Characteristics of fermented goat milk using combination of 
keducha and \textit{Lactobacillus casei} starters

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Abstract. Kombucha is potential for starter for milk fermentation process. This study was aimed to evaluate the effect of combination of \textit{L. casei} and kombucha starters on quality of fermented goat milk. In this study, control group (T0) used 5\% \textit{L. casei}; T1 and T2 groups used 2\% \textit{L. casei} combined with 5\% and 10\% kombucha, respectively. Data of microbiological (total of bacteria, probiotics, yeast, and acetic acid bacteria); chemical (pH, acidity, lactose, fat, protein, moisture, and fatty acid); antioxidant activity, viscosity, and sensory qualities were analysed using one-way ANOVA. The results showed that the addition 5 and 10\% of kombucha starter did not affect the total of bacteria and probiotic, but significantly affected (P<0.01) the total yeast and total acetic acid bacteria. Acidity, pH, lactose, fat, and protein were similar, whereas the linoleic acid was higher in T1 than T2 and T0. Moisture content and antioxidant activity of T2 were higher than T0 and T1. The viscosity of T1 and T2 were lower (P<0.05). Sensory acceptance of fermented milk of T0 and T1 were higher than T2. It is concluded that \textit{L. casei} combined with 5\% kombucha starters (T1) was the best proportion to ferment goat milk.

1. Introduction
Fermented milk is an acid process by the cultivation of starter culture and incubation on the optimal condition. There are many kinds of microorganisms that can be used for the milk fermentation process. Generally, groups as a milk starter are lactic acid bacteria (LAB) such as \textit{L. casei}, \textit{L. platarum}, \textit{Pediococcus sp}, etc [1]. Moreover, the alternative cultures could be used from different resources as long as they are safe for human, such as kombucha culture from tea fungus fermentation.

Kombucha is a sour slightly sparkling beverage prepared at home by fermenting sweetened black tea with a symbiotic culture which is called tea fungus. The tea fungus is a symbiosis of acetic acid bacteria (\textit{A. xylinum}, \textit{A. aceti}, \textit{A. pasteurianus}, \textit{G. oxydans}) and yeast (\textit{Saccharomyces sp.}, \textit{Zygosaccharomyces sp.}, \textit{Torulopsis sp.}, \textit{Pichia sp.}, \textit{Brettanomyces sp.}) [2,3]. Kombucha tea liquid contains acetic acid as the main fermentation product and other compounds such as ethyl-gluconate, glucoric, latic acid, oxalic acid, saccharic acid, keto-gluconic, carbonic acid as well as tea components (catechins, theaflavins, favonols) and cell metabolites (invertase, amylase, other oxidative enzymes) [4,5].

Using starter culture from kombucha tea combined with probiotic \textit{L. casei} is an alternative culture to be applied in milk fermentation technology (i.e., produced a good fermented milk product). Therefore, the objective of this study was to evaluate the effects of combination cultures between
kombucha tea and *L. casei* on the quality fermented goat milk including microbiological, phsyico-chemical and sensory quality.

### 2. Materials and methods

#### 2.1. Preparation of kombucha starter

Kombucha tea was made by Malbasa method with modification [6]. Starter kombucha was prepared by 1 l of boiled tap water with 70 g sucrose (forming 7% solution of sucrose) and 1.5 g of green tea. The tea was heated for 5 min at 100°C, tea leaves were removed by filtration and the obtained solution were cooled to room temperature. Cold tea was inoculated by 10% of fermentation broth and 2.5% of tea-celullose from previous kombucha fermentation and incubation at room temperature (±27°C) for five days.

#### 2.2. The process of fermented goat milk

Goat milk was pasturized at temperature of 80°C for 30 min and cooled at room temperature. Furthermore, cold milk was inoculated by bulk starter *L. casei* to 5% (T0) and 2% (T1 and T2) (v/v) and combined with 5% and 10% kombucha, respectively. Fermentation process was incubated at temperature 37°C for 12 hours [7].

#### 2.3. Microbiological analysis of fermented goat milk

Microbiologiscal analysis in fermented goat milk involved total bacteria, total probiotic, total acetic acid bacteria and yeast. Bacteria calculations used total plate count method (TPC) by different mediums for each bacterial types. Medium for total bacteria analysis used plate count agar (PCA) medium, a total probiotic used MRS (deMan Rogosa and Sharped) medium by added bile salt 0.15%, total acetic acid bacteria used Hestrin and Schram (HS) medium, and total yeast used malt extract agar (MEA) medium. After dilution each sampe, it was inoculated into the agar medium and incubated at temperature of 37°C for 24 h [8].

#### 2.4. Physico-chemical analysis of fermented goat milk.

Physical analysis measured was viscosity of fermented goat milk by viscometer. Chemical analysis included pH value, acidity test, lactose, Lowry’s protein, fat, fatty acid profile, water content, and antioxidant activity [9–11].

#### 2.5. Sensory analysis of fermented goat milk

Organleptic products used scoring method[9]. The number of panelist who involved in sensory test was 18 panelist. They given a scored for smell, taste, color and appearance for fermented goat milk [9].

