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Physicochemical, Microbiology, and Sensory Characterization of Goat Milk Kefir in Various Incubation Time

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ABSTRACT

This study aimed to analyze the characteristics of goat milk kefir based on the incubation time variations on physicochemical, microbial and organoleptic. The method used was an experimental method using a Completely Randomized Design with 3 various incubation time which were for 12 hours, 18 hours and 24 hours with five replications. Parameters measured were physicochemical characteristics (pH, lactic acid concentration, and ethanol content), microbial characteristics which as the total number of lactic acid bacteria (LAB) colonies and the organoleptic characteristics were color, aroma, taste and favorability level towards goat milk kefir. Physicochemical and microbial data were analyzed by Analysis of Variance (ANOVA) while the organoleptic data were analyzed descriptively. The results showed that the incubation time gave significantly difference effect ($P < 0.05$) on the pH, lactic acid concentration, the total number of LAB goat milk kefir and did not significant effect on the ethanol content. The organoleptic results showed that the incubation time didn't significant effect on color and aroma, however gave significant affects on the tastes and favorability towards goat milk kefir. It can be concluded that the best incubation time was 24 hours. The physicochemical characteristics showed the pH value at 4.16 ± 0.089 ; lactic acid concentration at $0.24 \pm 0.039\%$; ethanol content at $0.75 \pm 0.044\%$. The microbiology characteristics, the total number of Lactic Acid Bacteria at $1.24 \times 10^7 \pm 0.008$ CFU/ml. The characteristic of organoleptic color at 3.95 (white); aroma at 4.10 (kefir scent); taste at 4.25 (poor acid) and favorability at 4.15 (like).

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Introduction

Fermented milk is a processed product made by controlling the fermentation time to produce the desired acid and flavor (Ali, 2011). One of the fermentation products is kefir. Kefir is a fermented product produced by adding kefir grains to milk. Chen *et al.* (2005) stated that microorganisms contained in the kefir grains were *Lactobacilli* strains such as *L. brevis*, *L. cellobiosus*, *L. acidophilus*, *L. casei*, *lactococci* (including *Lc. Lactis subspecies*), *Streptococcus salivarius* ssp. *thermophilus*, *Leuconostoc mesenteroides* and *L. cremoris* and several types of fungi, such as *Kluyveromyces*, *Candida*, *Torulopsis*, and *Saccharomyces* sp.

The fermentation process caused an increase of microbial activity, a decrease in pH, and an increase of acidity in fermentation products (Afriani, 2010). The standard of goat milk kefir according to Chen *et al.* (2005) were consisted of the chemical contents of goat milk kefir were $2.96 \pm 0.00\%$ crude protein; $3.30 \pm 0.02\%$ crude fat, $2.45 \pm 0.58\%$ lactose; 1.52 ± 0.76 g/L L-Lactic acid,

1.44 ± 0.01 g/L D-lactic acid, 1.18 ± 0.01 g/L ethanol. Pogacic *et al.* (2013) found that kefir stored in cold temperatures for three days has a pH value of 4.2-4.7, lactic acid of 0.8-1.2%, ethanol of 0.5-0.7% and about 0.20% CO₂.

The characteristics of kefir are strongly influenced by the type of microbial starters, temperature, duration of fermentation, and raw materials added in the fermentation process (Haryadi *et al.*, 2013).

Materials and Methods

Goat milk kefir production

The goat milk in this study was obtained from Majene farms, Sulawesi Barat. Goat milk was pasteurized at 105°C for 5 minutes and then added with 2% kefir grains that were previously identified for the lactic acid bacteria population, which were *Lactococcus cremoris*, *Streptococcus cremoris*, *Lactobacillus plantarium* and the identified yeast was *Saccharomyces cerevisiae*. Goat milk that has been added with kefir grain

was then incubated at 37°C for 12 hours; 18 hours and 24 hours.

Kefir physicochemical quality measurement

Kefir which has been incubated at 37°C for 12 hours, 18 hours and 24 hours then measured for the pH value, lactic acid concentration, ethanol content, and weight grain kefir. The measurement of kefir pH refers to the method of Purnomo and Muslimin (2012) using a pH meter [Hanna], the percentage of lactic acid (Purnomo and Muslimin, 2012), and ethanol content (Yulianti, 2014).

