Properties of Goat Milk Kefir Supplemented with Glucomannan from Porang (*Amorphophallus oncophyllus*) Tuber

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ABSTRACT

Properties of kefir depends on the kinds of milk as raw material, grain sources and other components which to be added within the fermentation process. Glucomannan is known as stabilizer and thickener that to be added in food processing. The objectives of this study were to evaluated the effect of glucomannan supplementation in fermentation process on microbiological, chemical and physical characteristics of goat milk kefir. Goat milk kefir were prepared by goat milk; goat milk+whey protein concentrate (WPC); goat milk+ glucomannan; and goat milk+WPC+glucomannan. The results showed that there were no effect of WPC, glucomannan or combination of WPC and glucomannan supplementation on kefir characteristics including the total lactic acid bacteria, yeast and total count, moisture and alcohol contents, acidity, pH and viscosity. The viscosity of kefir supplemented with combination of WPC and glucomannan tend to have a lower viscosity compared to kefir supplemented with WPC or glucomannan individually, although it was not significantly different. In conclusion, supplementation of WPC and glucomannan from porang tuber in fermentation process had no adverse effect on microbiological, chemical and physical characteristics of goat milk kefir.

Keywords: Goat milk kefir, Microbiological quality, Chemical quality, Physical quality, Porang glucomannan.

INTRODUCTION

Food quality can be determined by microbial, chemical, physical and sensory quality, while the end product characteristics of fermented milk such as kefir may also be influenced by the kind of milk, the origin of kefir grain and other components are added. During kefir fermentation, the lactose will be metabolize to organic acids, alcohol and carbon dioxide, so that kefir aroma will be fresh, sour and slightly alcoholic (Adriana and Socaciu, 2008). Since kefir containing microorganisms which have proteolityc activity, often leads to reduced curd and change their viscosity. Therefore, for stability of kefir product was required a stabilizer that able to prevent syneresis.

Similar to konjac glucomannan, locally porang glucomannan (*Amorphophallus oncophyllus*) from porang tuber in Indonesia is also rich in glucomannan and high viscosity. According to Harmayani *et al*. (2014), porang glucomannan has 86.43% solubility, 34.50% water holding capacity (WHC), viscosity 5,400 cP, degree of polymerization 9.4, degree of acetylation 13.7% and purity 92.69%.

Whey protein and polysaccharides combination are used to form mixed gels in food industry to attain either a desirable texture or to perform polysaccharide-protein (meat/dairy product) interaction (Banerjee and Bhattacharya, 2012). Supplementation of whey protein concentrate (WPC) in goat milk for fermentation can increase the sensory and nutritive quality of products and it stimulates the growth of various strains of lactic acid bacteria (Tratnik *et al*., 2006). The effect of supplementation of porang glucomannan or combination of glucomannan and WPC in kefir fermentation so far has not been studied by a previous study.

The purpose of this study was to evaluate the supplementation of glucomannan from porang tuber (*Amorphophalus oncophyllus*) on the microbiological, chemical and physical quality of goat milk kefir.

MATERIALS AND METHODS

Porang tuber was obtained from the low light intensity of forest in Nglanggeran, Yogyakarta, Indonesia. Extraction of glucomannan was according to (Amanah, 1992) with modification.

Goat milk was obtained from Ettawah Crossbred goat in Yogyakarta Indonesia. Whey protein concentrate was given by Sari Husada Milk industry in Yogyakarta-Indonesia, whereas kefir grain was obtained from local supplier in Yogyakarta.

Kefir was divided into 4 groups base on raw material of kefir: 1) goat milk, 2) goat milk + 1.0% WPC, 3) goat milk+ 0.3% porang glucomannan, and 4) goat milk + 1.0 % WPC+ 0.3% porang glucomannan. Goat milk kefir was prepared traditionally according to Otles and Cagindi (2003) with slight modification. The raw materials of kefir were mixed, pasteurized at 75ºC for 15 min, and cooled at room temperature, respectively. Kefir grains (2%) were inoculated into pasteurized milk and incubated at room temperature for 18 h. After incubation, the kefirs were filtered to separate kefir grain. The microbial, acidity, pH, alcohol, moisture and viscosity analysis were done on 1 d of storage at 4ºC.

Microbiological analysis. Sample (1.0 mL) was homogenized with 9 mL of 8.5 g/L sterile physiological saline to make an initial dilution (10^{-1}). Serial dilutions were made for each sample and 0.1 mL of the appropriate dilution (10^{-4}, 10^{-5}, and 10^{-6}) were spread plates on medium (Liu *et al*., 2012). DeMann Rogosa and Sharpe (MRS) agar (Merck) containing 100 ppm NaN_3 and 100 ppm $CaCO_3$ were used for determination of lactic acid bacteria (LAB). Malt extract agar (MEA) (Oxoid) containing 100 ppm chloramphenicol was used for determination of yeasts, and plate count agar (PCA) was used for microbial culturing of total viable bacteria respectively (Mundt *et al.,* 1967; Hwanhlem *et al.,* 2011; Eissa *et al*., 2011; Liu *et al*., 2012; Wakil *et al*., 2014).

