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Study on kefir grain concentration and the different length of storage on the physicochemical of goat milk kefir

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Abstract. This study was about the effect of kefir grain concentration level and the different length of storage, as well as the interaction between kefir grain concentration level and the different length of storage on the fat content, pH and the viscosity of goat milk kefir (GMK). The ingredients used were goat milk from Pegumas farmers group and kefir grains from Perwira kefir. Data was analyzed by using the analysis of variance of 2x4 factorial and continued by Duncan's new multiple range test. The results showed that kefir grain concentration gave a significant effect (P<0.01) on the fat content, pH and the viscosity of GMK. The different length of storage gave a significant effect (P<0.01) on the fat content, pH and the viscosity of GMK. The interaction had no significant effect (P>0.05) on the fat content and there was an interaction effect (P<0.01) on the pH and viscosity of the GMK. The conclusion of this study was the increase of the concentration level of kefir grain up to 3% resulting on the lowest fat content and pH, and the highest viscosity; and that the length of storage up to 21 days decreases the fat content and pH, but increases the viscosity.

1. Introduction

Kefir is a dairy product that can be obtained by fermenting milk with kefir grains which consist of bacteria and yeast. Its origin is from the region of Caucasus mountains and Eastern European regions [9]. Kefir is a natural probiotic agent which has beneficial effects on immunity and gastrointestinal tract such as antimicrobial, enhancement of mucosal barrier integrity, immune modulation, cholesterol-lowering, allergy, wound healing, ACE inhibition, lactose intolerance-preventing, antimutagenic, anticarcinogenic and antimicrobial properties [5]. As a fermentation product, kefir has some main chemical components such as lactic acid, acetaldehyde, diacetyl, acetoin, ethanol, carbon dioxide and free fatty acids, for example acetic, propionic, butiric, hexanoic, and has the following characteristics: pH abort 4.0; alcohol from 0.5% to 2%; fat content depends on the type of milk used; taste is acid, and slightly yeasty [14]. Kefir also had compounds such as vitamins, minerals, essential amino acids, bioactive peptides, exopolysaccharides and bacteriocins [6]. The microbial symbiotic mixture of lactic acid bacteria, acetic acid bacteria, and yeast in the kefir grain make kefir has an acidic-alcoholic taste. The primary factors that influence the final quality of kefir are microbiological diversity of kefir grains, the ratio of grain to milk, incubation, agitation, and the storage conditions [8].

Kefir grain is the fundamental factor that can determine the kefir quality. Kefir grain is a group of the stable microbial community to produce organic acids, peptides, bacteriocins, and fatty acids. The

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most common species of kefir grains are *Lactobacillus* L. *kefiri*, L. *kefiranofaciens*, L. *kefirgranum*, and L. *parakefiri*; characteristic yeasts in kefir are *Saccharomyces* spp. and *Kluyveromyces marxianus* [5]. Kefir grains can establish the unique flavor properties of kefir. Different kefir grain causing different microbial groups on the formation of kefir aroma which can affect its final quality [4]. The composition of the microflora of kefir grains differs according to their origin of the grains and conditions on the production method [7].

This kefir has been produced from goat milk, although in Indonesia it is usually prepared from cow milk on the commercial scale. Goat milk has higher protein, non-protein N and phosphate. It gives greater buffering capacity compared to cow milk. Goat milk has also some physicochemical properties such as smaller fat globules, a higher percent of short and medium-chain fatty acids, and softer curd formation of its proteins are advantageous for higher digestibility and healthier lipid metabolism [12]. [11] reported that kefir from pasteurized fresh goat had the following chemical characteristic: pH 6.66, acidity 0.19%, fat 3.43%, protein 4.72%, and lactose 4.30%. This study was about the effect of kefir grain concentration level and the different length of storage, as well as the interaction between kefir grain concentration level and the different length of storage on the fat content, pH and the viscosity of goat milk kefir.

2. Materials and Methods

2.1. Materials

The materials used for making goat milk kefir were goat milk from Pegumas farmers group Purwokerto and kefir grains from Perwira kefir Klaten. The materials used for testing fat content, pH, and viscosity were buffer, ether, filter casa, condenser, soxhlet, pH meter and viscometer. The equipments used were glass jar, scale, thermometer, pot, stirrer, stove, and refrigerator.

2.2. Methods

Goat milks were pasteurized by using a low temperature long time method at 63°C for 30 min and divided into two different jars. Kefir was made by using kefir grains and milk that were cooled down to 25°C and inoculated with 1% and 3% (wt/vol). Kefir grains rate and incubated at 24°C for 20 hours. At the end of the incubation, kefir grains were separated by using a sieve and then the kefir samples were put into glass jars. Each concentration level of kefir was divided based on length of storage 0, 7, 14, and 21 days become 8 glass jars in total (K1= 1% grain kefir 0 day of storage, K2= 1% grain kefir 7 days of storage, K3= 1% grain kefir 14 day of storage, K4= 1% grain kefir 21 day of storage, K5= 3% grain kefir 0 day of storage, K6= 3% grain kefir 7 days of storage, K7= 3% grain kefir 14 days of storage, K8= 3% grain kefir 21 days of storage).

