Physiochemical and Organoleptic Features of Goat Milk Kefir Made of Different Kefir Grain Concentration on Controlled Fermentation

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Abstract. Kefir contains bacteria and complex yeast in protein and polysaccharide matrix formed during anaerobic growth. Kefir fermentation uses kefir grains as starter. This research was aimed to evaluate the physiochemical and organoleptic composition of goat milk kefir made of different kefir grain concentration at controlled fermentation. Materials used were 27 litres of Ettawah crossbred (PE) milk and kefir grains. The experimental research was subject to Completely Randomized Factorial Design with nine combined treatments namely kefir grain concentrations (1, 3, and 5%) and controlled pH fermentation (5.5, 5.0, 4.5) with three repetitions. The observed variables were total solids (%), kefir proximate (%), alcohol level (%), kefir grain profile (SEM) and kefir organoleptic semi-trained panel. Result demonstrated that kefir total solids in all treatments and interactions were generally equal but significantly affected kefir alcohol level, kefir protein percentage, fat content and ash content. Hedonic scale showed that different kefir grain concentration and pH in fermentation significantly affected goat milk kefir texture, flavor and aroma. It was concluded that 1% kefir grain concentration and 4.5 pH in fermentation produced the lowest alcohol level or 0.283% and had the most preferable flavor and aroma based on rank test.

Key words: kefir, concentration, fermentation, goat milk, kefir grains

Abstrak. Kefir mengandung bakteri dan ragi kompleks dalam protein dan matrik polisakarida yang terbentuk selama pertumbuhan anaerobic. Fermentasi kefir menggunakan biji kefir sebagai starter. Penelitian ini bertujuan mengkaji komposisi fisiokimiawi dan organoleptik kefir susu kambing yang terbuat dari biji kefir dengan konsentrasi berbeda pada fermentasi kontrol. Materi yang digunakan dua puluh tujuh liter susu kambing Peranakan Ettawah (PE) dan biji kefir. Penelitian menggunakan Rancangan Acak Lengkap pola faktorial dengan sembilan kombinasi perlakuan, yaitu konsentrasi biji kefir (1, 3, dan 5%) dan fermentasi pH kontrol (5.5, 5.0, 4.5) dengan tiga pengulangan. Peubah yang diamati adalah total padatan (%), perkiraan kefir (%), kadar alkohol (%), profil biji kefir (SEM) dengan panel organoleptik agak terlatih. Hasil penelitian menunjukkan bahwa total padatan kefir di semua perlakuan dan interaksi pada umumnya sama namun secara nyata mempengaruhi kadar alkohol kefir persentase protein kefir, kandungan lemak dan abu. Skala hedonik menunjukkan bahwa konsentrat biji kefir dan pH yang berbeda dalam fermentasi secara nyata mempengaruhi tekstur, rasa dan aroma kefir susu kambing. Disimpulkan bahwa 1% konsentrat biji kefir dan 4.5 pH dalam fermentasi menghasilkan kadar alkohol rendah atau 0.23% dan memiliki rasa serta aroma yang paling disukai berdasarkan uji peringkat.

Kata kunci: kefir, konsentrat, fermentasi, susu kambing, biji kefir

Introduction

Kefir is a drink produced from yeast and bacteria fermentation process with special flavor, is favorable and healthy. Kefir is one of functional drinks which by frequent intake will prevent and relieve some diseases. Microflora in kefir grains working on fermentation process consists of several lactic acids and yeast in polysaccharide matrix. Microflora composition in kefir grain is so complex that it is hard to obtain several starter grains with equal quality (Mainville et al., 2006). Kefir characteristic is influenced by microflora composition in kefir grains. Brazilian kefir is dominated by lactic acid bacteria (60.5%) and yeast (30.6%) and acetic acid bacteria (8.9%).

Kefir is composed of combined cultures of some yeasts from genus Kluyveromyces, Candida, Saccharomyces and of lactic acid bacteria from genus Lactobacillusin protein and polysaccharide matrix formed during anaerobic growth. Kefir grains have white yellowish irregular form, resembling cauliflower's granule (Guzel-Seydim et al., 2005). Lactic acid and yeast diversity used in kefir production will determine the characteristics, particularly acidity level, alcohol level, CO_2, and aroma resulted during fermentation.

