hours, respectively. Cocoghurts have ash as much as 1.10 - 1.31%, water 45.51 - 45.95%, and a solid total of 54.05 -54.19%.

Table 1. Quality parameters of probiotic cocoghurt produced using starters *Streptococcus thermophiles* and *Lactobacillus casei* subsp. *casei* R-68 during the fermentation process

Fermentation time (h)	**Protein** (%)	**Ash** (%)	**Moisture** (%)	**Total solid** (%)
T1 (fermentation time for 6 h)	5,07^{a1}	1.23^{a}	45,89^{a}	54.11^{a}
T2 (fermentation time for 10 h)	5,36^{ab}	1.27^{a}	45,94^{a}	54.06^{a}
T3 (fermentation time for 14 h)	5,58^{b}	1.10^{a}	45,81^{a}	54.19^{a}
T4 (fermentation time for 18 h)	6,09^{c}	1.20^{a}	45,51^{a}	54.49^{a}
T5 (fermentation time for 22 h)	6,30^{c}	1.31^{a}	45,95^{a}	54.05^{a}

[1]Means followed by lowercase letters in the same column indicated significant differences (p<0.05).

Table 2. Fat content and fatty acid profile in probiotic cocoghurt using the starter *Streptococcus thermophilus* and *L. casei* subsp. *casei* R-68 during the fermentation process

Fat content and fatty acid profile	Fermentation time				
	6 h	10 h	14 h	18 h	22 h
Fat contents (%)	31.62	31.59	31.37	33.80	28.83
Capliric acid	2.87	2.52	2.32	2.64	2.17
Capric acid	2.08	1.93	1.72	1.92	1.68
Lauric acid	13.76	13.86	11.85	12.65	11.74
Miristic acid	4.75	4.99	4.06	4.42	4.10
Pentadecanoic acid	ND	ND	ND	ND	ND
Palmitic acid	2.53	2.81	2.15	2.36	2.20
Stearic acid	1.13	1.30	0.96	1.03	0.97
Arachidic acid	0.02	0.02	0.01	0.02	0.02
Dodecanoic acid	0.005	0.01	0.004	0.004	0.003
Total saturated fatty acids	27.15	27.44	23.07	25.04	22.88
Miristoleic acid	ND	ND	ND	ND	ND
Palmitoleic acid	0.0002	0.002	0.002	0.003	0.003
Oleic acid	1.70	2.00	1.48	1.58	1.51
Linoleic acid	0.23	0.29	0.21	0.21	0.21
α-linolenic acid	ND	ND	ND	ND	ND
11-eicosanoic acid	0.01	0.01	0.01	0.01	0.01
Arachidonic acid	ND	ND	ND	ND	ND
EPA	ND	ND	ND	ND	ND
DHA	ND	ND	ND	ND	ND
Total unsaturated fatty acids (%)	1.94	2.30	1.70	1.80	1.73
Unknown fatty acids (%)	0.04	0.05	0.04	0.05	0.04
Total fatty acids (%)	29.13	29.79	24.82	26.90	24.66

ND = Not detected

Table 3. Quality of probiotic cocoghurt produced using starters *Streptococcus thermophilus* and *Lactobacillus casei* subsp. *casei* R-68

Sensory test parameters	Scores	Description
Colour	3.73	slightly white
Aroma	3.27	coconut milk aroma
Taste	4.07	sour and sweet
Texture	3.23	rather thick
Hedonic	2.68	liked by panelists

In general, fermentation time caused a slightly decreased in the fat levels of cocoghurts. This reduction is caused by a decreased in fatty acids, namely capliric acid, capric acid, lauric acid, miristic acid, palmitic acid, stearic acid, dodecanoic acid, oleic acid, and linoleic acid. In contrast, fatty acids such as arachidic acid, palmitoleic acid, and 11-eicosanoic acid were stable for 22 hours of fermentation (Table 2). Most of the fat content in the cocoghurt come from the pure coconut milk which used as the primary raw ingredient. A smaller percentage of the fat content is from the used skimmed milk.

The result of the sensory test under optimal starter conditions (3%) and fermentation time (10 hours) is presented in Table 3. The panelists like the

cocoghurt produced under these conditions because it had the characteristics of slightly white, flavored with coconut milk, sweet and sour, and slightly thick.

Discussion

Cocoghurt is an example of a locally produced fermented product with high economic potential. The amount of starter added to the fermentation medium is one of the essential factors in the fermentation process's success. The higher the concentration of the starter used, the lower the pH of cocoghurt and the increasing total lactic acid in cocoghurt. This fact is due to the increasing number of LAB at the beginning of the fermentation that breaks down simple sugars, especially lactose and sucrose, to produce lactic acid as the end of sugar metabolism. The formation of lactic acid during fermentation caused a decrease in pH value and increased total lactic acid in cocoghurt. The availability of sufficient nutrients in the fermentation medium also directly triggered the growth of LAB (Ganzle, 2015), so that the amount of LAB in cocoghurt increased with the increasing number of starters.