2.3. Parameter of Research

Research parameters consisted of Chemical were fat content and Physic were pH and viscosity

3.1. Fat Content

Fat content was determined by using Soxhlet extraction method [1]. Soxhlet fat extraction used ether for 16 hours or until the solution becomes clear. Fat is the material left in the soxhlet flask after dried at 105°C for 8 hours. Fat content was obtained by dividing the fat weight by the sample weight and multiplying by 100%.

2.3.2. pH

The pH value of the sample was measured with a pH meter calibrated with phosphate buffer pH 7.00 and phosphate buffer pH 4.00 by directly submerging the probe into homogenized kefir sample [7].

2.3.3. Viscosity

Viscosity of goat milk kefir was tested with Brookfield viscometer with 30 rpm of speed [16].

1569 (2020) 032001

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2.4. Statistical analysis

Data of chemical composition, and physical quality of goat milk kefir were analyzed by using analysis variance of 2x4 for torial, i.e 2 concentration level of grain kefir 1 and 3 % and 4 length of storage of 0, 7, 14, 21 day). The average differences were tested by Duncan's new multiple ranges test

3. Results and Discussion

Table 1. Physicochemical composition of Kefir Grain Concentrations And Different Length of Storage On Goat Milk Kefir

Variables	Kefir grain		Average			
	level (%)	0	7	14	21	
Fat content (%)	1	$2,77 \pm 0.17$	$3,17 \pm 0,11$	$2,44 \pm 0.12$	$2,90 \pm 0,13$	$2,82 \pm 0,30^{a}$
	3	$2,11 \pm 0.08$	$2,71 \pm 0.08$	$1,97 \pm 0.02$	$2,37 \pm 0,37$	$2,29 \pm 0,34^{b}$
	Average	$2,44 \pm 0.38^{qr}$	$2,94 \pm 0,27^{p}$	$2,21 \pm 0.27^{r}$	$2,63 \pm 0,38^{q}$	
pН	1	$6,13 \pm 0,06$	$5,65 \pm 0,09$	$5,77 \pm 0,06$	$5,20 \pm 0,10$	$5,69 \pm 0,35^{a}$
	3	$4,74 \pm 0,04$	$4,47 \pm 0,05$	$4,62 \pm 0.07$	$4,46 \pm 0,06$	$4,58 \pm 0,13^{b}$
	Average	$5,44 \pm 0,76^{p}$	$5,06 \pm 0,65^{\circ}$	$5,19 \pm 0,63^{q}$	$4,83 \pm 0,41^{s}$	
Viscosity (cP)	1	$65,0\pm 5,0$	$93,3 \pm 2,9$	$118,3 \pm 2,9$	$150,0 \pm 5,0$	106,7 ±32,9b
	3	136,7±2,9	163,3±2,9	$171,7 \pm 2,9$	$195,0 \pm 0,0$	166,7 ±21,9a
1	Average	100,8±39,4s	128,3±38,4 ^r	145,0 ±29,3 ^q	172,5 ±24,8 ^p	

a,b Different superscript at the same column indicated significant difference (P<0.01). **p,q,r,s** Different superscript at the same row indicated significant difference (P<0.01).

1 3.1. Fat Content

The statistical analysis showed that there was no interaction bet seen kefir grain concentration level and the length of storage on goat milk kefir. The fat content of goat milk kefir with kefir grain concentration level and the different length of storage ranged from 1.97 to 3.17% (Table 1). The fat contents were around normal according to National Standard of Indonesia (SNI) at amount of minimum of 0.6% [3]. The higest fat content of goat milk sefir was obtained on 1% kefir grain with 7 day length of storage treatment. The lowest fat content of goat milk kefir was obtained in 3% kefir grain with 14 days length of storage treatment.

The result showed that kefir grain concentration level had very significantly effect on fat content (P<0.01). The increase of the concentration of kefir grain up to 3% resulting on the lowest fat content. The higher concentration level of kefir grain made fat content of goat milk kefir decreased. This was due to the activity of lactic acid bacteria from kefir grain that synthesize fatty acid from carbohydrates with the help of an enzyme. The higer concentration level of kefir grain causing lactic acid bacteria increased and produced more lipase enzyme, so the fat that 2was hydrolized also increased and causing fat content decreased. In line with research [17] that used of higher kefir grain concentration level of 10%, produced less fat content compared to the one that used of 5% kefir grain concentration level. [2] reported that at the fermentation processed, carbohydrates, protein, fat, and nucleic acid could be broken into simple components. The first component that was attacked by bacteria is carbohydrates (lactose) then protein and fat to a simple components degradation. Lactose will be broken into glucose and galactose, protein will be broken into amino acid, and fat will be broken into fatty acid.