Kefir grain can be used in any kinds of milk, and milk fatty acid will affect kefir chemical composition. Goat milk is kefir main ingredient with special characteristics for specific products in many countries. In Europe and Mediterranean, goat milk is the main ingredient of kefir with high nutritional and economical value bearing specific composition and characteristics (Boyazoglu and Morand-Fehr, 2001; Dubeuf et al., 2004). Some researchers have conducted studies on kefir made of various ingredients, among which were cow milk (Irigoyen et al., 2005), goat milk (Chen et al., 2005), soy milk (Pourahmad et al., 2011), peanut milk (Bensmira dan Jiang, 2011).

General characteristic of kefir is containing 4.0 pH, 0.5 to 2% alcohol level and fatty acid depending on the type of milk (Irigoyen et al., 2005). Kefir grains produces several components namely lactic acid, acetate acid, CO_2, alcohol (ethyl 2 alcohol) and aromatic compounds that give kefir special feature of sour fresh flavor (Güzel-Seydim et al., 2000; Otles and Cadingi, 2003). This special flavor is derived from symbiotic metabolite of bacteria and yeast, including product of proteolysis and lipolysis enzyme degradation (Choi and Ng-Kwai-Hang, 2003). Kefir grain sources are Tibetan kefir (Zhou et al., 2009), Taiwanese kefir (Chen et al., 2005) and Russian kefir.

Kefir grains used in kefir production affects the physiochemical characteristics and organoleptic, while fermentation period will determine the product final pH. Some researchers suggested that kefir grain concentration be used at 1 to 5% within 20-hour incubation (Chen et al., 2005) to 24-hour incubation at room temperature to produce the optimal kefir (Sawitri, 2011). Kefir produced from sensory kefir grains is preferable than that from starter culture (Assadi et al., 2000); furthermore, kefir chemical composition is affected by the type of milk and fermentation condition.

Different kefir grain concentration at different pH fermentation or incubation will result in different kefir physiochemical and organoleptic characteristics; this research was therefore aimed to evaluate the physiochemical and organoleptic composition of goat milk kefir made of different kefir grain concentration at controlled fermentation.

Materials and Methods

Twenty seven litres of Ettawah crossbred goat milk and kefir grains were administered in this research, along with MRSA (de Man Rogosa Sharp Agar) and MRSB (de Man Rogosa Sharp Broth) media. The laboratory equipment used were Kefir maker, organoleptic testing apparatus, oven, glass tube, pH meter (Hanna) for laboratory analysis (Soxlet, Kjeldahl), SEM (Scanning Electron Microscope).

Research design. Experimental research was subject to Completely Randomized Design (Steel and Torrie, 1996). Treatments consisted of 9 combinations namely kefir grain concentration (1, 3, 5%) and controlled fermentation pH (5.5, 5.0, 4.5). Each treatment was repeated 3 times.

Data analysis. The obtained data were subject to statistical analysis of ANOVA. Further test, Duncan was conducted using SPSS version 17.0 with 5% standard deviation.

Kefir making procedure. Goat milk was pasteurized at 72^{O}C for 15 seconds, cooled to

20-28^{O}C, then added with kefir grains based on treatments (T1, T2, T3) and incubated at room temperature (28^{O}C) for 24–60 hours (according to controlled pH or 5.5, 5.0, and 4.5). Incubation wasceased after controlled pH was reached (5.5, 5.0, dan 4.5), then kefir was sievedusing fine sifter. Clear whey is ready-made and packed kefir product. The solids were kefir grains ready for multiple fermentation.

Variables measurement

Kefir composition. The analysis of total protein level was subject to micro Kjeldhal method (AOAC, 2005), water content with oven method (AOAC, 2005), fat content with soxlet extraction method (AOAC, 2005) and ash content with oven method (AOAC, 2005).

Total alcohol content in kefir. Twenty five ml sample added with 50 ml aquadest was stored in distillation flask, and 25 ml aquadest was poured into container. Distillation was done to obtain 50 ml volume in the container (James, 1995).

Kefir grain texture Electron Microscope (SEM) (Ali et al., 2009). Kefir grains were added to 4% glutaraldehyde w/v in phosphate buffer with 7.2 pH or 12 hours at 40^{O}C. Samples were then cleansed with 0,1 M cocodylate buffer three times in 10 minutes. The next stage, dehydration process, used ethanol gradually with concentration of 50, 70, 85, 95 and 100% v/v. Being dehydrated, the samples were freezer-dried, gold-coated then electron microscope-examined.

