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Chemical and microbiological qualities of soft cheese supplemented with porang flour and Lactobacillus rhamnosus during cool storage

Kualitas kimia dan mikrobiologis keju lunak dengan penambahan tepung porang dan Lactobacillus rhamnosus selama penyimpanan dingin

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ABSTRACT

Cheese is one milk-based livestock product that has a chance to be a functional food by combining probiotics and prebiotics. The study aimed to develop synbiotics cheese from cow milk with the addition of porang flour as prebiotic and *Lactobacillus rhamnosus* as a probiotic that will give health benefits to the host. The experimental study used a Completely Randomized Design (CRD) factorial pattern 4x3 with 3 replications. The first factors consisted of P0 (soft cheese without porang flour and *Lacobacillus rhamnosus*), P1 (soft cheese with 0.03% porang flour), P2 (soft cheese with 5% *Lactobacillus rhamnosus*), and P3 (soft cheese with 0.03% porang flour + 5% *Lactobacillus rhamnosus*). The second factors were storage T0 (0 days), T1 (7 days), and T2 (14 days) at a temperature rate of 4-10°C. Variables measured were pH value, total titratable acidity (TTA), free fatty acid (FFA), and a total of Lactic Acid Bacteria (LAB). Data were analyzed using analysis of variance (ANOVA) with post hoc test Duncan's Multiple Range Test (DMRT). It resulted that the addition of porang flour and *Lactobacillus rhamnosus* significantly affected (P<0.05) pH, TTA, FFA, and a total of LAB, in line with storage that also significantly affected all parameters measured (P<0.05). Interaction between the addition of porang flour and *Lactobacillus rhamnosus* was significantly affected (P<0.05) to pH, TTA, LAB, and a total of LAB. In summary, the addition of porang flour and *Lactobacillus rhamnosus* produced soft cheese that had the potency to be a functional food.

ABSTRAK

Keju sebagai salah satu produk hasil ternak berbasis susu berpotensi sebagai pangan fungsional dengan mengkombinasikan probiotik dan prebiotik didalamnya. Tujuan penelitian adalah mengembangkan keju sinbiotik susu sapi dengan penambahan tepung porang sebagai prebiotik dan Lactobacillus rhamnosus sebagai probiotik yang berpotensi sebagai pangan fungsional yang bermanfaat bagi manusia. Penelitian eksperimental menggunakan Rancangan Acak Lengkap pola faktorial 4x3 dengan 3 kali ulangan. Faktor pertama terdiri atas: P0 (keju tanpa tepung porang dan Lactobacillus rhamnosus), P1 (keju dengan 0,03% tepung porang), P2 (keju dengan 5% Lactobacillus rhamnosus), dan P3 (keju dengan 0,03% tepung porang+5% Lactobacillus rhamnosus). Faktor kedua berupa penyimpanan T0 (0 hari), T1 (7 hari), dan T2 (14 hari) pada rentang suhu 4-10°C. Variabel yang diukur yaitu nilai pH, total asam tertitrasi (TAT), asam lemak bebas (ALB), dan total bakteri asam laktat (BAL). Data dianalisis menggunakan analysis of variance (Anova) dan uji lanjut Duncan's Multiple Range Test (DMRT). Hasil penelitian menunjukkan bahwa penambahan tepung porang dan Lactobacillus rhamnosus berpengaruh nyata (P<0,05) terhadap nilai pH, TAT, ALB, dan total BAL, dan penyimpanan memberikan pengaruh signifikan terhadap semua parameter yang diuji (P<0,05). Interaksi antara penambahan tepung porang dan penyimpanan memberikan pengaruh signifikan (P<0,05) terhadap nilai pH, TAT, ALB, dan total BAL. Kesimpulan dari penelitian ini adalah penambahan tepung porang dan Lactobacillus rhamnosus menghasilkan keju yang berpotensi sebagai pangan fungsional.

Kata Kunci:
Keju
Lactobacillus rhamnosus
Sinbiotik
Tepung porang

INTRODUCTION

Cheese is made from various types of milk, such as cow milk as the most well-known commodity in Indonesia. Cheese is grouped into hard cheese and soft cheese, and soft cheese contains water content of not more than 80% (Wulandari et al., 2021). Semarang district is the second highest producers of cow milk after Boyolali, with a 25,963 population of dairy cows that produce 28,047,872 l of milk per year (Aisyah, 2012). High milk production allows diversifying products to prolong the shelf-life and biological dan economical value of milk. The rapid development of biotechnology in the food sector is one of the reasons for the development of functional food products by involving good microbes that have beneficial effects to host.

