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Characteristics of Goat Milk Kefir with Addition of Red Yeast Rice Extract During Storage

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ABSTRACT

Kefir is one of milk products, produced by the addition of bacteria and yeast-containing kefir starter. This study aimed to evaluate the microbial characteristic, antibacterial activity, chemical and physical characteristics, antioxidant activity, and organoleptic characteristic of goat milk kefir which treated with *angkak* (red yeast rice) extract supplementation during storage. The study was initiated with milling to produce *angkak* flour, which then was diluted on sterilized distilled water on 1:2 ratio. Kefir were obtained by fermenting goat milk using 3% kefir grain for 18 hours at room temperature. Different treatments on the study were *angkak* extract supplementations on level of 0; 2; 4; and 6% for three different storage periods, i.e., 0, 7, and 14 days. The parameter of microbial characteristics observed on this study were total of lactic acid bacteria, TPC, total yeast, and antibacterial activity. Chemical tests performed on this study included the value of acidity, pH value, alcohol content, and water content. Meanwhile, the physical evaluations included viscosity, color test (brightness value, red value, and yellow value), and followed with antioxidant activity test on DPPH method and organoleptic test. Data from chemical, microbial, and physical evaluation were analyzed on one way ANOVA for these following parameters: total count of lactic acid bacteria, TPC, antibacterial activity, value of acidity, pH value, alcohol content, water content, viscosity, brightness value, red value, yellow value, antioxidant activity, and sensory values (alcoholic taste and acceptability). The analysis were further continued on two way ANOVA for total count of lactic acid bacteria, TPC, antibacterial activity on E. coli and S. aureus, alcohol content, viscosity, brightness value, red value, yellow value, and antioxidant activity. Meanwhile, the organoleptic characteristics were analyzed on non-parametric Kruskal Wallis, followed by Duncan's multiple range test (DMRT). Data were analyzed on computer program of Software Statistical Product and Service Solution (SPSS) version 18 for windows. The study revealed that *angkak* extract supplementation on goat milk kefir during 0, 7, and 14 days of storage had significant effects ($P<0.05$) on total count of lactic acid bacteria, TPC count, antibacterial activity, value of acidity, pH value, alcohol content, and sensory qualities (alcoholic taste and acceptability). There was an interaction between *angkak* extract supplementation and storage period on total count of lactic acid bacteria, TPC, antibacterial activity on E. coli and S. aureus, alcohol content, viscosity, brightness value, red value, yellow value, and antioxidant activity. As summary, supplementation of *angkak* extract at 6% level and 14 days storage periods increase the microbiological quality, viscosity, alcohol content, antioxidant activity, as well as its alcoholic taste-associated acceptability, and inhibit the growth of pathogens (E. coli and S. aureus).

Keywords: Angkak, Goat milk, Kefir, Kefir quality

Introduction

Goat milk being processed into multiple products to improve its acceptability among consumers as well as extend their shelf-life due to their nature as perishable foodstuff that can be tainted by microorganism (Shiva *et al.*, 2011). Goat farming in Indonesia is limited only as meat-type livestock. Furthermore, the use of goat as milk producer is still limited. Peranakan Etawah (PE) is the common goat breed reared by farmers as it is one of the goats that produce milk to supply animal-based protein. PE has high productivity and excellent adaptability. Goat milk contains 3.4% protein, 4-7% fat, 4.5%, and 4.27% lactose. Lactose of milk sugar gives distinct sweet taste. Lactose is used the same way of carbohydrate, but firstly has to be broken down into glucose and galactose by an enzyme called lactase which available on digestive tract (Atasoglu *et al*., 2010).

Goat milk has natural antiseptic properties that helps to suppress bacteria growth in the body. This effect comes from fluorine availability

which is 10-100 times higher than cow milk (Karitas and Fatchiyah, 2013). Fluorine acts as natural antiseptic which prevent the growth of bacteria, thus, improve the body immunity (Milani and Wendorff, 2011). Goat milk also can be as a good source of bioactive peptides from milk protein, which can act as antihypertension agent (Moslehishad *et al.*, 2013), antibacterial, and antifungal agents (Dare *et al*., 2014). Antibacterial peptide works in a way that it develops interaction on bacteria membrane which followed by disruption, membrane physiological disturbance such as on cell membrane biosynthesis and cell division, or translocation through membrane to interact with targeted cell's cytoplasm (Almaas *et al.*, 2011).