Enumeration of lactic acid bacteria

The enumeration of total lactic acid bacteria population was based on the Total Plate Count (TPC) method and MRSA media (de man, rogosa sharpe agar) [Merck] medium. Kefir samples (1 mL) were transferred into 9 ml of sterile distilled water to make a serial dilution up to 10⁶, each sample was homogenized by vortex. Each dilution of 10⁵ and 10⁶ was pour-plated with MRSA media (0.1 mL). The plates were incubated at 37°C for 24 hours. The plate that showed 25-250 colonies was selected to calculate the total number of lactic acid bacteria content (Swanson *et al.*, 1992) with the following formula:

$$\frac{\text{colony}}{\text{gram} \left(\frac{\text{cfu}}{\text{ml}} \right)} = \text{the total number of colony} \times \frac{1}{\text{dilution factor}}$$

The sensory test

The sensory test in this study are the color formation, aroma, taste, and hedonic test. The sensory consist of 20 semi-trained panelists. Indicators of color, aroma, taste and favorability assessment can be seen in Table 1.

Data analysis

This study used a completely randomized single factor design with three incubation times which were for 12 hours, 18 hours and 24 hours, in five replications. Analysis of variance (ANOVA) analyzed pH data; lactic acid concentration; ethanol contents; weight gain of kefir grain; and the total of lactic acid bacteria and followed by the least significant difference (LSD) test (Steel and Torrie, 1993). Obtained the sensory data were

analyzed descriptively. All of the data were processed by using SPSS 19.0 for Windows and MS Office Excel 2007 software.

Result and Discussion

The pH value of goat milk kefir

The pH value of goat milk kefir was ranged at 4.16-5.40 (Table 2). The highest pH value was obtained after 12 hours of incubation which reached 5.40 while the lowest pH was obtained after 24 hours of incubation time. The result of analysis of variance showed that the incubation time has a significant effect ($P < 0.05$) on the goat milk kefir pH. Purnomo and Muslimin (2012) reported that the pH of goat milk kefir with the addition of 3% kefir grains (w/w) decreased along with 0 hour of incubation time (control), the pH was 6.17±0.01; at 18 hours of incubation the pH was 4.91±0.01; at 21 hours of incubation the pH was 4.83±0.02; and at 24 hours of incubation the pH was 4.77±0.02 respectively. Lengkey and Balia (2014) reported that kefir pH value with the addition of 5% kefir grains with an incubation time of 8 hours was 4.6; 16 hours was 4.5 and 24 hours was 4.3. According to Adesokan *et al.* (2011) the pH value was strongly related to the produced acid content. Increased acidity levels and decreased pH in milk fermentation with the lactic acid bacteria culture addition were seen during 24 hours incubation. Suriasih *et al.* (2012), pH of cow's milk kefir with 24-hour incubation was 4.35±0.08; 48 hours was 3.82±0.06 and 72 hours was 3.38±0.10. Motaghi *et al.* (1997), the pH value generated on cow's milk kefir with the addition of 5% kefir grains and added with 1% sucrose at 12 hours was 4.0, 24 hours was 3.89, 36 hours incubation time was 3.01, 48 hours was 3.0, 60 hours was 2.98 and 72 hours was 2.98.

The lactic acid concentration of goat milk kefir

The percentage of lactic acid goat milk kefir in the research was can be seen in Table 3. The percentage value of lactic acid goat milk kefir obtained in the range of 0.14-0.24%. The highest lactic acid concentration was found after 24 hours of incubation was 0.24% and the lowest

Table 1. Color, aroma, taste, and favorability

The organoleptic parameters	Scale				
	1	2	3	4	5
Color	Very yellow	Yellow	Slightly yellow	white	Very white
aroma	no kefir scent	little kefir scent	Slightly kefir scent	kefir scent	Highly kefir scent
Taste	Very acid	Acid	Slightly acid	poor acid	Not acid
likes	Very dislike	Dislike	Slightly like	Like	Very like

Table 2. The pH value of goat milk kefir obtained after various incubation time

Replications	Incubation (hours)		
	12	18	24
1	5.50	5.00	4.10
2	5.30	5.20	4.30
3	5.40	5.10	4.20
4	5.50	5.00	4.10
5	5.30	5.20	4.10
Mean	5.40±0.100 ^a	5.10±0.100 ^b	4.16±0.089 ^c

