Microbiological and organoleptic test of kefir from the balance of goat milk and cow milk with different fermentation time

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Abstract. This research aims to evaluate the process of making kefir with the correct method, as well as evaluate the type of milk that is best suited for the manufacture of kefir and the most appropriate fermentation to produce kefir based on physical, microbiological and sensory quality. The research was conducted for 3 months (October-December 2018). The draft used in this study of the complete randomized draft (RAL) consisting of two-factor 3 treatments and 3 repeats. The factor A is: Cow milk 100% (P1), goat milk 50% + cow milk is 50% (P2), and cow milk is 100% (P3). While Factor B is: fermentation 48 hours (S1), fermentation 60 hours (S2), and fermentation of 72 hours (S3). Results of the study showed the highest LAB contained in goat milk fermentation for 72 hours (8.69) hedonic value of the highest flavor found in the milk of fermented cow 72 hours (4.0889), hedonic value of the highest scent contained in the milk fermented cow 72 hours (4.4888), the highest value of hedonic texture found in fermented goat milk 72 hours (3.9778). Conclusion results of this research that is the longer fermentation time greatly affects the quality of kefir.

1. Introduction
Kefir is a fermented dairy product that has a specific flavor as the result of fermentation of lactic acid bacteria (LAB) and yeast that live together and mutually beneficial. The flavor of milk fermentation (kefir) is dominated by the acid flavor caused by the activity of lactic acid bacteria that grows in the fermentation process of kefir [1]. In Indonesia, kefir has not been popular by the public because kefir drinks are not drinks originating from Indonesia. Kefir comes from the Caucasus mountains between the Black Sea and the Caspian Sea, southwest Russia. Kefir has different names such as Kepi, Kippe, Kapov, and Kiaphir. This type of fermented milk has been widely consumed by some Asian and Scandinavian countries [2]. Goat milk as one of the raw materials of kefir has advantages over cow milk, among others, has distinctive sensory characteristics due to the scent of goat sourced from short-chain fatty acids [3,4]. It has better digestibility than cow milk because of its less fat globular [5,6]. The Kefir-based goat milk of Ettawa (PE) has the potential to be developed as a functional food in Kefir products as it does not only contain macro nutrients, but also be able to decrease significantly the lactose level of milk from 3.29 to 2.45% [7]. The decreasing in the amount of lactose indicates the role of lactic acid and yeast bacteria found in kefir seeds to produce a number of secondary metabolites that have a functional role to human health. The temperature and length of kefir storage are factors that must be considered to maintain the quality of kefir.
During the fermentation process, there is a decreasing in pH and an increasing in the amount of lactic acid produced by LAB, and the ethanol produced yeast (Khamir). Temperature, length of storage, starter and optimum conditions of fermentation will affect the physicochemical and sensory properties of kefir [8]. During storage there is a biochemical change in kefir, and storage temperature plays an important role in the biochemical process. In freezing temperatures, microorganisms in kefir are damaged, whereas in cold temperatures only changes in metabolic activity are slower than at room temperature. Therefore the purpose of this research is to study the quality of physical, chemical, microbiological, balance as well as the long time fermentation of kefir.

2. Materials and methods
The materials used in this research are goat milk, cow milk, stater kefir samples obtained from home industry in Medan. The tools used in this research include measuring cups, thermometer, kjeldhal, reaction tubes, auto clave, Soxhlet, stirrer, mug, spoon, sieve and stationery. The research method used is experimental using complete random draft (CRD) factorial pattern [9]. Factor 1: Milk type (goat milk 100%, cow milk 100%, and dairy goat milk mix 50:50); 2nd factor: Long storage (48 hours, 60 hours, and 71 hours) so that combination between 2 x 3 factors and each treatment is repeated 3 times, each replay using 100 ml kefir.

