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Research Article
Characteristics of Goat Milk Cheese Added with Liquid Smoke and Porang Glucomannan Ripened with *Lactobacillus rhamnosus*

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Abstract

Background and Objective: Spoilage by microorganism contamination often occurs during cheese processing and ripening, while syneresis often occurs during curd formation. Liquid smoke is often known as an antimicrobial, whereas, glucomannan has water binding capacity. The purpose of this study was to determine the effect of liquid smoke and porang (*Amorphophallus oncophyllus*) glucomannan addition in cheese processing on viability of lactic acid bacteria, physico-chemical characteristics and antioxidant activity of goat milk cheese ripened with *Lactobacillus rhamnosus*. **Materials and Methods:** Goat milk for cheese making was divided into 4 groups: Control (without liquid smoke and glucomannan), milk added with liquid smoke, milk added with glucomannan and milk added with a combination of liquid smoke and glucomannan. These cheese were ripened for 30 days in a refrigerator. **Results:** Liquid smoke and glucomannan had no effect on total lactic acid bacteria (10^8-10^9 CFU g^{-1}), acidity, pH, moisture, soluble protein, free fatty acid, total phenolic content and antioxidant activity of goat milk cheese. However, the texture of cheese treated with liquid smoke and/or glucomannan was softer ($p<0.05$) than the control cheese. After ripening, the acidity, moisture, soluble protein, free fatty acid (FFA), total phenolic and texture of cheese were increased significantly ($p<0.05$), whereas, the pH, moisture content and antioxidant activity were decreased significantly ($p<0.5$). **Conclusion:** Liquid smoke and glucomannan addition had no negative effect on viability of lactic acid bacteria, chemical characteristics and antioxidant activity, but may soften the texture of ripened goat milk cheese. After ripening, the phenolic content increased, but the antioxidant activity decreased. The ripened goat milk cheese with *L. rhamnosus* added with porang glucomannan has potential as a synbiotic cheese.

Key words: Goat milk cheese characteristics, liquid smoke, porang glucomannan, *Lactobacillus rhamnosus*, antioxidant activity

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The process of cheese making is often subjected to contamination and spoilage by other microorganisms during ripening. Liquid smoke is a substance known to give a typical flavor and able to maintain the quality and ensure food safety. The phenol component in liquid smoke contributes to the smoke flavor and liquid smoke color and also has antioxidant and antibacterial properties[1]. Through the distillation process, polycyclic aromatic hydrocarbons (PHA) known to be carcinogenic will be eliminated and the color of liquid smoke can be adjusted[1,2].

In a previous study, it has been shown that during cheese making by adding 0.015% carrageenan followed by heat treatment at 90°C for 5 min can increase yield by 13.6% due to increased recovery of whey protein and water retention in cheese[3]. In addition to carrageenan, glucomannan also has water retention properties. Similar to konjac glucomannan, locally extracted glucomannan from porang (*Amorphophallus oncophyllus*) tuber in Indonesia also has a high viscosity. Porang glucomannan has 86.43% solubility, 34.50% water holding capacity (WHC), viscosity 5400 cP, degree of polymerization 9.4, degree of acetylation 13.7% and purity 92.69% and has a prebiotic properties *in vivo*[4]. The soluble protein, free fatty acid, antioxidant activity and texture of ripened goat milk cheese added with porang glucomannan has not been studied.

The pickling of unripened Domiatti cheese in smoked whey decreased the total viable bacterial counts and the viable counts of proteolytic, lipolytic bacteria, molds and yeasts[5]. However, the effect of porang glucomannan or combination of liquid smoke and porang glucomannan addition in fresh goat milk as raw material of cheese has so far not been reported on viability of lactic acid bacteria in cheese ripened with *L. rhamnosus*. The purpose of this study was to determine the effect of the addition of liquid smoke and porang glucomannan in cheese processing on total lactic acid bacteria, chemical and physical quality and also antioxidant activity of cheese ripened with *Lactobacillus rhamnosus*. It was expected that liquid smoke and porang glucomannan did not decrease the growth of lactic acid bacteria and could improve the physicochemical properties of cheese.