^{ab}) superscript format showed the significant differences ($P < 0.05$).

percentage value of lactic acid at 12 hours incubation time was 0.14. The analysis of the incubation time showed a significant effect ($P < 0.05$) on the percentage of lactic acid. Purnomo and Muslimin (2012), reported that the acidity of goat milk kefir on the addition of kefir grains 3% (w/w), the increase acidity based incubation time, at the incubation time of 0 hours was $0.41 \pm 0.02\%$; 18 hours was 0.51 ± 0.1 ; 21 hours was 0.61 ± 0.01 and 24 hours was 0.70 ± 0.01 while the pH value obtained was 6.17 ± 0.01 ; 18 hours was 4.91 ± 0.01 ; 21 hours was 4.83 ± 0.02 ; and 24 hours was 4.77 ± 0.02 . Lengkey and Balia (2014) described that production of lactic acid after 5% kefir grains addition with an incubation time of 8 hours was 0.37%; 16 hours was 1.10%, and 24 hours was 1.24%. Suriasih *et al.* (2012) stated that the acidity level of cow's milk kefir at a concentration of 5% (w/v) with an incubation time for 24 hours was 0.89 ± 0.03 ; for 48 hours was 1.28 ± 0.04 and 72 hours of incubation time was 1.73 ± 0.02 . Motaghi *et al.* (1997) reported that the acidity (g/100 g) produced in cow's milk kefir containing 5% kefir grains, and 1% sucrose after 12 hours of incubation was 1.18, 24 hours was 1.47, 36 hours was 2.35, 48 hours was 2.40, 60 hours was 2.41 and 72 hours was 2.45.

The ethanol content of goat milk kefir

The obtained ethanol content in goat milk kefir was between 0.69-0.75% (Table 4). The highest ethanol content was obtained after 24 hours of incubation was 0.75% and the lowest ethanol content after 12 hours of incubation time was 0.69%. The results of analysis of variance showed that the incubation time did not significant effect ($P > 0.05$) on the produced ethanol content. The results were different from the results by Yusriyah and Agustini (2014), where the alcohol content had decreased in line with the time of fermentation process, where kefir with 1% alcohol after 24 hours fermentation was 10.404 mg/ml, 48 hours fermentation time was 7.913 mg/ml, and fermentation time of 72 hours was 6.777 mg/ml. The decrease of alcohol content due to the high

total acid content of *Lactobacillus bulgaricus* will inhibit the growth of microorganisms to ferment, including *Candida* yeast. Thus *Candida* kefir could not break down the substrate at the beginning of fermentation which causes a decrease in alcohol content starting at fermentation after 24 hours to 72 hours. In contrast (Purnomo and Muslimin, 2012), it was reported that ethanol content of goat milk kefir was decreased after 24 hours incubation compared to ethanol content obtained by control (0 hours incubation time), while the incubation time of 18 hours, 21 hours and 24 hours respectively were $0.60 \pm 0.03\%$; $0.86 \pm 0.02\%$; $0.84 \pm 0.02\%$ and $0.80 \pm 0.02\%$ in addition of 3% kefir grains (w/w). Chen *et al.* (2005) reported that the ethanol content of goat milk kefir was 1.18 g/L. yeast was the critical role in producing alcohol during the kefir production. The alcohol content was influenced by the type and number of culture starter; fermentation time; temperature; and type of raw material used. Motaghi *et al.* (1997), alcohol (% w/w) produced on cow's milk kefir with 5% kefir grains, 1% sucrose after 12 hours incubation time was 0.10, 24 hours was 0.15, 36 hours was 0.15, 48 hours was 0.18, 60 hours was 0.20 and 72 hours was 0.20.