Angkak is fermentation product of rice (Oryza sativa) by *Monascus purpureus* yeast. The fermentation produces yellow to red pigments (Indrawati *et al.*, 2010). *M. purpureus* produces non-toxic pigment that also does not disturb immunity (Kasim *et al.*, 2006). Angkak is widely used as natural food color. Furthermore, angkak also possess great health benefits, such as relieving digestion-related problem, improving blood circulation, strengthening lymph function, as well as enhancing drugs efficacy (Shi and Pan, 2011).

Angkak supplementation on kefir delivers double benefits, namely providing nutrient for the body yet has distinct taste. Angkak also has been proven to have antimicrobial properties on pathogenic bacteria on gastrointestinal tract. Study conducted by Indriani and Andayani (2012) showed that angkak supplementation on shrimp paste at level up to 1.5% can constrain microorganism growth. Moreover, Sulistyaningtyas *et al*. (2019) also indicated that the lactic acid and alcohol content of Kefir fermented with L. bulgaricus and Candida starter increased as an effect of angkak treatments. Silmi (2013) also reported that the color coming from angkak gives unique color on pasteurized milk receiving angkak more than 4% and stored at 4℃ for 3 days. However, studies on goat milk kefir have not been widely conducted, so that this study aimed to determine the effects of angkak extract supplementation and storage period on microbiological quality, antibacterial activity, physico-chemical characteristics, antioxidant activity, and organoleptic characteristics of goat milk kefir.

Materials and Methods

The study was performed during the period of June 8th to December 8th in 2015. The study was carried out at Laboratory of Milk and Egg Technology – Faculty of Animal Science and Laboratory of Food Technology – Faculty of Agriculture Technology of Universitas Gadjah Mada. Materials used on this study were fresh goat milk from Bumiku Hijau – Etawah Goat Farm located in Condong Catur Yogyakarta, angkak (*Monascus purpureus*) obtained from Beringharjo market, kefir grain obtained from kefir starter-home industry of Ibu Imas's in West Java, agarose nutrient (Merck), DeMann Rogosa Sharpe Broth (Merck), alcohol 70%, distillated water, spiritus, NaOH 0.1N, Phenopthalein (PP), pH buffer, $CaCO_3$, $NaNO_3$, saturated potassium carbonate, potassium dichromic sulfuric acid, Vaseline, agarose, NaCl, chloramphenicol, methanol, DPPH.

Production of angkak flour and extract. To produce angkak flour, 500 g of angkak rice was ground on grinder (100 mesh particle size) until the flour were obtained. The flour were then dissolved on sterile distillated water on 1:2 ratio. Extraction was performed by until the angkak flour were perfectly dissolved, then filtered on filtered cloth to yield filtrate and residue.

Production of kefir. Kefir grain was acquired from home industry Imas Kefir in West Java. Kefir was made following Chandan (2006) method. 250 ml of goat milk were transferred into sterilized glass. The kefir production was initiated with angkak extract supplementation on different levels, namely 0, 2, 4, and 6%, heated at 85℃ for 30 minutes, and cooled until reaching 22℃ temperature. Milk were added with 3% of kefir grain and incubated at room temperature ($\pm$28.5℃) for 10 hours until reaching a pH value of 4.2 to 4.6. Then, it was filtered to separate the result tested on this study and kefir grain. Kefir were then stored at 4℃ on different storage periods, namely 0, 7, and 14 days to evaluate the shelf-life of angkak-supplemented kefir.

Data analysis

Data were evaluated on one way ANOVA, for these following parameters: total count of lactic acid bacteria, TPC, antibacterial activity, value of acidity, pH value, alcohol content, water content, viscosity, brightness value, red value, yellow value, antioxidant activity, and sensory value (alcoholic taste and acceptability). Data analysis were followed with two way ANOVA test for total count of lactic acid bacteria, TPC, antibacterial activity on *E. coli* and *S. aureus*, alcohol content, viscosity, brightness value, red value, yellow value, and antioxidant activity. Meanwhile, the organoleptic characteristics were analyzed on non-parametric Kruskal Wallis analysis. Significant difference were further analyzed on Duncan's Multiple Range Test (DMRT). Data analysis were performed on Software Statistical Product and Service Solution (SPSS) version 18 for windows.