MATERIALS AND METHODS

This study was performed at Department of Animal Product Technology and The Integrated Research and Testing Laboratory-Universitas Gadjah Mada, Yogyakarta, Indonesia in the period of April-September, 2017.

Glucomannan extraction: Extraction of glucomannan was carried out according to Amanah[6] with modification. Briefly, porang flour (10 g) was mixed with 500 mL aquadest for 1.5 h at 55°C, while stirred periodically. Then, the mixture was filtered using a thin cloth to separate the sediment. The supernatant was mixed with 96% ethanol (supernatant:ethanol = 2:1) to extract glucomannan. The formed glucomannan as clouds was filtered and dried in a cabinet dryer at a temperature of 48°C for 8 h. Then, the dried glucomannan was blended and sieved 80 mesh.

Cheese preparation: Goat milk cheese was prepared from goat milk obtained from Ettawah Crossbred goat at Sleman, Yogyakarta, Indonesia. Liquid smoke of coconut shell commercially was obtained from Faculty of Agricultural Technology, Universitas Gadjah Mada Yogyakarta. Cheese in this study were divided into four groups of treatments: (1) Control cheese (goat milk without liquid smoke and porang glucomannan, (2) Goat milk+liquid smoke, (3) Goat milk+glucomannan and (4) Goat milk+liquid smoke+glucomannan. Liquid smoke and glucomannan were added 0.3 mL/100 mL milk (0.3% v/v) and 0.2 g/100 g milk (0.2% w/v), respectively. The four groups above were pasteurized at 72°C for 15 min and cooled to 40°C. Starter of lactic acid bacteria (*Lactobacillus rhamnosus* FNCC 0052, 8.0 log CFU g^{-1}) was inoculated into the pasteurized milk as much as 3% (v/v). The inoculated milk were incubated at 40°C for 60 min and then added with vegetable rennet (+QSO, Guatemala) as much as 3% (v/v) and incubated at 40°C for 90 min until the curd formed. Then, the curd was cut into pieces for drain whey and filtered to separate casein from whey using cheese cloth. For complete separation of curd and whey, the filtering process was left overnight in a refrigerator. Sodium chloride solution (10% w/v) was prepared and the curd was soaked for 60 min and then pressed under a weight of 1:1 (curd:weight) for 60 min and then weighed. Curd was wrapped in a sterile cloth and put in a ventilated container and then ripened in a refrigerator (4°C) for 30 day. The cheese quality was determined before and after ripening. In this study using 3 replicates for each treatment. In a previous study, liquid smoke were added 0.3, 0.4 and 0.5%, while porang glucomannan were added 0.1, 0.2 and 0.3%. The best curd (firm curd) was obtained from addition of liquid smoke 0.3% and glucomannan 0.2% (unpublished data).

Microbiological analysis: Samples of cheese (1.0 g) were diluted in 9.0 mL physiological NaCl and the procedure were continued to obtain a final dilution of 10^{-6}. About 0.1 mL of 10^{-5} and 10^{-6} dilutions (appropriate decimal dilutions) were

spread into each a sterile Petri dish on each medium. This was subjected to incubation at 37°C for 48 h. To determine LAB populations, the colonies formed were counted and expressed in log CFU g^{-1} [7]. Lactic acid bacteria were determined on modified deMan, Rogosa and Sharpe (MRS) agar (Merck) containing 100 ppm NaN_3 [8] and 100 ppm $CaCO_3$ [9].

Titratable acidity analysis and measuring of pH: The pH of cheese was measured using a pH-meter (HANNA-HI 98103) and acidity was analyzed by titratable acidity according to Hashim *et al.*[10] with slight modification. Titratable acidity was expressed as percentage of lactic acid and determined by titrating 9 g of cheese with 0.1 N NaOH using phenolphthalein as an indicator to an end-point of faint pink color.

Moisture and soluble protein analysis: For moisture analysis, cheese samples were dried at 105°C for 24 h and the water content of the samples were gravimetrically determined[11].