Organoleptic testing of Kefir (Rahayu and Nurosiyah, 2008). Kefir sample testing was performed by semi-trained panel on product preferability, observing flavor, aroma and texture. Testing was conducted using hedonic scale following a rank. Each sample was tagged with unique random three-digit number. Neutralizers after sensory evaluation were made available in that ground coffee for aroma attribute and a glass of water for flavor attribute. Panelists were asked to determine their preferability on each sample without comparing them.

Result and Discussion

Physiochemical Characteristics of Kefir

Macro nutrition is chemical composition determining food quality including kefir fermentation. Macro nutrition in kefir is almost equal to milk as the main ingredients. Some researcher stated that chemical composition of kefir depends on some factors namely type of milk and kefir grains during the process. Fat content in commercial fermented milk is affected by the product type and standardized process within the range of 0-10%, while kefir produced without standardized process had 3-4% fat content (Sady et al., 2007).

Chemical testing on goat milk kefir with 5.5, 5.0, and 4.5 pH fermentation and 1, 3 and 5% of total solids resulted in different average of alcohol level, protein level, fat content and ash content (Tabel 1). Table 1 presents that kefir total solids was 14.59%, within the highest and lowest point was 16.85% and 13.04%, respectively. Kefir grain concentration (1, 3 and 5%), controlled fermentation (pH 5,5; 5,0 dan 4.5) and the interaction generally showed equal total solid. It was in line with that of Sady et al. (2007) namely 9.69 to 13.69% and was still within range of total solid of fermented yoghurt or 11.6 to 13.9 (g/100g) (Ozer, 2006). Total solid of kefir product was affected by the quality of main ingredients, in that fortification milk would affect total solid and improve kefir consistency, flavor and aroma. Total solid of goat milk was 18.1% (Chandan 2006; Raynal-Ljutovac, 2008) and 13.41%.

Alcohol Level of Goat Milk Kefir

Starter used in kefir production was kefir grains composed of lactic acid bacteria and yeast to produce lactic acid, carbon dioxide, ethanol, acetaldehyde, diacetyl and acetone to make kefir special flavor and aroma (Beshkovaaet al., 2002).

Table1. Average value of chemical testing on different kefir grain concentration at controlled fermentation

Treatment	Chemical characteristics (%)				
	Total solids	Alcohol	Protein	Fat	Ash
1% pH 5.5	14.81±0.11	0.80±0.07^{d}	3.75±0.05^{bc}	6.66±0.09^{e}	0.04±0.00^{e}
1% pH 5.0	14.76±0.22	0.53±0.07^{bc}	3.83±0.11^{cd}	6.07±0.01^{d}	0.45±0.01^{d}
1% pH 4.5	13.04±0.04	0.28±0.04^{a}	3.66±0.02^{b}	5.35±0.02^{bc}	0.42±0.00^{c}
3% pH 5.5	14.55±0.10	0.60±0.07^{c}	4.18±0.05^{e}	5.21±0.07^{ab}	0.52±0.00^{f}
3% pH 5.0	14.50±0.08	1.18±0.04^{f}	3.96±0.04^{d}	6.82±0.07^{e}	0.48±0.00^{e}
3% pH 4.5	16.85±3.69	0.46±0.07^{b}	3.21±0.04^{a}	5.06±0.06^{a}	0.32±0.00^{b}
5% pH 5.5	15.42±0.41	1.48±0.07^{g}	3.84±0.02^{cd}	5.92±0.03^{d}	0.30±0.01^{a}
5% pH 5.0	14.67±0.06	1.79±0.10^{h}	3.70±0.03^{bc}	5.57±0.04^{c}	0.45±0.00^{d}
5% pH 4.5	13.11±0.05	1.00±0.07^{e}	3.72±0.03^{bc}	5.55±0.04^{c}	0.49±0.00^{e}
Average	14.59±1.169	0.90±0.49	3.76±0.03	5.80 ±0.06	0.43±0.07

Values bearing different superscript at the same column shows significant (P<0.05).