Chemical analysis. The pH of kefir was measured using a pH-meter (HANNA-HI 98103), and acidity was analyzed by titratable acidity according to Hashim *et al.* (2009) with slight modification. Titratable acidity is expressed as percentage of lactic acid, and determined by titrating 9 g of kefir with 0.1 N NaOH. The moisture content of kefir was analyzed according to AOAC (2000). Alcohol in kefir samples were analyzed by Conway microdiffusion method (MacLeod, 1949) with modifications.

Physical analysis. Kefir viscocity was measured by Viscometer (Brookfield RVT), using spindle number 3 with a speed of 10 rpm. Measured sample volume was 100 mL at room temperature.

Statistical analysis. The data of total LAB, yeast, total count, acidity, pH, alcohol and moisture contents were analyzed by one way ANOVA, using SPSS version 17.0.

RESULTS AND DISCUSSION

Microbiological quality. Based on Table 1, supplementation of WPC or glucomannan from porang has no effect on microbiological quality of goat milk kefir. This means WPC or glucomannan that added in kefir fermentation have no negative effect on microbial growth. The average number of LAB in kefir was higher than yeast. This result similar to a previous study that total LAB in goat milk kefir was higher than total yeast in order to 7 and 5.81 log CFU/mL, whereas the total count was 9.91 log CFU/mL (Nurliyani *et al*., 2014). The average of total yeast in the present study were also similar to a result study by Oner (2012) that goat milk kefir has a total yeast of 4.84 log CFU/mL.

Table 1. The average of total LAB, yeast, total count, acidity and pH of goat milk kefir supplemented with WPC and glucomannan

Kefir	LAB^{ns} (log CFU/mL)	Yeast^{ns} (log CFU/mL)	Total count^{ns} (log CFU/mL)	Acidity ^{ns} (%)	pH ^{ns}
GM	6.97±0.28	4.67±0.69	9.12±1.02	1.14±0.12	3.91±0.30
GMW	7.09±0.43	4.44±0.49	9.27±1.19	1.09±0.14	4.01±0.09
GMG	6.89±0.62	4.92±0.19	8.52±0.79	1.05±0.05	4.05±0.16
GMWG	6.95±0.13	5.25±0.17	9.11±0.95	1.04±0.08	3.92±0.25
Average	6.98±0.35	4.82±0.49	9.01±0.90	1.08 ±0.09	3.97±0.19

ns : not significant
GM : Goat milk kefir, GMW: Kefir made from goat milk + WPC, GMG: Kefir made from goat milk+glucomannan, GMWG : Kefir made from goat milk+WPC+glucomannan

Chemical quality. Based on Table 1, there were no differences in acidity between kefir type in the present study. Thus, supplementation of 1% whey or 0.3% glucomannan in kefir production had no effect on acidity due to the number of LAB was also not affected by whey or glucomannan (Table 1). In the milk fermentation, only 30% of lactose will be converted into lactic acid (Hui, 2007), so that supplementation of WPC which contains lactose had no effect on lactic acid production. While glucomannan is polymer of mannan and glucan that ccould not be degraded by microorganisms in kefir because have no mannanase which is secreted extracellularly. Actually, the yeast cell wall contains glucan, mannan and protein and also contains hydrolytic enzymes inherently present in the cell. The mannan in cell wall will be degraded when the cell undergo autolysis by the activity of P (1-3) glucanase and protease present within the cells, followed by the activity of P (1-6) glucanase and mannanase (Belem and Lee , 1998; Kwiatkowski and Kwiatkowski, 2012).

The kefir type also has no effect on pH value (Table 1). The pH of fermented milk was determined by a number of organic acids (not only lactic acid) produced during the fermentation and storage. Thus, when a significant production of organic acids during kefir fermentation or during storage it will lower the pH.

Supplementation of WPC or glucomannan in kefir fermentation has no effect on alcohol and moisture content of kefir (Table 2). The average of alcohol content in the present study was lower than the average of alcohol in the kefir prepared by conventional method (fermentation at 22ºC, 24 h) which has in average 1.3 to 1.36% (Yoo *et al*., 2013). According to Oner *et al.* (2010), the concentration of ethanol in kefir more affected by kefir microbial, and the amount of ethanol is largely determined by periode of storage time and the kind of culture. Theaverage of moisture content of kefir in this study was lower than a previous study by Oner *et al*. (2010)

Table 2. The average of alcohol, moisture and viscosity of goat milk kefir supplemented with WPC and glucomannan