Results and Discussion

Microbiological quality

Total count lactic acid bacteria (LAB). The statistical evaluation on this study confirmed that angkak extract supplementation for different storage period (0, 7, and 14 days) did not alter the total count of lactic acid bacteria (P>0.05) (appendix 1). This result indicates that angkak supplementation at different levels during

incubation period has no effects on LAB growth. According to Codex no. 243 (Codex Alimentarius Commission, 2003), the minimal LAB population for kefir is 107 CFU/g or 7 log CFU/g. LAB is anerobic bacteria and able to produce lactose enzyme as energy source. LAB bacteria are also able to synthesis proteolytic enzyme, so that they can acquire required amino acids for their growth from protein breakdown (Widodo, 2003).

LAB growth at level 2%, 4%, and 6% of treatment groups were marked with clear zone around colony after incubation. LAB produce lactic acid that will react with $CaCO_3$ after incubation period. A clear region will appear around colony on media as a result of Ca-lactate that is dissolved on the media. According to Farnworth and Edward (2005), goat milk contains 4.1% of lactose. LAB has lactate dehydrogenase enzyme to produce lactic acid from pyruvate, thus milk then has acidic taste (Ismaiel, 2011). The acidity gets stronger due to the availability of either lactic acid or propionic acid and acetic acid (Liu and Lin, 2000). Purnomo and Muslimin (2012) reported that biochemical alteration on substrate fermentation is a result of heterofermentative lactic acid activity and yeast.

Total yeast. This study shows that total yeast was not significantly affected by angkak extract supplementation on different storage period (0, 7, and 14 days) (P>0.05), see appendix 2. The average of total yeast on day- 0, 7, and 14 are 6.15±0.29; 6.84±0.62; 6.29±0.29 log CFU/ml. The average of final total yeast on goat milk kefir supplemented with different level of angkak are 6.35±0.58; 6.35±0.43; 6.50±0.59; 6.50±0.52 log CFU/ml.

Table 2 shows that the total yeast was not different since day 0 (6.15±0.29 Log CFU/ml), yet the value increases at day-7 (6.84±0.62 log CFU/ml) and day-14 (6.29±0.29 log CFU/ml). The reduction on yeast growth on day-14 was due to the yeast has entered death phase when cell autolysis occurs, thus at 6% concentration did not affect the yeast growth.

Total plate count. According to Table 3, the length of storage period affected the TPC of angkak-supplemented kefir. The bacteria growth decreased on day-14. Angkak supplementation can inhibit the total microorganism as it has antimicrobial properties. Henry *et al.* (2014), stated that angkak contains monaskin and ankaflavin (yellow pigment), monaskorubin and rubropunctatin (pink pigment), monaskorubramin and rubropunctamin (red pigment) which all can kill several microorganism. Extract supplementation did not reduce the TPC significantly. However, the TPC tends to increase on day-7, and decrease on day-14. It might be a result that there are still microorganism on kefir during the storage period.

Antibacterial activity

Escherichia coli EPEC. Table 4 indicates the statistical value of angkak extract supplementation at level of 0 %, 2 %, 4 % dan 6 % are 4.01 mm, 4.83 mm, 5.74 mm, and 5.75 mm. the interaction between angkak extract and antibacterial activity altered the clear zone. The biggest clear zone formed towards *E. coli* was observed at level of 4 %. E. coli is gram negative bacteria that has fewer peptidoglycan and more lipid content. The inhibition power of fermented

Table 1. Average of total LAB (log CFU/ml) on angkak-supplemented kefir during storage

Angkak concentration	Storage period (days)			
(%)	0	7	14	Average^{ns}
0	7.07±0.35^{abc}	7.43±0.57^{bc}	6.39±0.22^{a}	6.96 ±0.55
2	7.10±0.63^{abc}	6.56±0.50^{a}	6.91±0.22^{abc}	6.85±0.48
4	7.17±0.12^{abc}	6.82±0.54^{abc}	6.90±0.28^{abc}	6.96±0.34
6	6.72±0.31^{ab}	6.91±0.42^{abc}	7.57±0.49^{c}	7.07±0.53
Average^{ns}	7.02±0.35	6.93±0.54	6.94±0.52	

^{a,b}different superscripts on the same row indicates significant difference (P<0.05)
^{ns}nonsignificant

Table 2. Average of total yeast (log CFU/ml) on angkak-supplemented kefir during storage