Total lactic acid bacteria (LAB)

The obtained total lactic acid bacteria (LAB) obtained was $1.24-1.74 \times 10^{-7}$ CFU/ml (Table 5). The highest total LAB obtained after 18 hours of incubation time and the lowest total lactic acid bacteria was after 24 hours of incubation time. It was shown that after 24 hours of incubation, there was a decrease in total lactic acid bacteria (1.24×10^{-7} CFU/ml). It indicates that the number of LAB colonies after 24 hours of incubation was decreased was due to acid content increased that causes a decrease in LAB growth. The results of variance analysis showed a significant effect ($P < 0.05$) on the incubation time on the total LAB. The results of Chen *et al.* (2005) research on goat milk kefir with 3-5% inoculation level of Taiwan kefir grains incubated at 15°C; 17.5°C; 20°C and 22.5°C for 20 hours showed the number of

Table 3. The lactic acid concentration of goat milk kefir on different incubation times

Replications	Incubation (hours)		
	12	18	24
1	0.14	0.19	0.20
2	0.14	0.17	0.25
3	0.14	0.16	0.26
4	0.13	0.18	0.28
5	0.15	0.17	0.19
Mean	0.14 ± 0.007^a	0.17 ± 0.011^a	0.24 ± 0.039^b

^{ab}) superscript format showed significant differences ($P < 0.05$).

Table 4. The ethanol (%) of goat milk kefir on different incubation times

Replications	Incubation (hours)		
	12	18	24
1	0.69	0.75	0.83
2	0.69	0.69	0.73
3	0.69	0.72	0.75
4	0.68	0.70	0.73
5	0.68	0.65	0.72
Mean	0.69 ± 0.005^a	0.70 ± 0.037^a	0.75 ± 0.044^a

^{ab}) superscript format showed significant differences ($P < 0.05$).

Table 5. The total number of lactic acid bacteria (CFU/ml) (10^{-7}) of goat milk kefir on different incubation times

Replications	Incubation (hours)		
	12	18	24
1	1.65	1.75	1.24
2	1.64	1.74	1.25
3	1.63	1.73	1.23
4	1.65	1.73	1.24
5	1.64	1.74	1.25
Mean	1.64±0.008 ^a	1.74±0.008 ^b	1.24±0.008 ^c

^{ab}) superscript format showed significant differences ($P < 0.05$).

microflora was 10^6 - 10^7 CFU/ml. Safitri and Swarastuti (2011) reported that the total microorganism of skim milk kefir at 2.5% kefir grains concentration increase the total microbes in line with the fermentation duration of 8 hours, 16 hours, and 24 hours were 5.92; 6.21 and 6.70 (log CFU/g) respectively. Suriasih *et al.* (2012) noted that the total LAB in kefir was 10^8 - 10^9 CFU/ml. Arbangi *et al.* (2014) showed that the total microbes in goat milk kefir were increased after 8 hours of fermentation (9.1 ± 0.9) and fermentation time (9.5 ± 1.3). However, the total microbe was decreased also of 5% kefir grains after 8 hours incubation (9.0 ± 0.0) and 16 hours incubation (8.8 ± 1.2). Suriasih *et al.* (2012) reported that the period of incubation time has an effect on total lactic acid bacteria found in kefir that could increase a total lactic acid bacteria in line with the length of incubation. The total lactic acid bacteria at 5% kefir grains concentration (w/v) with 24 hours incubation time was 9.51 ± 0.18 , 48 hours was 9.31 ± 0.09 , and 72 hours was 8.99 ± 0.20 . The total LAB contained in kefir grains was 8.5×10^8 and 1.5×10^5 CFU/ml (Witthuhn *et al.*, 2004; Arslan, 2015).

The organoleptic test

The results of the organoleptic test obtained (Figure 1) showed that the kefir color has no difference compared to various kefir grains level and incubation times. The results showed that the kefir color was 3.95-4.0 (white). This

indicates that the duration of incubation does not affect the color of kefir produced, it was because, during the process of kefir production, there were no additional ingredients added which could change the color of kefir. The aroma of kefir was 3.50-4.10 (kefir scents). The results of this study indicated that the aroma of kefir was not influenced by the incubation times, but it has an effect towards the taste and the level of kefir fondness that produced. The taste of kefir based on incubation period was 4.10 (poor acid)-4.90 (not acid). This showed that the longer incubation has an effect on the sour taste produced. The level of favorability value obtained based on the incubation time was 3.50-4.15 (like), which considered to be favored. Kesenkas *et al.* (2011) stated that yeast has an important role in producing ethanol and carbon dioxide (CO_2) at the end of fermentation that results in a unique kefir flavor that was different from other fermentation products. Simova *et al.* (2002) reported that the activity of *K. Marxianus* in the production of cow's milk kefir was to utilize lactose to produce alcohol, flavors, and yeast-like aroma. The longer fermentation time decreases the level of preference for the smell and taste of goat milk kefir, where at 8 hours fermentation, the goat milk produces the most preferred taste and smell (Purbasari *et al.*, 2013). Tratnik *et al.* (2006) concluded that the acidity affects the sensory decline in kefir.

