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Chemical characteristics of pasteurised goat milk and goat milk kefir prepared using different amount of indonesian kefir grains and incubation times

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Abstract: The chemical characteristics of pasteurised goat milk and goat milk kefir prepared using different amount of Indonesian kefir grains and incubation times had been studied. Kefir samples were prepared using amount of kefir grains 3, 5 and 7% (v/v) with incubation times of 8, 21 and 24 hours, respectively, and controls were made for without kefir grains and before incubation. The results showed that pasteurised goat milk samples contain fat: 3.43%, protein 4.72%, lactose 4.30% , titratable acidity number expressed as lactic acid content 0.19% and pH value of 6.66. While the best chemical characteristics (pH 4.37; etanol content 0.91%; titratable acidity number 0.76%; and lactose content: 4.23%) was obtained from goat milk kefir prepared with 7% (w/v) kefir grains and incubation time of 24 hours.

Keywords: Goat milk, kefir grain, incubation time, chemical characteristics

Introduction

Kefir are commonly prepared by fermentation of milk with kefir grains which contain lactic acid bacteria mainly Lactobacilli, Lactococci and *Leuconostocs* species, acetic bacteria and yeast mixture bound together with milk casein and complex sugars where polysaccharide are as its matrix. (Koroleva,1991; Angulo *et al*., 1993). The existance yeast in kefir fermentation is to produce ethanol and carbondioxide, and the grains usually contain either lactose and non-lactose fermenting yeast (Koroleva,1988; Angulo *et al.*, 1993). Simova (2002) and Yöksedag *et al*. (2004) found that lactose fermenting yeast were *Kluyveromyces lactis* and *Kluyveromyces marxianus*,*Torula kefir*, while non-lactose-fermenting yeast was *Saccharomyces cerevisae*. However, as reported by Farnworth (2005) who study on microbial analysis of kefir originated from different locations showed different population of microorganisms.

This kefir can be produced from sheep or goat milk, although in European countries usually it is prepared from cow milk on a commercial scale. Although kefir could be prepared from either cow, goat, sheep as well as buffalo milk with its own characteristics and the chemical characteristics of such product is also affected by the methods used for preparation. (Wojtowski *et al*., 2003 and Irigoyen *et al*., 2005). The quality of kefir as reported by Cais-Sokolinska (2008) was depended on the quality and composition and also strain types of grains microorganisms as well as preconditions before inoculated. Irigoyen *et al*. (2005) in their study reported that kefir produced from cow, goat, sheep and buffalo milk had the following chemical characteristics such as pH about 4.0, alcohol from 0.55 to 2.0%, fat content depends on the type of milk used, and this fermented milk have an acid, prickly and slightly yeasty taste. They also found that acid and yeasty flavour together with prickly sensation were due to carbon dioxide as typical kefir flavour.

Several researches have reported that the chemical characteristics of goat milk are currently well recognized (Park *et al*., 2007; Ismaiel *et al*, 2011) and the typical characteristics and quality of kefir depend on the amount of kefir grains and incubation times (Motaghi *et al*.,1997; Otles and Cagindi, 2003, Chen *et al*., 2009). However the chemical characteristics of Indonesian pasteurised goat milk and goat milk kefir prepared using Indonesian kefir grains are not yet studied.

Hence the purpose of this study was to investigate the chemical characteristics of either pasteurised goat milk which will be used as raw material for goat milk kefir samples and kefir manufactured from goat milk added with different amount of Indonesian kefir grains and incubation times at room temperature.

Materials and Methods

Fresh goat milk and kefir grains

Fresh goat milk were obtained from Etawah filial goat of about 2 – 3 years of age from Milk and Animal

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Product Technology Training Centre, Songgoriti, Batu, East Java. Goat milk were pasteurised first at 85°C for 25 min and cooled down until it's temperature was 24°C before added with kefir grains as described by Motaghi *et al*. (1997). While local kefir grains were obtained from Animal Product Technology Laboratory, Faculty of Animal Husbandry, Brawijaya University, Malang, East Java. These kefir grains were propagated by transferring them into UHT cow milk and allowing them to grow for approximately 20 h at 24°C; then kefir grains were separated from milk and stored at 4°C for 30 min before added in pasteurised goat milk.

Kefir samples preparation

Goat milk kefir were prepared using 500 ml pasteurised goat milk added with 3, 5 and 7%(w/v) kefir grains then incubated at 24°C for 18, 21 and 24 h, respectively. Control were samples with kefir grains before incubation were carried out.