Soluble protein was analyzed by Lowry method[12]. Lowry A solution was prepared with phosphotungstate and phosphomolybdate (1:1). Lowry B solution was prepared by mixing of 2% Na_2CO_3 in 100 mL NaOH 0.1N and then added with 1.0 mL cupric sulfate 1% and 1.0 mL potassium tartrate 2%. Cheese samples (1.0 g) were added with 5.0 mL Lowry B, vortexed and incubated for 30 min. Furthermore, absorbance of sample was read on Spectrophotometer at 750 nm (Spectronic 21D). Soluble protein was calculated based on the standard curve of bovine serum albumin.

Free fatty acid (FFA) analysis: Free fatty acid of cheese was analyzed by adding of heated ethanol, titration with natrium hydroxide and expressed as oleic acid percentage according to FSSAI[13]. The FFA was calculated by formula as follow:

$$\text{FFA (\%)} = \left[\frac{(\text{mL NaOH} \times \text{N} \times \text{MW of oleic acid})}{\text{Sample weight} \times 1000}\right] \times 100$$

Where:

N = Normality of NaOH

MW = Molecular weight

Texture analysis: The sample of cheese was cut into a cube and then the texture were analyzed by using Texture Analyzer (No. M08-372-E0315) at temperature of 27°C.

Determination of total phenolic content and antioxidant activity: The content of total phenolics was analyzed spectrophotometrically (Spectronic 200) using the Folin Ciocalteu colorimetric method with standard curve of gallic acid[14].

The antioxidant activity of cheese upon 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) radicals was estimated according to Liu *et al.*[15], by dilution sample with methanol and addition of DPPH. The capability of the test material to scavenge DPPH radicals was calculated as:

$$\text{DPPH (\%)} = 1 - \left[\frac{\text{Absorbance of the sample at 517 nm}}{\text{Absorbance of the control at 517 nm}}\right] \times 100$$

Statistical analysis: The data of microbiological, chemical, physical and antioxidant activity of cheese were expressed as Mean±standard deviation and comparison between treatment were analyzed by two-way ANOVA (cheese group vs ripening time), followed by Duncan's Multiple Range Test (DMRT). Statistical analysis were performed using the program Statistical Package For Social Sciences (SPSS), version 17.

RESULTS

The Table 1 showed the average curd yield (%) of cheese made with different treatment preparation. Cheese prepared with addition of 0.3% liquid smoke or 0.2% glucomanan and also combination of liquid smoke and glucomannan showed no significantly differences in wet curd yield or in dry curd yield.

Total lactic acid bacteria: Addition of liquid smoke and porang glucomannan in cheese processing had no effect on total amount of lactic acid bacteria (LAB) in ripened cheese by *L. rhamnosus* (Table 2).

Table 1: Average of curd yield (%) of goat milk cheese treated with liquid smoke and porang glucomannan

	Cheese treatment				
Curd yield	Control cheese	Goat milk+liquid smoke	Goat milk+glucomannan	Goat milk+liquid smoke+glucomannan	Average
Wet (%)[ns]	25.57±1.04	21.89±1.10	24.63±3.94	24.85±3.62	24.23±2.78
Dry (%)[ns]	63.65±2.54	50.92±2.61	63.60±8.78	66.73±10.79	61.22±8.83

ns: Not significant

Table 2: Average of LAB (Log CFU g^{-1}), acidity (%) and pH of goat milk cheese treated with liquid smoke and porang glucomannan on 0 and 30 days of ripening

	Cheese treatment				
Ripening (day)	Control cheese	Goat milk+liquid smoke	Goat milk+glucomannan	Goat milk+liquid smoke+glucomannan	Average[ns]
LAB					
0	8.25±0.35	8.60±0.54	9.04±1.17	8.68±0.44	8.64±0.67
30	8.41±0.93	8.10±0.43	9.13±0.25	8.30±0.05	8.49±0.60
Average[ns]	8.32±0.64	8.37±0.50	9.09±0.76	8.49±0.34	
Acidity					
0	0.47±0.17	0.57±0.29	0.25±0.08	0.31±0.08	0.40±0.20[a]
30	0.58±0.02	0.49±0.07	0.57±0.18	0.67±0.26	0.58±0.15[b]
Average[ns]	0.53±0.12	0.53±0.19	0.41±0.21	0.49±0.26	
pH					
0	6.52±0.04	6.26±0.19	6.30±0.11	6.21±0.07	6.31±0.16[a]
30	5.90±0.06	5.81±0.24	5.76±0.06	5.89±0.12	5.84±0.13[b]
Average[ns]	6.20±0.34	6.03±0.31	6.02±0.30	6.04±0.19	

ns: Not significant, [a,b]Means with different letters within the same column indicate significantly different ($p<0.05$)