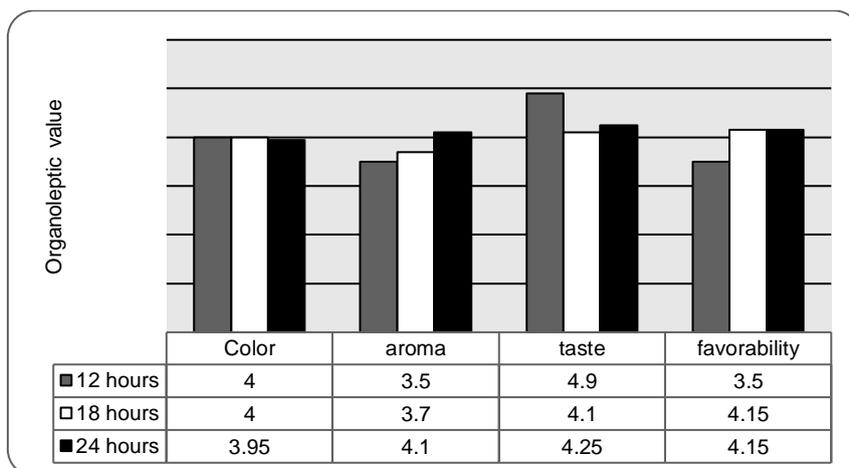


Figure 1. The organoleptic characteristics of goat milk kefir obtained at incubation time. The organoleptic description: **color** : (1) very yellow, (2) yellow, (3) slightly yellow, (4) white, (5) very white. **Aroma**: (1) has no kefir scent, (2) little kefir scent, (3) slightly kefir scent, (4) kefir scent, (5) highly kefir scent. **Taste**: (1) very acid (2) acid (3) slightly acid (4) poor acid (5) not acid. **Favorability**: (1) very dislike, (2) dislike, (3) slightly like, (4) like, (5) very like.

Conclusions

The standards according to the Codex Standard for Fermented Milks CODEX STAN 243-2003 kefir contents at least minimal 2.7% m/m protein, minimal <10% m/m fat, acidity at least minimal 0.6%, 10^7 CFU/g total microbes and the total yeast at least 10^4 cfu/g. The best goat milk characteristics of kefir was obtained after 24 hours of incubation time, with physicochemical and microbial characteristics were 4.16 ± 0.089 pH; $0.24 \pm 0.039\%$ lactic acid; $0.75 \pm 0.044\%$ ethanol; $1.24 \times 10^7 \pm 0.008$ CFU/ml total LAB; and the sensory characteristics value were 3.95 point for color (white); 4.10 points for aroma (kefir scent); 4.25 points for taste (poor acid) and 4.15 points (like) for favorability.