Chemical analysis

pH values of either pasteurised goat milk and goat milk kefir were measured by following the method of Kebede *et al*. (2007), while titratable acidity and lactose content were determined using methods No. 947.05 (Anonymous, 2000) and 896.51 (Anonymous, 2000). Fat and protein content of pasteurised goat milk were measured using Babcock method AOAC No – 988-04 (Anonymous,2000) and Kjeldhal method No 920.105 (Anonymous, 2000). Ethanol content of goat milk kefir samples were determined by following the method as described by Kartika *et al*. (1992).

Statistical analysis

The data of chemical characteristics of pasteurised goat milk obtained are the average of three independent (triplicate) experiments (batches) and triplicate of analysis and completed by standard deviation (+ s.d.). While the data of goat milk kefir samples obtained also from triplicate independent experiments were statistically tested using ANOVA analysis (Microsoft Excell, 2007), and if there were significant differences the analysis was continued using Duncan Multiple Range Test (DMRT) as desribed by Yitnosumarto (1993).

Results and Discussion

The chemical characteristics of goat milk obtained from Etawah filial of about 2-3 years old and then pasteurised were presented in Table 1. It is interesting to note that the fat content was 3.43% which is similar to the result reported by Jenness (1980) i.e in the range of 3.40 – 7.76%, although the protein content 4.72% was slightly higher than as reported by Jenness (1980) i.e. 3.40%. These differences could be possibly due to different species, lactation period and feeding management of goat or sheep. While lactose content (4.30%), pH (6.66) and acidity (0.19%) of pasteurised goat milk samples were similar as reported by Park *et al*. (2007) where their samples also had lactose content of 4.10%. Ju'arez and Ramos (1986) also found that average pH value and acidity number in their goat milk samples were in the range of 6.50 – 6.80 and 0.14– 0.23%, respectively.

Table 1. Chemical characteristics of pasteurized goat milk*

pH	6.66 ± 0.085
Acidity (% lactic acid)	0.19 ± 0.025
Fat (%)	3.43 ± 0.152
Protein (%)	4.72 ± 0.195
Lactose (%)	4.30 ± 0.190

*Means of three replications of experiments and three replications of analysis ± standard deviation.

Table 2 shows the values of the main chemical characteristics of goat milk kefir prepared by using different amount of kefir grains and incubation times. The difference of pH value, acidity number and lactose content of pasteurised goat milk (6.66; 0.19%; 4.30%) and innoculated pasteurised goat milk before incubation with different amount of kefir grains (6.03 – 6.17; 0.41 – 0.45%; 5.05 – 5.58%), were possibly due to lactic acid and lactose contents in kefir grains itself.

Table 2. Chemical characteristics of goat milk kefir produced by using different amount of Indonesian kefir grains and incubation time*

Kefir grains (% v/w)	Incubation time (hours)	pH	Acidity (%)	Lactosa (%)	Ethanol (%)
3**	control	6.17±0.01^{k}	0.41±0.02^{a}	5.58±0.003^{j}	0.69±0.003^{a}
	18	4.92±0.01^{h}	0.51±0.01^{c}	5.16±0.002^{l}	0.86±0.002^{g}
	21	4.83±0.02^{f}	0.61±0.01^{f}	5.06±0.002^{h}	0.84±0.002^{f}
	24	4.77±0.02^{e}	0.70±0.01^{g}	4.98±0.003^{f}	0.80±0.002^{d}
5	control	6.10±0.02^{j}	0.44±0.02^{b}	5.17±0.002^{i}	0.72±0.001^{b}
	18	4.86±0.03^{g}	0.54±0.01^{d}	5.05±0.002^{g}	0.91±0.002^{i}
	21	4.73±0.03^{d}	0.63±0.01^{f}	4.90±0.003^{d}	0.87±0.002^{h}
	24	4.63±0.01^{c}	0.71±0.02^{g}	4.78±0.000^{c}	0.82±0.002^{e}
7	control	6.03±0.00^{i}	0.45±0.01^{b}	5.05±0.003^{g}	0.74±0.003^{c}
	18	4.76±0.03^{e}	0.58±0.01^{e}	4.95±0.001^{e}	0.97±0.002^{l}
	21	4.53±0.03^{b}	0.62±0.02^{f}	4.67±0.003^{b}	0.94±0.003^{k}
	24	4.36±0.02^{a}	0.76±9,91^{h}	4.23±0.002^{a}	0.91±0.003^{j}

*Means of three replications ± standard deviation
**Means with different superscript in the same column showed significant differences ($p \leq 0.01$).
control: before incubation