Table 3: Average of soluble protein content (%) and FFA (%) of goat milk cheese treated with liquid smoke and porang glucomannan on 0 and 30 days of ripening

	Cheese treatment				
Ripening (day)	Control cheese	Goat milk+liquid smoke	Goat milk+glucomannan	Goat milk+liquid smoke+glucomannan	Average
Soluble protein					
0	24.26±0.88	24.91±5.14	30.93±2.60	24.10±4.16	26.05±4.25[a]
30	42.39±5.13	34.93±7.43	39.64±3.55	35.90±4.09	37.83±5.23[b]
Average[ns]	31.51±10.27	29.92±7.92	35.28±5.52	30.00±7.44	
FFA					
0	24.26±0.88	24.91±5.14	30.93±2.60	24.10±4.16	26.05±4.25[a]
30	42.39±5.13	34.93±7.43	39.64±3.55	35.90±4.09	37.83±5.23[b]
Average[ns]	31.51±10.27	29.92±7.92	35.28±5.52	30.00±7.44	

ns: Non significant, [a,b]Means with different letters within the same column indicate significantly different ($p<0.05$)

Acidity (% lactic acid) and pH: There was no statistically differences in the acidity or pH of goat milk cheese in all treatments. Thus, the cheese acidity and pH not affected by liquid smoke, glucomannan, or combination of liquid smoke and glucomannan addition. However, after 30 days of ripening the acidity increased significantly ($p<0.05$) (Table 2). There was no statistically differences in the interaction between the cheese treatment factor and ripening time factor on cheese acidity or pH.

Soluble protein: The addition of liquid smoke and porang glucomannan in cheese preparation had no effect on their soluble protein (Table 3). However, soluble protein in cheese increased significantly ($p<0.05$) after 30 days of ripening at 4°C. There was no statistically differences in the interaction between the factor of cheese treatment and ripening time factor on soluble protein in the cheese.

Free fatty acid (FFA): Addition of liquid smoke or porang glucomannanin cheese processing had no effect on FFA (Table 3). After 30 days of ripening the FFA in cheese increased significantly ($p<0.05$). There was no statistically differences in the interaction between the factor of cheese treatment and ripening time factor on FFA in the cheese.

Moisture content: There was no significant difference among cheese treatments, but the ripening time had an effect on moisture content in the cheese. There was no interaction between cheese treatment and ripening time on the cheese moisture content (Table 4).

Total phenolic: During the 30 days of ripening, there was a significant increase in phenol content ($p<0.05$). However, the antioxidant activity decreased significantly ($p<0.05$) after 30 days of ripening (Table 4). The addition of liquid smoke and porang glucomannan did not significantly affect on the content of cheese phenolic (Table 4).

Cheese texture: The texture of cheese without the addition of liquid smoke and glucomannan (control cheese) had a greater value than the texture value of cheese with the addition of liquid smoke and glucomannan (Table 4). After 30 days of ripening, the cheese texture become harder ($p<0.05$) than before ripening (0 day).

Table 4: Average of moisture content (%), total phenolic (%), texture (N) and antioxidant activity of goat milk cheese treated with liquid smoke and porang glucomannan on 0 and 30 days of ripening