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References

- Adesokan, I. A., B. B. Odetoyinbo, Y. A. Ekanola, R. E. Avanrenren, and S. Fakorede. 2011. Roduction of Nigerian nono using lactic starter cultures. *Pakistan J. Nutrit.* 10: 203-207.
- Afriani. 2010. Pengaruh penggunaan starter bakteri asam laktat *Lactobacillus plantarum* dan *Lactobacillus fermentum* terhadap total bakteri asam laktat, kadar asam dan nilai pH dadih susu sapi. *Jurnal Ilmiah Ilmu-Ilmu Peternakan* 13: 279-285.
- Ali, A. 2011. Isolation and identification of lactic acid bacteria isolated from traditional drinking yoghurt in Khartoum State, Sudan. *Current Research in Bacteriology* 4: 16-22. <http://doi:10.3923/crb.2011.16.22>.
- Arbangi, Z., T. Setyawardani, and M. Sulistyowati. 2014. Jumlah bakteri asam laktat (bal), mikroba, dan kadar air kefir susu kambing dengan konsentrasi biji kefir dan waktu fermentasi berbeda. *Jurnal Ilmiah Peternakan* 2: 87-93.
- Arslan, S. 2015. A review: chemical, microbiological and nutritional characteristics of kefir. *CyTA-Journal of Food*. 13: 340-345. <http://doi:10.1080/19476337.2014.981588>.
- Chen, M. J., J. R. Liu, C. W. Lin, and Y. Y. Tzu. 2005. Study of the microbial and chemical properties of goat milk kefir produced by inoculation with Taiwanese Kefir grain. *Asian-Aust. J. Anim. Sci.* 18: 711-715.
- Haryadi, Nurliana, and Sugito. 2013. Nilai pH dan jumlah bakteri asam laktat kefir susu kambing setelah difermentasi dengan penambahan gula dengan lama inkubasi yang berbeda. *Jurnal Medika Veterinaria* 7: 4-7.
- Kesekas, H., N. Dinkci, K. Seckin, O. Kinik, and S. Gonc. 2011. Antioxidant properties of kefir produced from different cow and soy milk mixtures. *Journal of Agricultural Sciences*. 17: 253-259.
- Lengkey, H. A. W. and R. L. Balia. 2014. The effect starter dosage and fermentation time on pH and Lactic acid production. *Biotechnology in Animal Husbandry* 30: 339-347. <http://doi:10.2298/BAH1402339L>.
- Motaghi, M., M. Mazaheri, N. Moazami, A. Farkhondeh, M. H. Fooladi, and E. M. Goltapeh. 1997. Short communication: kefir production in Iran. *World Journal of Microbiology and Biotechnology* 13: 589-581.
- Pogacic, T., S. Sinko, S. Zamberlin, and D. Samazija. 2013. Microbiota of Kefir Grains. *Mljekarstvo* 63: 3-14.
- Purbasari, N. A., D. R. Hantoro, and S. Wasito. 2013. Pengaruh konsentrasi biji kefir dan waktu fermentasi terhadap viskositas dan penilaian organoleptik kefir susu kambing. *Jurnal Ilmiah Peternakan* 1: 1021-1029.
- Purnomo, H. and L. D. Muslimin. 2012. Chemical characteristics of pasteurized goat milk and goat milk kefir prepared using a different amount of Indonesian kefir grains and incubation times. *International Food Research Journal*. 19: 791-794.
- Safitri, M. F. and A. Swarastuti. 2011. Kualitas kefir berdasarkan konsentrasi kefir grain. *Jurnal Aplikasi Teknologi Pangan* 2: 87-92.
- Simova, E., D. Beshkova, A. Angelov, Ts. Hristozova, G. Fregova, and Z. Spasov. 2002. Lactic acid bacteria and yeasts in kefir grains and kefir made from them. *J. Indust. Microbiol. Biotechnol.* 28: 1-6.
- Steel, R. G. D. and J. H. Torrie. 1993. Prinsip dan Prosedur Statistika: Suatu Pendekatan Biometric. Alih Bahasa: B. Sumantri. Gramedia Pustaka Utama, Jakarta.
- Suriasih, K., W. R. Aryanta, G. Mahardika, and N. M. Astawa. 2012. Microbiological and chemical properties of kefir made of Bali cattle milk. *Food Science and Quality Management* 6: 12-22.
- Swanson, K. M. J., F.F. Busta, E. H. Peterson, and M. Jonhson. 1992. Colony Count Methods: In Compendium of Methods for The Microbiology Examination of Foods. 3rd. Edited by C. Vanderzant, D.F. Splittsoesser. Compiled by the APHA Technical Commite on Microbiology Methods for Foods.
- Tratnik, I., R. Bozanic, Z. Herceg, and I. D. A. Drgalic. 2006. The quality of plain and supplemented kefir from goats and cows milk. *Int. J. Dairy Technol.* 59: 40-46.
- Witthuhn, R. C., T. Schoeman, and T. J. Britz. 2004. Isolation and characterization of the

- microbial population of different South African Kefir Grains. *Int. J. Dairy Technol.* 57: 33-37. <http://doi:10.1111/j.471-0307.2004.00126.x>.
- Yulianti, C. H. 2014. Uji beda kadar alkohol pada tape beras, ketan hitam dan singkong. *Jurnal Teknik* 6: 531-536.
- Yusriyah, N. H. dan R. Agustini. 2014. Pengaruh waktu fermentasi dan konsentrasi bibit kefir terhadap mutu kefir susu sapi. *UNESA Journal of Chemistry.* 3: 53-57.