Cheese as one of the livestock products will give functional value when combined with probiotics and prebiotics into a synbiotic product. Synbiotics are defined as a combination of probiotics and prebiotics, a substance that affects the health of the digestive tract. Requirements for probiotics in food to meet synbiotics are 105-107 CFU/g Lactic Acid Bacteria (LAB) so that it s expected to have a beneficial effect on the host (FAO & WHO, 2002; Rodrigues, Rocha-Santos, Gomes, Goodfellow, & Freitas, 2012). One of carrier of probiotics is livestock products because it contains excellent nutrition value that required to maintain microbial live. There are many kinds of milk-based products, such cheese, yoghurt, concentrated yoghurt, or kefir. Cheese is a good carrier of probiotics (Setyawardani, Sumarmono, & Widayaka, 2019) because of its higher pH and fat content also the consistency of cheese are solid so that probiotics are better protected.

In the production of ripened cheese, probiotics' roleise as a starter that will cover the cheese matrix during ripening (Setyawardani, Rahardjo, & Sulistyowati, 2017). Genus of Lactobacillus mostly have been applied to develomilk-baseded functional food, which species of *Lactobacillus rhamnosus* and *Lactobacillus plantarum* mostly applied in ripened cheese (Setyawardani et al., 2017; Setyawardani, Rahayu, & Palupi, 2016; Langa et al., 2019). Microbes reach optimal growth in a sufficient nutrition source, such dietary fiber named prebiotic. Prebiotic selectively could stimulate the growth of probiotics in colon (Setiarto, Jenie, Faridah, & Saskiawan, 2015). Homayouni, Alizadeh, Alikhah, & Zijah (2012) stated that commercial prebiotics met in market were oligosaccharides-group like FOS (fructo-oligosaccharide), GOS (galacto-oligosaccharide), rafinosa, and inulin studies about synbiotics cheese or explorations of porang flour had been done to evaluate properties of physical, chemical, microbiologies, or sensory and benefit of porang flour to functional foods (Langa et al., 2019; Harmayani, Aprilia, & Marsono, 2014; Aryanti, Kharis, & Abidin, 2015; Zhang et al., 2020; Kavas et al., 2021).

One of Indonesian local plants that contains high fiber is porang, a source of glucomannan. The glucomannan is composed a combination of polysaccharides from galactomannan, heteroglycan, glucan, arabinogalactan that could modulate imun (Zia et al., 2016). Combination of glucomannan and probiotics bacterial may produce synbiotic cheese to functional food that could increase quality and health benefits to host. The study aimed to develop synbiotics cheese from cow milk with the addition of porang flour a as prebiotic and *Lactobacillus rhamnosus* as probiotic that will give health benefits to host.

MATERIALS AND METHODS

Materials

Cow milk was purchased from smallholder dairy farm in Gedhanganak, Ungaran Timur, Semarang. Culture of *Lactobacillus rhamnosus* was provided from Center for Food and Nutrition Studies, Universitas Gadjah Mada, Yogyakarta. Porang flour obtained from CV Shitaki, Malang, Jawa Timur.

Methods

This experiment study was used Completelly Randomized Design (CRD) factorial pattern 4x3. The first factor was the use of porang flour and Lactobacillus rhamnosus, and the second factor was length of storage. Treatments were repeated 3 (three) times. Treatment consisted of: first factor, P0: soft cheese without porang flour and *Lactobacillus rhamnosus*, P1: soft cheese with 0.03% porang flour, P2: soft cheese with 5% *Lactobacillus rhamnosus*, P3: soft cheese with 0.03% porang flour + 5% *Lactobacillus rhamnosus*. Second factor are T1: soft cheese stored 0 day in temperature range 4-10°C, T2: soft cheese stored 7 days in the temperature range 4-10°C, and T3: soft cheese stored for 14 days in the temperature range 4-10°C

Starter culture preparation

The culture of *Lactobacillus rhamnosus* in broth media was pipetted 100 µl into 100 mL sterile skimmed milk and incubated 45°C for 18 hours (mother culture). Mother culture then inoculated again to sterile skimmed milk, incubated 45°C for 18 hours and ready to use as starter.

