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Development of Local Functional Food Made Of Buffalo Milk Improved With Culture Probiotics (Lactobacillus casei)

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A B S T R A C T

The research was conducted to develop functional food products of milk-based livestock origin (Semi hard-type cheese), with the addition of pure culture Lactobacillus casei as a probiotic agent, and citric acid and *Mucor meihei* as milk coagulants. The research material was semi-hard type cheese made of approximately 35 liters of buffalo milk from West Sumbawa Regency as a basic ingredient with the probiotic pure culture. The results showed that the pure culture of probiotic (*Lactobacillus casei)* at levels of 10% and 15% can survive and develop quite well in semi-hard cheese during aging, from 1 day, 7 days, and 14 days, respectively (3.79 – 5.92) and (4.91 – 6.31) log cfu g^{-1}. While the 0.025% rennet of the volume of milk from Mucor miehei gives a pretty good result, it can be seen from the product recovery which can reach (34.30 + 0.32) %. During aging for 14 days, and organoleptic quality which includes aroma, color, and texture was getting better, with the criteria of a semi-hard aroma, yellowish-white color, and semi-hard texture. It can be concluded, that semi-hard type cheese can be used as one of the functional foods of probiotic carriers. To get the therapeutic effect, this probiotic should be consumed at least 100 grams per serving.

1. INTRODUCTION

1.1. Research Background

Consuming functional foods is beneficial to support an immune response so that it can maintain and improve the health of the body. Some types of functional foods are available in the market such as Yogurt and Yakult which are known to contain probiotics. Probiotics are dietary supplements in the form of living microbes that benefit the host individual by improving the balance of the gut microbes [1]. More details explained that consuming foods or drinks that contain probiotics, has benefits including 1) as a source of nutrition. The nutritional benefits of probiotics have been widely studied in dairy-based products fermented with *Lactobacilli* and *Bifidobacterium*. 2) Health value or therapeutic effects. Effects on intestinal disorders and infections. 3) Stimulation of the immune system. 4) Potential anti-tumours activity. The habit of consuming probiotic foods can reduce the contamination of carcinogen chemicals by ingested carcinogen toxins, then probiotics also function to change the atmosphere in the intestines by reducing population or bacterial metabolic activities that can produce carcinogenic compounds. 5) Anti-hypertensive effects / lower cholesterol levels.

Some types of functional dairy-based foods except Yakult and Yogurt can also be milk candy and semi-hard cheese types. Semi-hard cheese can be made from cow's milk or buffalo milk. Semi-hard cheese made from cow's milk is not as smooth as semi-hard cheese made from buffalo milk. With a little innovation in the addition of probiotics, it will increase the value and function of buffalo milk products which will eventually stimulate buffalo farmers, especially in this region of West Nusa Tenggara Province, whose population is starting to decline. Based on this explanation, research on the characteristics of Semi-hard cheeses Made from Buffalo Milk enriched with Probiotic Bacteria as a Functional Food has been successfully conducted, This research is expected to be useful in the development of functional food products of milk-based origin. The addition of *Lactobacillus casei* is intended as a probiotic culture and *Mucor meihei* is used as a coagulant of milk.

1.2. Literature Review

Milk is a complex fluid in terms of physiologically and biologically, water is the main component, then protein, lactose, fat and inorganic, compounds as its principle constituents. It is

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consider as simple access of food and has quantity of nutrients generously, minerals and vitamins. Percentage of proteins, lactose, lipids and mineral in milk is 3.2%, 5%, 4%, and 0.7% respectively [2]. Moreover, Ref. [3] stated that milk from different species has its own characteristics. Buffalo milk is liquid having very smooth texture, especially higher in calcium and low in cholesterol than cow milk, goat and sheep milk. Cow milk contains 43% more cholesterol, 40% and 58% less protein and calcium respectively than buffalo milk. Acidity and chemical composition of raw milk used for the production of probiotic semi hard cheese and after adding probiotic its changed, the fat level increased just because of probiotics from lactic acid bacteria family decreased the pH level which is used to enhance the shelf-life of cheese [4].

Cheese is a fermented food product that is rich in calcium and has benefits for the body. The product derived from the coagulation of milk protein. Apart from casein (milk protein), other components of milk such as fat, minerals and fat-soluble vitamins are also carried in clumps of casein particles. The water-soluble components of milk are left in the residual solution from the agglomeration of casein called whey [5]. One type of probiotic bacteria is *Lactobacillus casei. Lactobacillus casei* helps limit the growth of pathogenic bacteria in the gut [6]. Judging from the high nutritional value of semi hard cheese and the benefits of probiotics for health, this can make the processed food one of the typical functional foods locally in West Nusa Tenggara Province.

1.3. Research Objective

The research aimed to find out the viability of probiotic bacteria in semi hard cheese, the characteristics of semi hard cheese, and the organoleptic value of semi hard cheese during aging.