2.1. The variables of interest

2.1.1. Sensory test. Kefir sensory testing was carried out by 15 semi-trained student panelists. Kefir samples of 50 ml are served in glass cups and randomly coded with three-digit numbers. The number of samples tested as many as 27 samples and panelists tested with the scoring method for texture, flavour and aroma attributes [10]. For sensory test scores is available in table 1 [11].

| Table 1. Score test of texture, flavour and scent. |
|-----------------------------------------------|
| **Scent test**                        |
| Very acidic                              | 5 |
| Acidic                                   | 4 |
| Kefir scent                              | 3 |
| Smells a bit                             | 2 |
| Smells                                   | 1 |
| **Flavour test**                        |
| Very acidic                              | 5 |
| Acidic                                   | 4 |
| A little acidic                          | 3 |
| Rather acidic                            | 2 |
| Not acidic                               | 1 |
| **Texture Test**                        |
| Very smooth                              | 5 |
| Smooth                                   | 4 |
| Rather rough                             | 3 |
| Rough                                    | 2 |
| Rather rough                             | 1 |

2.1.2. The total test of lactic acid bacteria. In total bacterial testing conducted based on the test of the number of bacterial starter according to [12], taken 1 ml of the sample solution inserted in the reaction tube containing the aquades 9 ml then homogenized using a vortex (dilution 10-1). Dilution is carried out until the dilution factor of 10-5. From dilution is each taken 1 ml from the dilution level.
10-3 to 10-5 and poured into the petri dish sterile duplo. Then poured 12 ml to 15 ml of the still liquid MRS media into each petri dish. The petri dish is carefully shaken until the sample and the seeding medium are mixed evenly and compacted. Blanco examination by mixing the thinning water for each examined sample, then allow it until compact. Furthermore, all Petri bowls are inserted with a reverse position into the incubator at a temperature of 37 °C for 3 days. Colony growth in each petri dish containing 30 colonies to 300 colonies is recorded after 3 days. The total test process is done by taking 1 ml of the sample, then diluted in aquades and done by spreading method plated above YGC media (yeast Extract Glucose chloramphenicol for FIL-IDF). Petri bowls are incubated at 25 °c for 5 days. Then the number of growing colonies are calculated using the Total Plate Count method and expressed in units of CFU/ml or log cfu/ml. The Data obtained will be analyzed using a variation (Anova).

2. Research implementation

2.2. Equipment preparation. such as a spatula is sterilized by skipping the appliance over the fire Bunsen until glowing. The tools are widely used by the use of ovens such as Petri bowls, beaker cups, elemeyer and test tubes at 170oC for 2 hours. The autoclave is a precision tool such as a measuring cup, a volumetric pipette, at a temperature of 121oC for 15 minutes [13].

2.2.2. Making kefir culture. One sample using milk as much as 250 ml has been sterilized, then added to the starter kefir as much as 3 G. Then fermented during 48, 60 and 72 hours. Storage is done in containers that are tightly sealed (airtight). After storage is done stirring sufficiently. Then kefir starters are filtered and separated from the new kefir. Starter kefir and results are inserted into closed containers and stored before using.

2.2.3. Making kefir. Kefir making in this research is do by traditional methods [14]. Before making Kefir, first done sterilization tools used in the process of making Kefir. The making of Kefir begins by inserting 500 ml of milk on each sterile jar. The milk jar is then inserted into the plastic for easy retrieval after pasteurization process. Then pasteurized milk is about 80-90 °c for 15 minutes. Then the milk is cooled by temperature of 18-22 ° C.

The next step is inoculation. The kefir Starter is inserted into each of the milk jars according to the treatment, then labeled the treatment on each of the milk jars. Subsequently incubated at room temperature for 48 hours, 60 hours and 72 hours according to the treatment. After that it is done filtering to separate kefir and kefir seeds. Kefir obtained from the screening is taken for quality test, namely physical test (scent, flavor and texture) and chemical test (lactic acid levels, fat content and protein levels). The step is done again for the next replay.