Alcohol level of goat milk kefir made of different kefir grain concentration and pH fermentation had dissimilar average as well. Analysis result showed that kefir grain concentration with different pH fermentation significantly affected (P<0.05) the average of alcohol level of goat milk kefir. Alcohol level of 5% kefir grain concentration resulted differently from that of 1 and 3%. Alcohol produced by yeast was affected by the amount or concentration of kefir grain and fermentation period (Simovaet al., 2002; Farnworth and Mainville, 2003).

The highest alcohol was 1.79 at 5% kefir grain concentration with 5.0 pH fermentation, while the lowest alcohol level or 0.283 was at 1% kefir grain concentration with 4.5 pH fermentation. The more kefir grains the more yeast added so the alcohol produced by the yeast also increased (Chen et al., 2005). Average alcohol level was 0.90%, higher than that of Sawitri (2011) using 1-3% kefir grain concentration that resulted in 0.16 alcohol level. Alcohol level in kefir product was affected by fermentation period, while ethanol amount was affected by fermentation temperature and starter culture. Alcohol amount in kefir from commercial starter culture would rise along with the temperature rise (Bakhshandeh et al., 2011). Yeast was essential in kefir fermentation because yeast produced ethanol and CO_2 (Irigoyen et al., 2005).

Protein Level of Goat Milk Kefir

Kefir chemical composition indicated product's nutritional value. The average kefir protein level in this research range between 3.21 and 4.18%, averaging 3.76%. Kefir grains and pH fermentation influenced kefir protein percentage. The highest protein level was detected at 3% kefir grain concentration and 5.5 pH fermentation, while the lowest protein level at 3% kefir grain concentration and 4.5 pH fermentation. Protein level increased was followed by total solid increase (Magalhães et al., 2011).

Protein level of goat milk was 3.02%, while protein level of goat milk kefir was 2.96% (Chen et al., 2005). Fermentation process would increase protein level. At 0 hour fermentation, protein fermentation level was 2.12%, and after 4 hours it reached 3.91% (Magalhaes et al., 2011). Protein level of Etawah goat milk was 5.59% (Chandan et al., 2006; Raynal-Ljutofac, 2008).

Fat Content of Goat Milk Kefir

Total average of fat content in this research was 5.80% in which the lowest was 5.06 at 3% kefir grain concentration and 4.5%, while the highest was at 3% kefir grain concentration and

5.0 pH fermentation. Kefir grain concentration and pH fermentation affected final fat content.

Treatments of 1 and 3% kefir grain concentration at 5.5% pH fermentation resulted in different fat content, while 1 and 5% share equal fat content. Kefir grain concentration of 1 and 3% at 4.6 pH fermentation showed different fat content. Accordingly, different fat content in kefir grain was prevalently affected by kefir grain concentration due to its connection with microbe ability to degrade butterfat to a less complex form. Different fat content was due to lipase produced by kefir grains during fermentation.

Fat content reported in this research was higher than that of previous study, namely 3.51 and 3.60 g/100 ml after 24-hour fermentation. Former researcher stated that one of the kefir nutrition compositions was fat content equal to fat content in milk. Other researchers, Ching-Yun and Ching-Wen (1999) reported that kefir fat content was lower than that of milk main ingredients. Fat content of goat milk was 6.82% (Chandan et al., 2006 and Raynal-Ljutovac, 2008).

Ash Content of Goat Milk Kefir

Nutrition serving as kefir chemical components are vitamin, protein and mineral which during fermentation will increase kefir nutrition profile (Sarkar, 2007). Mineral content in kefir was specified to the measured ash content. Analysis result showed that kefir grain concentration with different pH concentration significantly affected ($P<0.05$) ash content whose total average was 0.43% with lowest average of 0.30 at 5% kefir grain concentration and 5.5 pH concentration and highest average of 0.52 at 3% kefir grain concentration and 5.5 pH fermentation.

During fermentation, vitamin B1, B2, calcium, amino acid, folic acid and vitamin K increased in kefir (Otles and Cadingi, 2003). Mineral level in kefir obtained in this research was lower than that by Ozer and Ozer (1999) namely 0.7% (Ozer and Ozer, 1999). Kefir contain macro and micro mineral, in which macro mineral consisted of potassium, calcium, magnesium and phosphor, while micro mineral consisted of copper, zinc, iron, manganese, cobalt and molybdenum (Liut-Kevicius and Sarkinas, 2004).