2. MATERIALS AND METHODS

2.1. Research Material

. The research material was semi hard cheese made of buffalo milk as a basic ingredient from West Sumbawa Regency (KSB), the probiotic culture used was *Lactobacillus casei* purchased from the Center for Food and Nutrition Studies, University of Gadjah Mada, Yogyakarta. While the milk coagulant was a microbial enzyme of Mucor meihei. Other ingredients were cheese-cloth; salt; calcium chloride ($CaCl_2$); MRSA (de Man Rogosa and Sharpe Agar) as culture media; BPW (Buffered Peptone Water) for serial dilutions; Gram stain solution; and sterile distilled water.

2.2. Research methods

2.2.1. Starter culture preparation [7]

Sixty (60) g skim milk powder was dissolved into 500 ml of water then pasteurized at 72 0C for 15 seconds. The temperature was lowered to reach 37 0C then 2 ml of pure *Lactobacillus casei* from the broth culture was added. The milk then incubated for 24 hours at room temperature; a probiotic culture starter is ready to use.

2.2.2. Manufacturing of probiotic semi hard cheese [8]

Buffalo's Milk was added by 3.5 % salt (NaCl) then pasteurized at 72 ºC for 15 minutes. Then, the milk was allowed to cool (about 35–40 ºC). Once cool, the probiotic culture was mixed into the milk while stirring evenly. The pan was covered and let stand for 5 minutes. The addition of probiotic cultures also plays a role in the acidification process to facilitate the curd formation process. Calcium chloride ($CaCl_2$) solution as much as 0.04% of the volume of milk and Mucor miehei enzyme as much as 0.025% of the volume of milk were added to pasteurized milk, then allowed to stand for 15 to 30 minutes.

After the milk coagulates with a firm like tofu, the curd was cut into cubes. If the lump was still too Semi hard like porridge, don't cut it too fast, it needs to be left for about 1 hour to increase the elasticity. After the curd was cut into pieces, then drain (separate) the whey (liquid) and leave a third of it. The first stage of curd washing process with the addition of hot water of 65 ºC. Then stirring gently for 15 minutes, followed by standing for 5 minutes so that the curd can be filtered.

Separation of whey from curd as much as 0.5 the volume of whey, then the second stage curd washing process with an addition of 75 ºC hot water, then stirred for 15 minutes. Physical examination of the curd whether it was chewy and ripe enough to be filtered. if it feels not chewy enough and cooked, then the stirring was continued in the hot water so that the curd was cooked. Followed by standing for 5 minutes then the curd can be filtered.

Separate the whey from the curd until it runs out using the cheese cloth, followed by pressing the curd using a weight for 15 minutes. The process of turning the cheese, then doing the molding process or pressing the cheese using a load twice the weight of the cheese for 3 hours. Cheese was removed from the mold and continued with the souring process for 4 hours at room temperature. Drying process by turning the cheese twice a day in the ripening room/refrigerator. The process of ripening or fermentation of cheese for 1 day, 7 days, 14 days with a temperature (11-13) ºC.

2.3. Data analysis

2.3.1. Observed variables

The variables observed in this study were viable probiotic bacteria count, products recovery in various treatments, and organoleptic value as supporting variables.

2.3.2. Microbiological analysis [9]

Total probiotic bacteria count in semi hard cheese samples using the Plate Count method. To report the results of microbiological analysis, the following procedure was carried out: The number of colonies that meet the counting requirements is between 25 and 250. Several colonies that are joined into one large colony, can be counted as one colony. A row of colonies which is visible as one solid line can be counted as one colony.

3. RESULTS AND DISCUSSION

3.1. Total probiotic bacterial count

Results of the observations on the content of probiotic bacteria in semi hard cheese were listed in Table 1. The cheese was a semi-hard type cheese in which the manufacturing process

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needs to be ripped for several weeks [10]. The ripening process of the cheese takes place in the refrigerator at a temperature of ± 20 oC, for 2 weeks which was divided into 3 observations. The observations were made at day 1, day 7, and day 14. The results of research on the use of *Mucor miehei* enzymes and probiotic starters and their viability in the cheese can be seen in Table 1.