3. Results and discussion

3.1. Total lactid acid bacteria

The results of various analyses showed that the type of milk, length of fermentation and its interactions gave a noticeable effect (P < 0.05) to BAL kefir, as can be seen in table 2. Based on table 2 it is known that the lowest rate of LAB content for all types of milk is in fermentation for 48 hours, and the highest LAB value is in fermentation for 72 hours. During Fermentation time 48, 60 and 72 hours showed a good increasing it was on kefir using cow milk, goat milk and a mixture of both types of milk. The results also showed that the content of LAB in goat milk kefir and cow milk between 8.61 to 8.69 where based on the standard Codex for fermented milk, kefir minimum containing LAB IE 7 log CFU mL-1. The number of lactic acid bacteria on all treatments is a good limit for consumption in a probiotic drink product that ranges between 107-109 CFU/ml. The minimum concentration of probiotic bacteria that is effective when consumed is 107 cfu/ml, it is to anticipate the occurrence of the decline during storage and where the Kefir sample fully meets the criteria of the specified LAB count [2,8]. In the results of the study also showed that the longer
fermentation time then the content of LAB on kefir will be increased. An increasingly long-lasting accelerator then the breeding of LAB will be increasing and causing the resulting lipase enzyme more and more so that hydrolyzed fat is also more and more, resulting in a decrease in fat levels [15].

Table 2. Total lactic acid bacteria kefir milk of goats and dairy cows on different fermentation periods.

| Counter Balance | Fermentation Duration (hour) | Average |
|-----------------|-----------------------------|---------|
|                 | 48                          | 60      | 72      |
| Cow milk        | 8.61                        | 8.63    | 8.65    |
| Goat and cow milk| 8.61                       | 8.63    | 8.66    |
| Goat milk       | 8.63                        | 8.66    | 8.69    |
| Average         | 8.62A                       | 8.64AB  | 8.66B   |

Description: * Superscript with uppercase letters in the column shows different very real (P < 0.01).

3.2. Hedonic flavour value

The results of various analyses showed that the type of milk, length of fermentation and its interactions provided a noticeable effect (P < 0.05) on the value of the hedonic flavors of kefir, as can be seen in table 3.

Table 3. The value of the scale of the hedonic flavor kefir milk of goat and cow’s milk in different fermentation periods.

| Counter Balance | Fermentation Duration (hour) | Average |
|-----------------|-----------------------------|---------|
|                 | 48                          | 60      | 72      |
| Cow milk        | 3.6667b                     | 3.8222b | 4.0889c |
| Goat and cow milk| 3.7778b                    | 3.7778b | 3.8000b |
| Goat milk       | 3.4889a                     | 3.7333b | 3.8000b |
| Average         | 3.6444                      | 3.7778  | 3.8963  |

Description: * Superscript different lowercase letters show different real (P < 0.05).

Based on table 3 indicates that the value of the rate Hedonic goat milk kefir, cow milk and the mixture of both types of milk against the fermentation of the first highest is kefir cow milk, the second is kefir mixed goat and cow milk, the third is goat milk. So it can be concluded that the value of the average hedonic flavor of all kefir types have acid creteria in both cow milk and goat milk and a mixture of both kefir. Based on the results The study showed that kefir with cow milk and goat milk with different fermentation periods showed real influential results (P. < 0.05) to the value of the hedonic flavor. Interactions between different types of milk and other fermentation periods also show significant results (P. < 0.05) against hedonic taste values. The grading attribute of kefir by panelist has the highest average score on fermentation for 72 hours with a score of 4.0889, with acid creteria. The assessment made by panelist showed that the kefir that was fermented during the study resulted in sour taste, the sour flavor sharpness will produce a princkly flavor where the flavor is produced by the presence of carbon dioxide by yeast that produces a distinctive flavor of kefir [16].