The fermentation time significantly influenced the pH, total lactic acid, and total LAB in the cocoghurt (p<0.05). Longer fermentation times significantly decreased the pH from 5.48 to 4.67, and the total lactic acid increased significantly from 1.17 to 2.45%. The decrease in pH was caused by the LCR-68 hydrolysis of lactose and sucrose into monosaccharides. The monosaccharide compounds were subsequently metabolized by LAB into organic acids, especially lactic acid. Results showed that the longer the fermentation process, the greater the total amount of lactic acid metabolized from sucrose and lactose. Lactic acid production is closely related to the length of the fermentation time. The longer the fermentation time, the more lactic acid is produced and accumulated in the fermentation medium. *S. thermophilus*, classified as a homofermentative bacterium, produces only lactic acid while LCR-68, classified as a heterofermentative bacterium, produces lactic acid, acetic acid, alcohol, and CO_2 as the final products of carbohydrate metabolism. This statement is supported by Ganzle (2015). The decreasing pH values and the increased total value of the lactic acid were due to the significant growth of both LAB strains, i.e., LCR-68 and *S. thermophilus* used in the starter medium. The total LAB content increasing that started at 6 hours fermentation until its optimum point at 14 hours. After that, from hours 18 to 22 of the fermentation process, there was a decreased in the total LAB content. The decreased in LAB content may be attributed to the accumulation of primary metabolites, mainly lactic acid, in the medium, causing the medium to be more acidic and for the pH to decrease, resulting in inhibition of LAB growth itself. The results of this study were differed from the results reported by Widagdha and Nisa (2015),

who reported that the optimal fermentation time for yogurt produced with grape juice was 12 hours. Meanwhile, Imam *et al*. (2015) reported that the optimal fermentation time for milk is 6 hours. The difference in fermentation time was most likely caused by the different starters. Widagdha and Nisa (2015) used a combination of *L. bulgaricus* and *S. thermophilus*, and Imam *et al*. (2015) only used *Enterecoccusfaecalis* UP-11 as the single starter. Meanwhile, the present study used a combination of LCR-68 and *S. thermophilus* as the starters for cocoghurt fermentation. The results were slightly differed from the results of previous research.

The decreased in sucrose and lactose presented in the media was in line with a longer fermentation time which indicated by LAB. The lactose content is decreased faster than sucrose from the beginning to the end of fermentation. LCR 68 was isolated from *dadih*, a traditional fermented product from buffalo milk. Hence, this strain was able to grow in a medium containing lactose. This fact is the main reason for the higher decrease in lactose compared to sucrose. This statement is following the results previously reported by Imam *et al*. (2015). Yoghurt culture bacteria, especially *S. thermophilus*, is reported to be able to preferentially metabolize and transport specific mono and disaccharides such as lactose, sucrose, maltose, glucose, and to a lesser extent, galactose (Hutkins *et al*., 1985; Poolman *et al*., 1988; Sobowale *et al*., 2011). Compared to glucose and fructose, the higher rate of sucrose utilization may be due to the different uptake rates (Hutkins and Morris, 1987). Unlike *S. thermophylus*, besides using simple sugars as energy sources, *L. casei* also used oligosaccharides (Hadisaputro *et al*., 2014; Roberfroid *et al*., 1998), fructooligosaccharides, galactooligosaccharides, isomaltooligosaccharides, and the digestible disaccharide isomaltose as energy sources (MacFarlane *et al*., 2008; Seibel and Buchhol, 2010). *L. casei* can also use citrate as an energy source to produce pyruvate, acetate, and acetoin (Diaz-Muniz *et al*., 2006; Mortera *et al*., 2013).

The needed nutritional compounds include carbohydrates, fats, protein, vitamins, and minerals. The results showed that the duration of the fermentation process significantly influenced the protein content but that it did not significantly affect the ash, moisture, and total solid content of the probiotic cocoghurt ($p<0.05$). The ash levels in cocoghurt did not change over the entire duration of the fermentation process. The ash levels in food refer to the inorganic materials present in food, such as minerals. The number of minerals presented in the medium was sufficient for the growing needs of both types of LAB. Minerals in the medium are used by LAB in the metabolic processes and /or the formation of cell components. Thus, the mineral content at each fermentation stage remained relatively the same. The total amount of solids in

cocoghurt did not change during the entire fermentation process, with the amounts ranging between 54.05 and 54.49%. LAB used only a small amount of solids in lactose, sucrose, and the other compounds needed for growth. Hence the total solids remained abundant during all of the fermentation time treatments. The total solids are mostly made up of carbohydrate compounds, proteins, fats, minerals derived from coconut milk, skimmed milk, and sucrose which are used as the main ingredients for making cocoghurt. Similarly, the moisture content in all treatments showed almost no difference between the treatments. The moisture content may be attributed to the quantity of coconut milk used, which was relatively equal in all treatment mediums.