	Cheese treatment				
Ripening (day)	Control cheese	Goat milk+liquid smoke	Goat milk+glucomannan	Goat milk+liquid smoke+glucomannan	Average
Moisture					
0	59.81±0.41	57.00±1.18	61.28±2.53	62.64±2.74	60.18±2.75[a]
30	45.07±2.35	46.78±6.90	40.89±8.96	43.12±9.17	43.97±6.69[b]
Average[ns]	52.44±8.21	51.89±7.13	51.08±12.62	52.88±12.28	
Phenolic					
0	0.02±0.00	0.02±0.00	0.02±0.00	0.02±0.00	0.02±0.00[a]
30	0.44±0.15	0.44±0.02	0.44±0.15	0.56±0.19	0.50±0.17[b]
Average[ns]	0.23±0.25	0.23±0.23	0.23±0.25	0.29±0.32	
Texture					
0	36.00±4.92	66.66±8.02	28.83±14.57	23.66±13.25	38.79±19.75[a]
30	761.16±173.2	38.25±8.13	538.75±415.42	341.50±79.90	457.83±336.83[b]
Average	398.58±412.11[p]	55.30±17.05[q]	232.80±348.21[q]	150.80±178.85[q]	
Antioxidant activity					
0	20.21±3.63	21.18±1.35	23.55±1.81	21.90±1.38	21.71±2.30[a]
30	3.09±0.00	4.46±0.59	4.21±0.15	5.97±0.74	4.43±1.15[b]
Average[ns]	11.65±9.65	12.82±9.20	13.93±10.65	13.93±8.77	

ns: Non significant, Different letters within the same column (a, b) or row (p, q) indicate significant difference ($p<0.05$)

Antioxidant activity: The ripening of cheese for 30 days at 4°C significantly reduced antioxidant activity of all cheese treatment groups ($p<0.05$) (Table 4). The increasing in phenol content followed by a decreasing in antioxidant activity after ripening (Table 4).

DISCUSSION

The wet or dry curd in all cheese treatments (liquid smoke, glucomannan, liquid smoke+glucomannan) was not significantly different. Thus, the treatment of liquid smoke had no effect on the curd yield that the same as other treatments. The smaller yield of curd in liquid smoke treatment (although not significant) due to no water binding component (i.e., glucomannan).

The curd yield in the present study (Table 1) different from the Domiati cheese that enhanced as the carboxymethylcellulose (as a hydrocolloid) addition increased[16]. However, the effect of combination of liquid smoke and glucomannan on curd yield was in accordance with the study by Santos *et al.*[17], that the wet or dry yield of curd prepared by using different calcium addition showed no differences in Minas cured cheese.

The high viability of this bacteria in the present study (Table 2) was not affected by liquid smoke and porang glucomannan. This result was in accordance with a previous study by Cichosz *et al.*[18], that the *L. rhamnosus* HN001 was still viable after 6-10 weeks of ripening in Dutch-type and Swiss-type cheeses which amount were 8 log CFU g^{-1}. On Table 2, the total lactic acid bacteria of cheese in around of 8.32-9.09 log CFU g^{-1} or 2.09×10^{8}-1.23×10^{9} CFU g^{-1}. Different from a previous study, lactobacilli increased during the 90 days of ripening in Tulum cheese, whereas, the others (enterococci, lactococci, leuconostocs and pediococci) did not change a significant amount. This profile seems to be common in most cheese varieties, due to lactobacilli was usually found in cheeses with longterm ripening[19].

During ripening, lactic acid bacteria was able to degrade lactose into lactic acid, then the pH of cheese decreased (Table 2). According to Ong and Shah[20], the degree of changes in cheese acidity during ripening and storage was feature of secondary fermentation.

Degradation of cheese protein by *Lactobacillus rhamnosus* during ripening could increase the soluble protein (Table 3), due to the bacteria had proteolytic activity[21]. However, it was contrary to the finding of Smiljanić *et al.*,[22] who showed that there was decreasing soluble protein in cheese ripened in salt solution due to proteolysis and diffusion of protein into salt solution.

The role of lactic acid bacteria in dairy products contribute to free fatty acids (FFAs) production, such as butyric acid and linoleic acid, by lipolysis of milk fats[23]. However, the presence of liquid smoke had no effect on the growth of lactic acid bacteria, whereas stabilizers (such as tragacanth, carboxymethyl cellulose, locust bean, propylene glycol alginate, xanthan, microcrystalline cellulose, guar and arabic) had no effect on milk lipase[24-26]. The increasing FFA after 30 days ripening (Table 3), was probably due to the occurence of lipolysis by *L. rhamnosus* during ripening. This was explained by a previous study that *L. rhamnosus* isolated from ovine milk cheese had lipolytic activity[27].