#### 2.6. Statistical analysis

The data were analyzed stastically by one way ANOVA operated by SPSS16 while sensory quality was analyzed by Kruskals-Wallis.

### 3. Results and discussions

#### 3.1. Microbiological quality of goat milk

This research showed that the total bacteria and probiotic were not different on fermented goat milk, but it appearance in total yeast and acetic bacteria by addition on kombucha starter (Table 1).
Table 1. Microbiological quality of fermented goat milk (log cfu/ml)

| Parameter            | T0     | T1     | T2     |
|----------------------|--------|--------|--------|
| Total bacteria       | ns     | 11.32 ± 0.64 | 11.99 ± 0.54 | 11.41 ± 1.17 |
| Probiotic            | 7.68 ± 0.62 | 7.60 ± 0.19 | 8.22 ± 0.37 |
| Yeast                | 0 a    | 5.44 ± 0.43 b | 5.07 ± 0.13 b |
| Acetic acid bacteria | 0 a    | 7.95 ± 0.46 b | 9.36 ± 0.23 c |

ns not significant at the same row table (P>0.05)
abc there were significant differences at the same row table (P<0.01)

The growth of acetic acid bacteria and yeast in fermented goat milk came from starter culture which dominated in kombucha tea. Moreover, the total acetic acid bacteria by addition 10% kombucha had the highest in fermented goat milk, whereas total yeast resulted about 5 log cfu/ml as well as reported by Malbaša [12].

3.2. Physico-chemical quality of goat milk

Effect of kombucha addition on physico-chemical quality of fermented goat milk can be seen in Table 2. Level of kombucha addition in fermented goat milk were not different on pH value, acidity, lactose, fat, and protein, whereas it had significant different on moisture content, antioxidant activity and viscosity. Moisture content and antioxidant activity in fermented goat milk added with 10% kombucha starter were higher than kombucha starter 0% and 5%. Moreover, the greatest viscosity of fermented goat milk was produced by 0% kombucha starter (Table 2).

Table 2. Physico-chemical quality and antioxidant activity of fermented goat milk

| Parameter                        | T0     | T1     | T2     |
|----------------------------------|--------|--------|--------|
| pH value                         | 4.49 ± 0.06 | 4.47 ± 0.11 | 4.37 ± 0.11 |
| Acidity (%)                       | 1.09 ± 0.12 | 1.06 ± 0.04 | 1.02 ± 0.48 |
| Lactose (%)                       | 2.72 ± 0.08 | 2.86 ± 0.46 | 2.91 ± 0.24 |
| Fat (%)                           | 3.12 ± 0.44 | 2.77 ± 0.80 | 2.97 ± 0.19 |
| Moisture (%)                      | 87.79 ± 0.59 a | 88.45 ± 1.09 a | 89.81 ± 0.47 b |
| Protein (%)                       | 2.83 ± 0.68 | 2.84 ± 0.75 | 3.11 ± 0.47 |
| Antioxidant activity (% DPPH)     | 6.16 ± 2.64 a | 6.25 ± 0.93 a | 9.46 ± 1.78 b |
| Viscosity (cP)                    | 891.6 ± 355.49 a | 433.26 ± 191.23 b | 518.23 ± 0.23 b |

ns not significant at the same row table (P>0.05)
abc there were significant differences at the same row table (P<0.01)

Some studies reported that pH value of fermented milk by starter combination kombucha tea and yogurt starter was about 3.5 to 4.5 [13]. Another result from fermented milk used only kombucha starter has pH 4.4 even though its incubation time will be twice longer than lactic acid bacteria culture [14,15]. Therefore, pH value from this study was still in accordance to the previous report (Table 2).

Kombucha tea contains a high of water that the greater level of kombucha starter in fermented goat milk, it will increase significantly water contains in final product. Several studies reported that milk-based beverage fermentation by 10% kombucha tea as a starter culture has water content 89.5% and 89.1% which was similar result from this research [14,15]. This study supposed that addition kombucha starter started from 5% in products will decline viscosity. Moreover antioxidant activity was increased by 10% kombucha due to leaf tea has a great antioxidant compounds such as vitamin C, B2, polyphenol and primarily catechins [12].

Furthermore, fatty acid analysis in fermented goat milk were found eight fatty acids which were lauric acid, myristic acid, pentadecanoic acid, palmitic acid, palmitoleic acid, linoleic acid, arachidonic acid and docosahexanoic acid (data not shown). The goat milk fermentation by addition kombucha starter
10% and 5% were significantly different on palmitic acid and linoleic acid by respectively which also has the highest fatty acid.

3.3. Sensory quality of fermented goat milk
Organoleptics analysis showed that fermented goat milk by addition kombucha starter 0% and 5% had sensory acceptance higher than by 10% kombucha (data not shown). It happened due to addition kombucha starter by 10% had more watery appearance which was not preferred by almost of all panelist.

4. Conclusion
To conclude our finding, the best quality of fermented goat milk was produced by combination of 2% \textit{L. casei} and 5% kombucha starters.

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