Most of the fat content in the cocoghurt come from the pure coconut milk that used as the primary raw ingredient. A smaller percentage of the fat content is the skimmed milk used. This fact is due to the formula of making cocoghurt that used coconut milk as its primary raw material with a bit of addition of skimmed milk. The content was 57 g in 240 g coconut milk (Spritzler, 2018), and in skimmed milk was 0.6-1.25% (US Dairy Export Council, 2005). The fat levels decreased slightly from 31.62% in the 6-hour fermentation process down to 28.83% after 22 h of fermentation, despite a slight increased in the total fat at 18 h fermentation. The results decreased total fat and some fatty acids, namely capliric acid, capric acid, lauric acid, miristic acid, palmitic acid, stearic acid, dodecanoic acid, oleic acid, and linoleic acid, during the fermentation process. This phenomenon may be due to these fatty acids forming cell components such as the cell membrane and wall of LAB. This statement was supported by Johnsson *et al*. (1995). They found that the cell membranes of 10 strains of *Lactobacillus* sp and *Lactococcus* sp were composed of saturated fatty acids such as myristic acid, palmitic acid, stearic acid, *cis*-9-octadecenoic acid, *cis*-11-octadecenoic acid, and unsaturated fatty acids such as sapienic acid and oleic acid. Besides the fatty acids reported by Johnsson *et al*. (1995), Guerzoni *et al*. (2001) reported other fatty acids in the cell membrane of *Lactobacillus helveticus* such ascapric acid, decanedioic acid (sebacic acid), lauric acid, *cis*-9-hexadecenoic acid or palmitoleic acid, *cis*-9-octadecenoic acid, *cis*-11-octadecenoic acid, *cis*-12-octadecadienoic acid, *cis*-11, 12-methyleneoctadecanoic acid, *cis*-icos-9-enoic acid and vernolic acid (*cis*-12,13-epoxy-*cis*-octadec-9-enoic acid). The latter fatty acids were only found in *L. helveticus* in response to acid, oxidative stress, and thermal stress during growth. Oleic acid is one example of an unsaturated fatty acid that can stimulate the growth of *L. delbuecki (*Partanen *et al.*, 2001). Linoleic acid contributes to the structure and function of the cell membranes, and it plays a part in the regulation of gene activity inside the cell (Orsavova *et al*., 2015; Jandacek, 2017). In our study, the oleic acid level in the fermentation medium

was relatively high due to its high content in coconut milk. This finding is thought to be one factor triggering the growth of LCR-68 and *S. thermophilus*. Hence the high levels of LAB from the beginning until the end of the fermentation process.

The color of cocoghurt was rather white, derived from coconut milk and skim milk. The sweet taste was caused by sucrose in the fermentation medium, and the sour taste was caused by lactic acid, which is formed during the fermentation. The dominant aroma of cocoghurt was due to pure coconut milk, which contains methyl nonyl ketones. This statement is supported by the National Center for Biotechnology Information (2020), which reports that methyl nonyl ketone is found in coconut and palm kernel. Cocoghurt in this study showed a thick texture because the decrease in pH of the medium caused protein coagulation. The cocoghurt is made using a 3% starter, and panelists preferred fermentation for 10 hours. This phenomenon might due to it has a sweet and slightly sour taste, a texture that resembles the texture of yogurt and has a coconut flavor, a distinctive aroma not possessed by other fermented milk products.

In conclusion, based on the results, it can be concluded that 3.0% starter *Lactobacillus casei* subsp. *casei* R-68 and *Streptococcus thermophilus* resulted in the optimal growth of LAB in cocoghurt. Fermentation time was significantly affected pH, total lactic acid, total LAB, and the protein content but did not significantly affect ash, moisture, fat, and the total solid content. The fatty acid profile of the probiotic cocoghurt consisted mainly of medium-chain saturated fatty acids, followed by long-chain saturated fatty acids and unsaturated fatty acids. The best cocoghurt was produced using skimmed milk 3.0% starter and fermentation time for 10 hours with the characteristic of being slightly white, tasting sour and sweet, with an aroma of coconut milk; the texture was relatively thick and preferred by the panelists.

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