Soft Cheese making

Soft cheese making used the method following (Setyawardani et al., 2019) with modification on pasteurized milk. Milk was pasteurized at 72°C for 15 seconds (High Temperature Short Time) and cooled until 45°C. Pasteurized milk was then inoculated by *Lactobacillus rhamnosus* starter to acidify and incubated 45°C up to 2 hours until pH decreased to 6.1 (P2 and P3 treatments). Control treatment (P0) and P1 used acetic acid to direct acidification until pH decreased into 6.1. The next step was clumping used 0.06 ml animal rennet/ liter then rested for 2 (two) hours until gell forming. Gell was cut and rested 10-15 minutes to syneresis, then heated into 40°C for 30 minutes and filtered to separated curd and whey using cheese cloth. Further, the curd formed is called cheese. Porang flour (P2 and P3 treatments) was added after filtering process. Soft cheese was wrapped by aluminium foil and stored on refrigerator with temperature range 4-10°C for 0, 7, and 14 days.

Measurement of pH, Total Titritable Acidity (TTA), and Free Fatty Acid (FFA)

pH value was measured using digital pH meter (Hanna Instrument, USA), 5 g of samples were mashed and added by 10 mL of aquadest. Calibrated digital pH meter then dip into samples, and pH value was appeared. Total titratable acidity (TTA) followed Sumarmono, Setyawardani, & Santosa (2020). Soft cheese of 10 g were mashed and added 10 ml aquadest and 2 drops phenolpthalein 1%. Then, titrated using NaOH 0.1 N until solution changed into pink colour and rested until 30 seconds. Free fatty acid (FFA) measured following Sudarmadji, Haryono, & Suhardi (2007). Samples of 10 g mashed and put into erlenmeyer, then added 50 ml 96% heated ethanol and 2 ml phenolpthalein indicator 1%. Sample titrated using NaOH 0.1 N until solution changed into pink colour and rested until 30 seconds.

Lactic Acid Bacteria (LAB) Count

Lactic Acid Bacteria counted using total plate count following Nurliyani, Harmayani, & Sunarti (2017). A 25 g sample was mashed and diluted into 225 mL sterile physiological saline (0.85%) to make initial dilution (10-1). Sample of 1 mL was pipetted into 9 ml sterile physiological saline to make 10-2 dilution, then serial dilution were conducted to each sample up to 10-8. Appropriate dilution of 0.1 ml were pipetted into deMann Rogosa Sharpe Agar (Merck, Germany), then spreaded using sterile driglaski glass. Inoculation carried on aseptic condition and incubated with 38°C for 2x24 hours.

Statistic Analysis

Data was analyzed using analysis of variance (ANOVA) used Completelly Randomized Design (CRD) factorial pattern 4x3 and post with post-hoc test using Duncan’s Multiple Range Test (DMRT) (Riadi, 2014).

RESULTS AND DISCUSSIONS

pH Value and Total Titratable Acidity (TTA)

Value of pH and total titratable acidity (TTA) could be used to look probiotics activity during storage. Addition of porang flour and *Lactobacillus rhamnosus*, cold storage, and interaction between porang flour and *Lactobacillus rhamnosus* and cold storage were significantly affected ($P<0.05$) to pH and TTA of soft cheese. Data of pH value and TTA is showed in Tabel 1.

The highest average pH was 6.60 and the lowest was 5.30. The highest average of TTA was 0.19% and the lowest was 0.17%. The value of pH was higher than recent study conducted by Leeuwendaal et al. (2022) that cheddar cheese containing probiotics has pH 4.6-5.6, then Setyawardani et al. (2019) stated that soft goat cheese during storage has pH 4.34-5.29. The average pH was contrary with TTA, the lower pH, titrated acid was higher, so that soft cheese more acid. The highest pH and

Table 1. The average of pH, TAT, and FFA of soft cheese with addition of porang flour and *Lactobacillus rhamnosus* during storage