The result showed that the length of storage had very significantly effect on fat content (P<0.01). The length of storage up to 21 days decreased the fat content. Goat milk kefir with 7 days length of storage produced the higest of fat content and decrease reached the lowest of fat content for 14 days length of storage. Based on this result, the highest fat content is on the early length of storage. It might have been caused by less of bacteria that produced lipase enzyme so fat will be broken after carbohydrate and protein. The activity of bacteria that produced lipase enzyme will increase during the

1569 (2020) 032001

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storage until its peak in 7 days length of storage, and then will decrease in 14 days length of storage and move up in 21 days length of storage. Accordings to [10] length of storage would decrease fat content of kefir because of lipolysis during the process.



The statistical analysis showed that there was a interaction between kefir grain concentration level and the length of storage on goat milk kefir. pH of goat milk kefir with kefir grain concentration level and different length of storage ranged from 4.46 to 6.13% (Table 1). The higest pH of goat milk kefir was obtained on 1% kefir grain with 0 day length of storage treatment. The lowest pH of goat milk kefir was obtained in 3% kefir grain with 21 day length of storage treatment.

The result showed that kefir grain concentration level had very significantly effect on pH (P<0.01). The increase of the concentration of kefir grain to 3% resulting on the lowest pH content. The higher concentration level of kefir grain made pH of goat milk kefir decreased. The higher kefir grain used the more lactic acid bacteria contained. Lactic acid bacteria change lactose on milk into lactic acid and causing acidity and decreased of pH. In line with research [9] pH of the kefir, will more acid when the percentage of the dosage of starter used more higher. Kefir with 5% starter had a higher pH than the kefir with 25% of starter. When the dosage of starter is higher, the acidity of the kefir will more acid. [8] reported that an increased of kefir grain associated with increasing the amount of lactic acid bacteria which produced lactic acid and made pH of goat milk kefir decreased.

The result showed that length of storage had very significantly effect on pH (P<0.01). The length of storage up to 21 days decreased the pH. Goat milk kefir with 0 day length of storage produced the higest of pH and the lowest of pH was for 21 days length of storage. The longer storage of goat milk kefir the more lactic acid bacteria produced made become more acid and decreased of pH. Kefir grain concentration level of 1% and 3% for 14 days of storage showed pH increased. This might have been caused by the decline in activity of lactic acid bacteria to the 14th day because of the increase in activity of other bacteria. Previous research [14] reported sheep kefir with different kefir grain during storage 7, 14, 21 days of storage produced the lowest pH at 21 days of storage.

Viscosity

The statistical analysis showed that there was an interaction between kefir grain concentration level and the length of storage on goat milk kefir. Viscosity of goat milk kefir with kefir grain concentration level and different length of storage ranged from 65,0 - 195,0 cP (Table 1). The higest viscosity grain milk kefir was obtained on 3% kefir grain with 21 days length of storage treatment. The lowest pH of goat milk kefir was obtained in 1% kefir grain with 0 day length of storage treatment.

The result showed that kefir grain concentration level had very significantly effect on pH (P<0.01). The increase of the concentration of kefir grain up to 3% resulting on the higest viscosity. The higher concentration level of kefir grain made the viscosity of goat milk kefir increased. The higher concentration level of kefir, the more amount of kefir grain used and caused the more lactic acid bacteria contained, so the lactic acid is formed. It will increase and aggregates those protein that affected viscosity. [15] said viscosity from fermented milk product was affected by aggregates of protein because an acid produced during the fermentation. Acid produced in the form of lactic acid that destabilise the protein and causing aggregates so fermented milk product would be condensed.

The result showed that the length of storage had very significantly effect on pH (P<0.01). The length of storage up to 21 days increased the pH. Goat milk kefir with 0 day length of storage produced the lowest of viscosity, and the higest of viscosity was for 21 days length of storage. The longer storage of goat milk kefir the more lactic acid bacteria produced, causing the decreased of pH and become more acid. Acid would aggregates of protein and made goat milk kefir viscosity's more condensed. [13] reported the kefir containing lactic acid bacteria and yeast that would improve viscosity product. Lactic acid bacteria produced texturing agents eksoselular exopolysaccharide and when interact with protein can improve viscosity.

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4. Conclution

The conclusion of this study was the increase of the concentration level of kefir grain up to 3% resulting on the lowest fat content and pH, and the highest viscosity; and that the length of storage up to 21 days decreases the fat content and pH, but increases the viscosity.

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Study on kefir grain concentration and the different length of storage on the physicochemical of goat milk kefir

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