Cheese ripening in this study, could reduce the moisture content (Table 4). Decrease in moisture content during ripening improved the texture of the cheese (the texture becomes rather hard) (Table 4). These cheeses were wrapped without vacuum packaging to allow for moisture evaporation from cheese structures through a cheese wrap that allows ventilation. The decrease in moisture content during ripening in this study was consistent with a previous studies on cheese produced from pasteurized milk that decreased in moisture content. The decreased in moisture content in pasteurized milk cheese was higher than the cheese produced from raw milk or treated pressure. Differences in water binding may indicate a change in the cheese-matrix structure due to the technological treatment applied to milk and/or physicochemical or biochemical differences (NaCl, proteolysis, lipolysis, etc.)[28].

The increase in phenol followed decrease in antioxidant activity after ripening (Table 4). The presence of phenolic compounds in the milk and cheese is a result of their transfer from plant to milk. According to Hilario *et al.*[29], pasture plants are rich and significant source of bioactive components and they can be transferred into the milk and cheese. In addition, the increased in phenolic compound (Table 4) during cheese ripening in the present study may be due to aromatic amino acid metabolism by *L. rhamnosus* resulting in phenol. According to Dunn and Lindsay[30], some lactobacilli can catabolism aromatic amino acids to produce Strecker-type compounds (phenylethanol, phenylacetaldehyde, p-cresol and phenol), causing unclean-type flavour in Cheddar cheese.

The decreased in texture of ripened cheese treated with liquid smoke and glucomannan in the present study compared to the control cheese (Table 4), due to dilution by the liquid smoke and the water binding by glucomannan. Glucomannan is water-soluble fiber and viscous with high water binding capacity (WBC) and swelling capacity (SC)[31]. Similar to a previous study that rheological properties were lower in cheeses prepared with carboxymethylcellulose as hydrocolloid[16]. The harder texture after 30 days of ripening compared to before ripening (0 day), due to decrease in moisture contents from cheese structure (Table 4). Texture changes during ripening due to solubilization of calcium phosphate, hydrolysis of the casein matrix, changes to water binding within the curd and loss of moisture caused by evaporation from the cheese surface[32].

After 30 days of ripening, the antioxidant activity of ripened cheese decreased (Table 4). This result was in accordance with a previous study, that cheese ripening for 90 days decreased antioxidant activity compared to controls measured by DPPH scavenging method[28]. The decline of antioxidants in this study was contrast to increased phenol during ripening. Therefore, antioxidant activity was possible not to be influenced by the presence of relatively small of phenols. The possibility of antioxidant activity was derived from peptides in the milk formed during ripening. The decreased antioxidant activity during ripening due to proteolytic activity resulting peptides that had lower scavenging activity. According to Namdari and Nejati[33], antioxidant activity of peptides may be higher or lower after proteolytic degradation. In a previous study, the antioxidant activity (by DPPH assay) of raw, pasteurized, boiled cow and buffalo milk showed decreased after 6 days of storage periode in refrigerator[34]. Different from the present study, Mexican Cotija cheese produced peptides with high antioxidant activity which enhanced during ripening time[35]. The level of their antioxidant formation depends on ripening conditions and ripening stage[36].

CONCLUSION

Liquid smoke and porang glucomannan had no adverse effect on the growth of lactic acid bacteria, chemical quality and antioxidant activity, but could soften the texture of goat milk cheese ripened with *Lactobacillus rhamnosus*. Combination of *Lactobacillus rhamnosus* and porang glucomannan has a potential to produce synbiotic cheese. Ripening process during 30 days could increase the phenolic content but decrease the antioxidant activity of the cheese.

SIGNIFICANCE STATEMENT

Ripened cheese with soft texture can be produced by addition of 0.2% porang glucomannan which is a local Indonesian prebiotic. Goat milk synbiotic cheese can be prepared by adding porang glucomannan and *L. rhamnosus* in the cheese processing. However, to produce synbiotic ripened cheese which has a high antioxidant activity, it is better to use selected probiotics that can produce bioactive peptides with high antioxidant activity during cheese ripening, or enriched with antioxidants from plant sources. This study will help the researcher to produce synbiotic ripened cheese which has a soft texture.

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