Treatment	Storage (days)	pH	TAT (%)	FFA (%)
P0 (0% porang flour + 0% L. rhamnosus)	T1 (0)	6.57±0.06^{h}	0.21±0.01^{g}	1.17±0.00^{g}
	T2 (7)	6.10±0.00^{f}	0.57±0.02^{f}	1.30±0.10^{fg}
	T3 (14)	6.37±0.06^{g}	0.62±0.01^{f}	1.58±0.08^{e}
P1 (0.03% porang flour)	T1 (0)	6.60±0.00^{h}	0.19±0.02^{g}	1.27±0.09^{fg}
	T2 (7)	5.90±0.00^{e}	0.54±0.00^{f}	1.28±0.03^{fg}
	T3 (14)	6.40±0.00^{g}	0.62±0.01^{f}	1.56±0.06^{e}
P2 (5% L. rhamnosus)	T1 (0)	5.80±0.00^{d}	0.62±0.03^{f}	3.60±0.11^{c}
	T2 (7)	5.57±0.06^{c}	1.09±0.02^{d}	2.81±0.45^{d}
	T3 (14)	5.40±0.00^{b}	1.42±0.04^{b}	4.12±0.14^{b}
P3 (0.03% porang flour + 5% L. rhamnosus)	T1 (0)	5.60±0.00^{c}	0.71±0.24^{e}	4.38±0.28^{b}
	T2 (7)	5.43±0.06^{b}	1.28±0.34^{c}	2.82±0.07^{d}
	T3 (14)	5.30±0.00^{a}	1.70±0.50^{a}	4.92±0.11^{a}
Addition		*	*	*
Storage		*	*	*
Addition×Storage		*	*	*

Note: means in the same column with different superscripts were significantly different (P<0.05); * = significant (P<0.05)

lowest TTA were soft cheese without porang flour and *Lactobacillus rhamnosus* (P0) and soft cheese with 0.03% porang flour (P1). The lowest pH and highest TTA soft cheese with 0.03% porang flour and 5% *Lactobacillus rhamnosus* (P3). It is because P0 and P1 were not added by *Lactobacillus rhamnosus* as acidifier but using acetic acid to decrease pH to 6.1. Adding acidifier on soft cheese making aimed to decrease the pH of milk, then the milk was easy to clump, and soft cheese was formed. The presence of *Lactobacillus rhamnosus* and its activity on soft cheese could be observed by looking the change in pH value (Triana Setyawardani et al., 2019). Length time of storage affected to decrease pH but increased TTA. Decreasing of pH also occured when added Lactobacillus rhamnosus (P2) and combination of porang flour and *Lactobacillus rhamnosus* (P3) during storage. The low pH of soft cheese during storage could help to against developing pathogenic and spoilage bacteria because inappropriate environment (Triana Setyawardani et al., 2019). It also indicated that *Lactobacillus rhamnosus* still conducting metabolisms, then producing lactic acid that resulted decreasing of pH during storage. Setyawardani et al. (2016); Prastujati, Hilmi, & Khirzin, (2018) stated that addition of lactic acid bacteria (LAB) culture could decrease pH as a mechanism of degradating lactose into lactic acid, the higher lactose content, pH would be lower. Value of pH on soft cheese without *Lactobacillus rhamnosus* was higher than soft cheese with addition of that culture, but it decreased on 7 days and increased on 14 days storage. It was because metabolic activity of yeast and mold that carried acid as carbon source and proteolysis by releasing alkaline component in a large amount (Setyawardani et al., 2016). Value of pH without *Lactobacillus rhamnosus* (P0 and P1) were still normal based on Musra, Yasni, & Syamsir (2021) that average of pH was 6.19-7.24.

Treatment P0 and P1 had the same TTA (P>0.05) on 0 day storage, and increased during storage. It related with Setyawardani et al. (2017), during storage, soft cheese made with heated milk was increase in TTA and changing pH value. TTA on P2 and P3 were higher because activity of *Lactobacillus rhamnosus* culture that able to produce acid during fermentation. Setyawardani et al. (2016)

mentioned that microbes had to adapted in stomatch environment and intestine so it could give balancing of intestinal microbes population.

Free Fatty Acid (FFA)

Addition of porang flour and *Lactobacillus rhamnosus*, cold storage, and interaction between porang flour and *Lactobacillus rhamnosus* and cold storage were significantly affected (P<0.05) to FFA. The highest FFA was 4.92% and the lowest was 1,17%. FFA in this study was lower than Setyawardani et al. (2019) that cheese with probiotics (*Lactobacillus plantarum* TW14 and *Lactobacillus rhamnosus* TW2) in 15 days storage has FFA 10.28%. The difference may be due to the use of mixed culture. TW 1 Treatment P0 and P1 had same FFA, but it increased during storage. Data of FFA was showed in Table 1. addition of *Lactobacillus rhamnosus* (P2) and combination (P3) had different FFA. Amar, Marwati, & Damang (2017) stated that lipase enzyme on LAB could react on mono and digliseride to release free fatty acid. Free fatty acid held an important properties of soft cheese flavour because mono and digliseride could be hydrolize by carboxy esterase or mono and digliseride lipase. Free fatty acid on treatments P2 and P3 on 7 days storage decreased and increased again in the end of storage. Dopieralska et al. (2020) stated that during storage, fatty acid composition changed into certain variety because of lipid oxydation, especially unsaturated fatty acid that very susceptible. It affected on changing portion of each fatty acid. The highest FFA was P3 on 14 days storage. Interaction between porang flour and Lactobacillus rhamnosus were significantly different (P<0.05). This study agreed with Amar et al. (2017), the longer storage, FFA of soft cheese would increase. Enhancement of FFA during storage indicated that lipolysis as mechanism of lipase enzyme from rennet, milk, and/ or starter culture (Setyawardani et al., 2017). The highest FFA was on P3, it related to studied concted by Rodrigues et al (2012) that FFA of cheese that ripened 60 days was significantly higher in synbiotcs than in probiotics cheese.