Table 1. Total probiotic bacterial during storage period (*log. cfu. g^{-1}*)

Level of *Lb. casei*	Storage Period (day)		
	1 st	7 th	14 th
10 %	3.798 [a]	4.548 [b]	5.916 [c]
15 %	4.912 [d]	6.264 [e]	6.306 [f]
LSD	0.362		

Note: The different Superscripts within raw and column were significantly different (P<0.05)

The results of the analysis of variance in the 3 x 2 factorial pattern and the results of LSD (Least Significant Difference) test on product recovery and average bacterial content in cheese given probiotic bacterial cultures at different concentrations were presented in Table 1, and Table 2. A comparison of the bacterial content in the cheese can be seen in Figure 1. Storage time of all days and probiotic levels were significantly affect the total probiotic bacteria of the cheese (P<0.05), with the viability tend to increase during storage (aging). The use of 10% probiotic cultures tend to increase significantly during aging. On the other hand, using 15% probiotic cultures increase slightly in day 14 as compared to 10% (Figure 1). Results show that the addition of 3.5% salt for semi hard cheese was still feasible, due to the inhibitory effect on microorganisms. It was proven that during storage the probiotic culture still survives quite well (Table 1, Figure 1). The types of bacteria observed were rod-shaped lactic acid bacteria. To determine the type or group of bacteria, whether belonging to the lactic acid bacteria group or not, the Gram staining process and catalase test was carried out previously.

The probiotic culture of the cheese was observed to be rods shape, Gram positive, catalase negative, which was consider belonging to lactic acid bacteria. In general, lactic acid bacteria (LAB) are Gram-positive and Catalase-negative, usually grow microaerophilic or absolutely under anaerobic conditions, and do not form spores [7]. It is characterized by the shape of the bacteria in the form of a rod. Lactobacillus ferment carbohydrates with pyruvate as an electron acceptor, and lactic acid as the final product. Physiological characteristics that allow it to function as a probiotic are: resistance to low pH and growth at body/room temperature. This allows lactic acid bacteria to survive in the digestive tract. The shape of lactic acid bacteria resembles a rod, derived from the addition of a probiotic starter, namely from a pure culture of *Lactobacillus casei. Lactobacillus casei* in functional food products on the market found in Yakult has benefits for body health.

According to Ref. [11] that some of the benefits of consuming milk and or probiotic milk products other than as a supply of nutrients for the body, also have important roles, including 1) stimulation of the immune system. The immune system provides an important defence against pathogenic microbes that have entered our bodies. The immune system is very complex, involving both cell-based and antibody-based, 2) potential anti-tumour activity. Cancer-causing chemicals (carcinogens) are produced by the metabolic activity of microbes living in the digestive system, 3) potential anti-tumour activity. Cancer-causing chemicals (carcinogens) can be used or produced by the metabolic activity of microbes that live in the digestive system. It has been suspected that the habit of consuming probiotic foods can reduce the contamination of carcinogenic chemicals by ingesting carcinogenic poisons and then changing the atmosphere in the intestines to reduce the population or metabolic activity of bacteria that can produce carcinogenic compounds. 4) anti-hypertensive effect or lowering cholesterol levels. Clinical studies show that the consumption of milk containing probiotics in people with high blood cholesterol can reduce blood cholesterol levels.

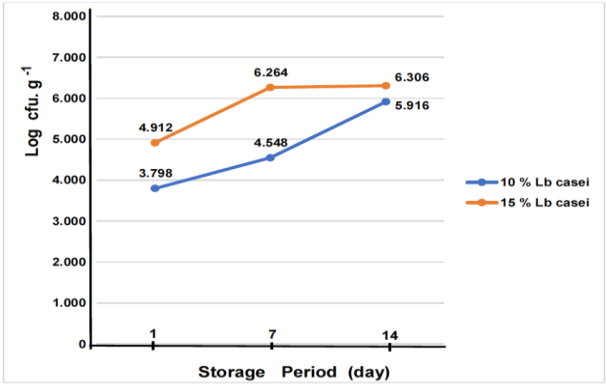

Figure 1. Total Probiotic Count of Semi-hard Cheese

In this study, the results obtained with the average viability probiotic bacteria in semi hard cheese at 10% and 15% level of (5.916 and 6.306) log cfu g^{-1} for 14th respectively. It was still below the recommended concentration standard, which is at least 6 log cfu g^{-1}, with an optimum daily dose of 8 to 9 log cfu g^{-1} (ml-1), and realized through the intake of 100 grams of probiotic products [12]. It can be seen that semi hard cheese can be used as a probiotic carrier food (functional food). However, one type of probiotic carrier food has been found in the context of food diversification from locally basic ingredients (buffalo milk from West Sumbawa Regency).

To be able to meet the minimum standard (6 log. cfu ml^{-1} or cfu g^{-1}) with a therapeutic effect, the intake of this probiotic semi hard cheese was at least 100 grams. The viability of the probiotic culture (*Lactobacillus casei*) was due to the food supply and the temperature in which the media was grown which was suitable and comfortable for these organisms. The food supply obtained from probiotic semi hard cheese has many nutrients and is indispensable for microbial growth. This can be seen in Table 1 and Figure 1 for the 7th day of observation. The concentration of probiotic bacteria and aging period have a significant increase on the total probiotic bacteria content of semi hard cheese (P<0.05). Moreover, that the aging period and the concentration level of *Lactobacillus casei* cultures were in line in terms of influencing the total *Lactobacillus casei* in the product (probiotic semi hard cheese).