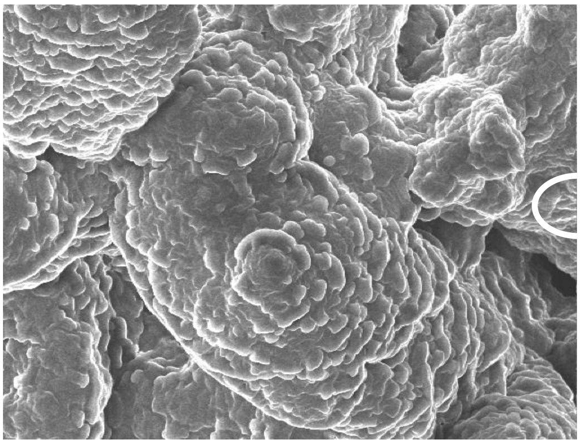

Figure 1. Scanning electron microscope (SEM) of kefir grain (5000 x)

SEM depicted kefir grain as cauliflower, with irregular form covered by biofilm so microbe composing kefir grains to blur. It was in line with the previous study that kefir grain had complex form with biofilm covering while the inner part had irregular form (Guzel-Seydim et al., 2005; Rea et al., 1996). Kefir grain as reported by Fanworth (2005) was cauliflower-like, small, measuring 1-3 cm long, white or yellowish white globe with mucous but elastic texture (Farnworth, 2005).

Organoleptic of goat milk kefir

Kefir texture, flavor, and aroma are the criteria of consumers' preferability related to the quality of kefir product. Testing preferability was performed by 26 semi-trained panelists consisting of undergraduate students. Overall testing result based on rank test is presented in Table 2.

Table 2 showed that the best texture mostly preferred by the panelists was goat milk kefir made of 5% kefir grain concentration with 5.5 pH fermentation (conversion value was 0.416)

Table 2. Panelists preferability on goat milk kefir

Treatments	Texture	Flavor	Aroma
1% pH 5.5	-0.81±0.936[a]	-0.796±0.770[a]	-0.358±1.161[a]
1% pH 5.0	-0.28±0.708[b]	-0.017±0.551[abc]	-0.092±0.666[a]
1% pH 4.5	0.11±0.788[b]	0.475±0.876[e]	0.603±0.940[b]
3% pH 5.5	0.19±0.867[bc]	-0.477±0.838[a]	-0.104±0.795[a]
3% pH 5.0	0.21±0.566[bc]	0.195±0.664[de]	-0.091±0.491[a]
3% pH 4.5	-0.16±0.955[b]	0.180±0.940[de]	0.205±1.077[ab]
5% pH 5.5	0.47±0.751[c]	0.056±0.525[de]	-0.310±0.577[a]
5% pH 5.0	0.13±0.800[bc]	-0.135±0.856[bc]	-0.306±0.834[a]
5% pH 4.5	0.199±0.969[bc]	0.418±1.025[e]	0.453±0.723[b]

Values bearing different superscript at the same column shows significant (P<0.05).

followed by 3% kefir grain concentration with 5.0 pH fermentation, and the least preferable was goat milk kefir made of 1% kefir grain concentration with 5.5 pH fermentation. Table 2 also showed the preferability level on flavor and aroma of goat milk kefir, in which the most preferable was 1% kefir grain concentration with 4.5 pH fermentation (each conversion score was 0.475 and 0.603), while the least preferable flavor and aroma of goat milk kefir was at 1% kefir grain concentration with 5.5 pH fermentation (conversion score was – 0.796 and – 0.358).

Result showed that kefir grain concentration and pH fermentation significantly affected (P<0.05) panelists' preferability level on texture, flavor and aroma of goat milk kefir. The higher kefir grain concentration, the more preferable it became due to the thicker the texture. Based on panelists assessment, the lower pH fermentation the higher the preferability on goat milk kefir flavor. Accordingly, the lower pH fermentation the more preferable the aroma because it was less goat-smelled. Aroma and flavor sensation of goat milk kefir was more affected by pH fermentation.

Conclusion

It was concluded that 1% kefir grain concentration at 4.5 pH controlled fermentation produced goat milk kefir with the lowest alcohol content namely 0.283%, bearing the most preferable flavor and aroma based on rank test.

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