3.3. Hedonic scent value

The results of various analyses showed that the type of milk, length of fermentation and its interactions gave a noticeable effect (P < 0.05) to the value of the hedonic aroma in kefir, as can be seen in table 4.
Table 4. The hedonic scale value of kefir milk in goat and cow milk in different fermentation periods.

| Counter Balance | Fermentation Duration (hour) | Average  |
|-----------------|-----------------------------|----------|
|                 | 48     | 60     | 72     |          |
| Cow milk        | 3.4444b | 3.7778cd | 4.4888e | 3.9037  |
| Goat and cow milk| 3.4888b | 3.6444cd | 4.2888e | 3.8074  |
| Goat milk       | 3.1333a | 3.6000bc | 3.8444d | 3.5259  |
| Average         | 3.3556 | 3.6741 | 4.2074 |

Description: * Superscript different lowercase letters show different real (P < 0.05).

Table 4 shows that the value of the smell of kefir milk for goat, cow milk and the mixture of both types of milk against the fermentation of the first highest is kefir cow milk, the second is kefir milk mixture of goat and cow, the third is goat milk. So that it can be concluded that the value of scent Test average of all types of kefir has acid criteria in both the milk kefir cow and goat milk and mixture of both kefir type. The value of hedonic scent is real effect on the type of milk and the length of fermentation then the interaction where kefir that has the highest value is kefir cow milk with a long period of fermentation for 72 hours is a score of 4.4888, with acid scent criteria. Based on that data, it is known that the highest value of hedonic aroma for all types of milk is in fermentation for 72 hours, and the lowest hedonic value of scent is in fermentation for 48 hours, it is also known that the longer the fermentation time then the scent is increasingly changed followed by the increasingly high acid flavor. The assessment of kefir scent has the highest average score in fermentation for 72 hours with cow’s milk kefir with a score of 4.4888, with an acidic scent criterion. Some factors that affect the formation of a distinctive scent kefir, fermented acid and ethanol [17].

3.4. Hedonic texture value
The results of various analyses showed that the type of milk, length of fermentation and its interactions gave a noticeable effect (P < 0.05) of the hedonic texture of kefir, as can be seen in table 5.

Table 5. The hedonic scale value of kefir texture of goat milk and cow milk at different fermentation periods.

| Counter Balance | Fermentation Duration (hour) | Average |
|-----------------|-----------------------------|---------|
|                 | 48     | 60     | 72     |         |
| Cow milk        | 3.4889a | 3.6444b | 3.6667b | 3.6000  |
| Goat and cow milk| 3.5111a | 3.7333b | 3.8444c | 3.6963  |
| Goat Milk       | 3.6667b | 3.7333b | 3.9778d | 3.7926  |
| Average         | 3.5556 | 3.7037 | 3.8296 |

Description: * Superscript different lowercase letters show different real (P < 0.05).

Table 5 shows that the hedonic value of the kefir goat milk scent, cow milk and the mixture of both types of milk against the fermentation of the first highest of the goat milk kefir, the second is kefir the milk mixture of goats and cows, the third is cow milk. So that it can be concluded that the value of a texture test of all kefir types have a subtle criteria either to the milk kefir cow and goat milk and a mixture of both kefir. The assessment of kefir texture has the highest score value in fermentation for 72 hours using goat milk kefir with a score of 3.9778 with fine texture criteria. Where the texture of the fermented product results is influenced by viscosity, which relates to the Exopolysaccharide product produced by the starter culture. Some strains of Streptococcus thermophilus are able to produce high molecular weight polysaccharides [8]. Some researchers state that the texture of
fermentation products are influenced by the length of storage those are assessed in sensory. Lactic acid bacteria such as Lactobacillus Bulgaricus and Streptococcus thermophilus contribute to composition, texture and sensory [18].

4. Conclusions
Kefir with goat milk is better than cow Kefir is reviewed from microbiological and sensory quality. With fermentation for 48 hours able to produce a quality kefir. The type of milk and the length of fermentation used to influence the quality of LAB and sensory trials. Where kefir goat milk with fermentation for 48 hours is the best and preferred by panelists.

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