3.2. Products recovery

Product recovery is the weight of the final product that can be obtained from a series of making a product. In the product (semi hard cheese), the final weight was obtained after curing for each treatment (Table 2).

Table 2. Product Recovery of Probiotic Cheese (%)

Number of Observations (N)	Concentrations of Probiotic Culture	
	10 %	15 %
1	31.4	34.0
2	30.8	33.8
3	30.7	34.4
4	29.8	34.6
5	30.6	34.5
Average	$(30.66 \pm 0.57)^{a}$	$(34.30 \pm 0.32)^{b}$
LSD	0.67	

Note: The same Superscripts within raw were significantly different (P<0.05)

The average product recovery of semi hard cheese given probiotic bacterial cultures at different concentrations were presented in Table 2. Based on the analysis of variance, the concentration of probiotic culture increased the product recovery significantly ($P < 0.05$). The high product recovery indicates that the buffalo milk from West Sumbawa has a high total solid content, especially the fat content. This indicates that buffalo cattle in Sumbawa, especially West Sumbawa Regency, are good enough to be developed as a center for buffalo. In addition, the salt concentration (3.5%) also affects the moisture content of the cheese so that it's also affects the level of product recovery. According to Ref. [13], the salt content in the curd at the time of pressing will be retained 6% -18% on the surface layer so that the surface layer becomes hard and cheese handling becomes easier.

3.3. Organoleptic value

Organoleptic quality is the power of consumer acceptance of food product/ foodstuff based on the level of preference and willingness [14]. In this study, the organoleptic qualities observed were the aroma, color and texture of semi hard cheese which were presented to 20 panelists who gave their assessments of all parameters.

The addition of probiotics had a very significant effect at the 5% level on the aroma of semi hard cheese containing (*Lactobacillus casei)* probiotic bacteria. The highest average generally was found in the treatment of 15% *Lactobacillus casei.* The higher concentration of probiotic culture given to each treatment caused the aroma of semi hard cheese tend to be disliked because probiotic bacteria including lactic acid bacteria (LAB) which produce lactic acids make the cheese aroma tend to be sour. The buffalo milk used in this study caused the cheese to have a distinctive aroma. A total of 20 untrained panelists preferred semi hard cheese with a concentration of 10% *Lactobacillus casei* because of its sour aroma which was not too strong.

Table 3. Organoleptic Value in Scoring Method (at 5 % significant level)

Parameter	Treatments					
	1st day		7th day		14th day	
	10%	15%	10%	15%	10%	15%
Aroma	2.26	2.64	2.32	2.83	2.48	2.92
Color	1.37	1.90	1.65	1.98	1.82	2.14
Texture	2.14	3.80	3.13	3.90	3.83	4.12

Aging period has a significant effect on the level of preference for the aroma, color and texture of semi hard cheese containing Lactobacillus casei. Table 3 shows that the value of the level of preference for the aroma, color and texture of the cheese tends to increase during ripening 1 day, 7 days and 14 days at different concentrations ranging from (2.26 – 2.92; 1.37 – 2.14; 2.14 – 4.14) respectively, for semi hard cheese added with Lactobacillus casei. The average indicates that the panelists liked the semi hard cheese added with Lactobacillus casei. The aroma can change because during ripening chemical reactions occur such as fat oxidation which can strengthen the rancid aroma of cheese and lactic acid bacteria which produce sour aroma. In addition, the addition of LAB can affect the pH value, taste and characteristics of cheese [15]. The results showed that the longer the aging time, the more favorable the aroma of semi hard cheese.

4. CONCLUSION

The semi hard cheese was manufactures using Lactobacillus casei culture and ripening time for 14 days. It was concluded that: Pure culture of probiotic (*Lactobacillus casei*) at level of 10% and 15% can survive and develop quite well in semi-hard cheese during aging, from 1 day, 7 days, and 14 days, respectively (3.79 – 5.92) and (4.91 – 6.31) log cfu g^{-1}. Aging time and culture concentration level were in line in influencing the total development of probiotic bacteria in the cheese. The pH value of cheese with the addition of probiotic bacteria was relatively constant during ripening with the highest average being 6.5 and the lowest being 6.4. Organoleptic quality which includes aroma, color and texture was getting better during aging, with the criteria of a semi hard aroma, yellowish white color and semi hard texture. The 0.025% rennet of the volume of milk from *Mucor miehei* gives a pretty good result, it can be seen from the product recovery which can reach (34.30 + 0.32) %. Semi hard-type cheese can be used as a functional food carrier for probiotics. To get a therapeutic effect, a minimum of 100 grams of probiotic semi hard cheese should be consumed per serving.

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