The average pH value of goat milk kefir were in the range of 4.36 – 6.17, and pH value of 4.36

was found in goat milk kefir produced by addition of 7%(w/v) kefir grains. It seems that this pH value was typically the isoelectric point of protein of goat milk kefir, it was also noted by Lee and Lucey (2004) that at pH value ≤ 4.6 curd formation or coagulation will occur as a result of interaction of denatured whey protein and casein micelles which followed by lactic acid production. Motaghi *et al*. (1997) reported that kefir manufactured by addition 5% Iranian kefir grains and incubation times of 12, 24, 36, 48, 60 and 72 hours had pH values in the range of 2.98 – 4.00. While Ismaiel *et al*. (2011) reported that in their study the final pH values were in the acidic side i.e. 2.91 – 4.04. Magalhaes *et al*. (2011) reported that before incubation the pH value of Brazilian kefir was 6.61±0.02 and after 24 hours of fermentation at 25°C was 4.42±0.01.

The highest acidity number of samples was found in goat milk kefir prepared using 7% kefir grains (0.76%) and according to Anonymous (2001) minimum acidity number expressed as lactic acid content was 0.60%. While Witthuhn *et al*. (2004) noted that the low acidity number in kefir was possibly due to the microorganisms of kefir grain was too long in the log phase condition. While Motaghi *et al*. (1997) found that acidity number of their samples were in the range of 1.18 – 2.45%. Bozamic and Tratnik (2001); Cais-Sokolinska *et al.* (2008) and Abdalla and Ahmed (2010) noted that the increase in the acidity of 'mish' (Sudanese fermented dairy product) might be due to increase of production of lactic acid by lactic acid bacteria. Magalhaes *et al*. (2011) in their study found that acidity of Brazilian kefir samples after 24 hours fermentation at 25°C were in the range of 2.10 – 2.73 mg/ml.

Lactose content of goat milk kefir decrease with higher amount of kefir grains added during preparation as well as longer incubation time is shown in Table 1. The decreasing amount of lactose were as the result of fermentation. Lactose play an important role as one of nutrient for microorganisms to grow, especially for lactose fermenting microorganisms. According to Ismaiel *et al*. (2011), lactose found as the most effective nutrient for growth of kefir grains microorganisms compared to other complex polysaccharides. Furthermore, it was found that increasing of lactose concentration will also increase the amount of kefir biomass, viscosity and kefiran. Gracia-Fontan *et al*. (2006) also noted that lactose content in their study were decreased from 4.92% to 4.02% during the first 24 hours of fermentation and the pH also decrease to 4.24. The differences between lactose content of goat milk kefir samples in this study and the one reported are possibly due to goat milk quality used, kefir grains microorganisms composition, and temperature and also incubation time. According to Motaghi *et al*. (1997) the reduction of sugar during kefir fermentation was also possibly due to its incubation time.

In regards of ethanol content it was found that higher amount of kefir grains caused increasing its ethanol content. The addition of 7% (w/v) kefir grains and incubation time of 18 hours gave the highest amount of ethanol (0.97%). According to Alm (1982) that ethanol and CO_2 produced were as the result of symbiotic fermentation of yeast and lactic acid bacteria namely Lactobacilli and Streptococci species. Libudzisz and Piatkiewicz (1990) noted that kefir prepared using Polish kefir grains containing ethanol of 0.035 – 2.000%. Kuo and Lin (1999) also reported that solids, lactose and ethanol contents of three different batches of kefir prepared using 5% Taiwanese kefir grains and incubated at 21°C showed significant differences and these diferences were predicted due to variations of microorganisms in kefir grains. While Otles and Cagindi (2003) also reported that ethanol content of 0.90 g/100g sampel of their traditional kefir; and Magalhaes *et al*. (2011) reported that the final content of ethanol of Brazilian kefir after 24 h fermentation at 25°C reached about 0.5 mg/ml .

The differences of chemical characteristics of kefir produced at different location are possibly due to difference of goat milk quality which is depend on goat species, feeding management, lactation period, and processing methods include amount of kefir grains used, time and temperature of incubation as well as the of microorganisms species found in the kefir grains. However, Wszolek *et al*. (2001) noted that kefir characteristics were more affected by the milk quality rather than the starter or innoculum itself.

Conclusion

Pasteurised fresh goat milk used as raw material for kefir production had following chemical characteristic: pH 6.66 ,acidity 0.19%,fat 3.43%,protein 4.72% and lactose 4.30%. The best quality of goat milk kefir was produced by addition with 7% Indonesian kefir grains and incubated at room temperature for 24 h. It was characterized by pH 4.63; acidity 0.73%; lactose content 4.23% and ethanol content 0.92%.

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