Kefir	Alcohol (%) ns	Moisture (%) ns	Viscosity (cP) ns
GM	0.77±0.51	83.20±0.85	6,266±2,349
GMW	0.81±0.29	82.92±1.21	7,946±2,247
GMG	0.58±0.51	84.21±1.49	3,720±5,785
GMWG	0.66±0.16	84.51±1.01	1,613±1,476
Average	0.71±0.35	83.71±1.21	4,886±3,843

ns : not significant
GM : Goat milk kefir, GMW: Kefir made from goat milk + WPC,
GMG : Kefir made from goat milk+glucomannan, GMWG: Kefir made from goat milk+WPC+glucomannan

that goat milk kefir had a moisture content of 86.98%. This differences in moisture content was probably due to differences in raw milk material used to produce kefir.

Physical quality. Shown on Table 2, the viscosity variation among types of kefir in around of 1,613 to 7,946 cP, but statistically not significance differences. Kefir made from goat milk+ 0.1% WPC+glucomannan (0.2 to 0.4%) in the present study had a viscosty similar to kefir prepared by conventional fermentation method according to a previous study by Yoo *et al*. (2013) i.e 1,250 to 1,277 cP.

Related to the increasing viscosity of kefir could be characterized by the formation of clusters from the milk fat globules, which are active due to the presence of phospholipids and proteins on their surface, and they interact with each other even before they form crosslinks with whey proteins and casein micelles (Cho *et al*., 1999). The increasing viscosity of kefir during fermentation could be caused by the increasing the acidity and then curd would be formed and trapped fat globules within the protein matrix. This curds are composed of aggregated casein micelles, whereas the trapped fat globules interact with each other due to the presence of phospholipids and proteins on their surface. Furthermore, the milk fat globules cluster form crosslinks with whey proteins and casein micelles (Cho *et al*., 1999; Everett, 2003). Viscosity of a mixture of biopolymers is affected by molecular weight, total biopolymer concentration, hydration capacity, pH, ionic strength, and presence of other functional groups (Gupta *et al*., 2012).

In the case of the present study showed that WPC could increase the viscosity of goat milk kefir, since whey was a protein that insusceptible to proteolytic activity. Whey proteins are generally more susceptible to heat treatment, unaffected by pH condition and are relatively more resistant to proteolytic activity compared to caseins (Alhaj *et al*., 2007). However, when WPC combined with glucomannan will reduce the kefir viscosty (Table 2), although statistically not significant. In a previous study showed that cow or goat milk kefir supplemented with WPC was more viscous than kefir supplemented with skim milk powder or inulin during 5 and 10 d storage at 5ºC. This is could be explained by the binding between positive charge of protein in WPC and water molecules, so that increases the kefir viscosity (Tratnik *et al*., 2006). Furthermore, interaction of WPC and glucomannan that supplemented in kefir will reduce the capasity to bind the water molecules. According to Herceg *et al.* (2002), the binding of ion pairs between carboxymethyl cellulose and whey protein had negative effect on the rheological characteristic of model solutions due to the decreasing number of active groups of hydrocolloid that associate with water molecules.

The decreased in viscosty of goat milk kefir that supplemented with glucomannan (Table 2) could be caused by proteolytic activity on casein during kefir fermentation and storage, thereby reducing the networking between casein and glucomannan that traps water molecules. Finally, the curd shrinkage and showed a liquid whey. Lactic acid bacteria and yeast in kefir have proteolytic activity, so that kefir milk prepared under normal conditions contained peptides from proteolysis of α-Lactalbumin and κ-, α-, and β-caseins (Ferreira *et al*., 2010). In other study, selected of 23 strains from kefir have been proven for their proteolytic activity, and *L. plantarum* KM4-mr1 produced the highest proteolytic activity (Kivanç and Yapici, 2015).

Decreasing in viscosity in kefir could also be caused by cytoplasmic glycohydrolase after certain incubation times that able to degrade exopolysaccharides (EPS) (Rimada and Abraham, 2001). According to Pham *et al*. (2000), incubation of EPS with glycohydrolases resulted from *Lactobacillus rhamnosus* R were capable in lowering the viscosity of the polymer as well as liberating some reducing sugars. Glucose, galactose and unidentified oligosaccharide at long fermentation process had been detected in whey fermentation by kefir grain. When extended incubation during 27 h, the reduce of viscosity was increased up to 33% (Pham *et al*., 2000; Rimada and Abraham, 2001).

CONCLUSION

The raw materials and the complex activity of various microorganisms during kefir fermentation will determine the characteristics of final product. However, the addition porang glucomannan in fermentation process had no effect on microbiological, chemical and physical characteristics of goat milk kefir. Supplementation of WPC which resistant to proteolytic activity seems to be suitable for kefir stabilizer. However, combination of WPC and porang glucomannan is not suitable for increasing the viscosity or stability of kefir.

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