Total of Lactic Acid Bacteria (LAB)

Addition of porang flour and Lactobacillus rhamnosus, cold storage, and interaction between porang flour and Lactobacillus rhamnosus and cold storage were significantly affected (P<0.05) to total of LAB. The highest total of LAB was 7.17 log CFU/g and the lowest was 3.91 log CFU/g. Total of LAB on P0 increased during storage, whereas P1 decreased. Addition of porang flour was not effective on soft cheese without starter culture. Addition of Lactobacillus rhamnosus (P2 and P3) had same total of LAB, but on 7 days storage, total of LAB increased, then decreased on 14 days storage. Data of LAB with addition of porang flour and Lactobacillus rhamnosus during storage is showed in Figure 1.

Enhancement of total LAB indicated activity of LAB that fermented lactose of milk. During cold storage, LAB kept growing and

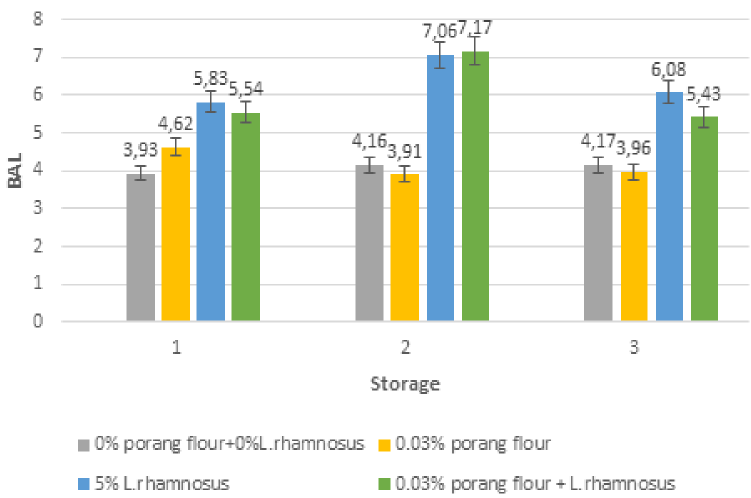

Figure 1. The average of total of LAB with addition of porang flour and *Lactobacillus rhamnosus* during storage

produced soft cheese substrates and metabolites as lactic acid. It contributed to decrease pH (Triana Setyawardani et al., 2019). Degradation of total LAB showed that inability of LAB to survive in matrix cheese during storage. It was in contrast with study conducted by (Setyawardani et al., 2016) that during storage for four weeks, total of LAB probiotic cheese decreased because of lactose reduction during separating whey, so LAB could survive on cheese during ripening.

The highest total of LAB were P3 and P2 on 7 days storage. Harmayani et al (2014) stated that porang glucomannan has ability to increase fermentation capacity by produce higher acetic acid than propionic and butiric acid. Porang flour was belong to prebiotics that selectively could increase probiotics growth (Langa et al., 2019), so that the addition of porang flour could increase the growth of *Lactobacillus rhamnosus* in cheese. Cárdenas et al. (2014) declared that addition of probiotics on cheese manufacture could improve flavour properties, quality, and promote health. Microbes on cheese must be able to survive in stomach condition and digestive tract to make sure that it is useful to maintain the balance population of intestinal microbes (T. Setyawardani et al., 2017).

CONCLUSION

In conclusion, addition 0.03% porang flour and 5% Lactobacillus rhamnosus could produce a good soft cheese. Storage also contributing factor affected to pH, TTA, FFA, and total of LAB. Soft cheese during 14 days storage still have good quality and had a potency to be a functional food.

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