Angkak concentration	Storage period (days)			
(%)	0	7	14	Average^{ns}
0	6.25±0.39	6.78±0.77	6.01±0.39	6.35±0.58
2	5.99±0.47	6.81±0.16	6.63±0.05	6.35±0.43
4	6.23±0.11	6.96±0.93	6.30±0.15	6.50±0.59
6	6.13±0.08	6.81±0.79	6.57±0.27	6.50±0.52
Average^{ns}	6.15±0.29	6.84±0.62	6.29±0.29	

^{ns} non significant

Table 1. Average of TPC (log CFU/ml) on angkak-supplemented kefir during storage

Angkak concentration	Storage period (days)			
(%)	0	7	14	Average^{ns}
0	7.70±0.09^{bc}	7.80±0.60^{bc}	8.19±0.14^{cd}	7.89±0.38
2	7.73±0.16^{bc}	7.91±0.33^{bcd}	7.60±0.02^{ab}	7.74±0.23
4	7.99±0.01^{bcd}	8.32±0.39^{d}	7.21±0.17^{a}	7.84±0.53
6	8.08±0.07^{bcd}	7.75±0.26^{bc}	7.59±0.50^{ab}	7.80±0.25
Average^{ns}	7.87±0.19	7.94±0.42	7.65±0.37	

^{a,b}different superscripts on the same row indicates significant difference (P<0.05).
^{ns}nonsignificant.

Table 4. Average of antibacterial activity of angkak-supplemented kefir against *E coli* (mm) during storage

Angkak concentration (%)	Storage period (days)			
	0	7	14	Average
0	4.46±0.57^{bc}	4.33±0.75^{b}	3.23±0.05^{a}	4.01±0.77^{p}
2	4.53±0.28^{bc}	4.66±0.05^{bc}	5.30±0.52^{cd}	4.83±0.46^{q}
4	6.83±0.60^{e}	5.16±0.23^{cd}	5.23±0.40^{cd}	5.74±0.90^{r}
6	5.66±0.46^{d}	5.90±0.26^{d}	5.70±0.43^{d}	5.75±0.36^{r}
Average	5.37±1.09^{y}	5.01±0.71^{xy}	4.86±1.05^{x}	

^{p,q,r,s} different superscripts on the same row indicates significant difference (P<0.05).

Table 5. Average of antibacterial activity of angkak-supplemented kefir against *S. Aureus* (mm) during storage

Angkak concentration (%)	Storage period (days)			
	0	7	14	Average^{ns}
0	4.10±0.70^{abc}	4.86±0.40^{bcd}	4.76±0.40^{bcd}	4.57±0.57
2	3.63±1.15^{ab}	5.40±0.26^{cd}	5.60±0.51^{d}	4.87±1.13
4	3.33±0.20^{a}	5.06±0.63^{cd}	7.13±1.20^{e}	5.17±1.78
6	3.56±0.70^{ab}	4.46±0.60^{abcd}	5.43±0.85^{cd}	4.48±1.02
Average	3.65±0.71^{x}	4.95±0.71^{y}	5.73±1.13^{z}	

^{a,b,c} different superscripts on the same row indicates significant difference (P<0.05).
^{ns} non significant.

milk on negative grame bacteria is a result of antimicrobial agents in form of organic acids (Herreros *et al*., 2005). LAB produce lactic acid and acetic acid that has inhibition spectrum.

Angkak contains tannin, a phenolic compound that also known to have antiseptic property that kills several bacteria, easily dissolved in water, easily forming complex protein, and sensitive on enzyme oxidation. At low level phenolic compounds work by disrupting cytoplasm membrane to cause leak and eventually the bacterial lysis occurs.

Staphylococcus aureus FNCC 0047. Table 5 shows the increase and the presence of antibacterial activity to *S. aureus* during fermentation process. The antibacterial activity comes from lactic acid on kefir. Table 5 indicates that statistical value of extract angkak supplementation at level of 0 %, 2 %, 4 % dan 6 % are 4.57 mm, 4.87 mm, 5.17 mm, 4.48 mm which give significant difference effect (P<0.05). It might be a result of antibacterial properties of several pigments as well as other compounds in angkak that can disrupt either negative or positive gram bacteria. The yellow pigment can inhibit *B. subtilis* and *Staphylococcus aureus* (Miao *et al.*, 2016). The bigger clear zone against *S. aureus* was observed at level 4 %. S. aureus is positive gram bacteria that has more complex peptidoglycan and fewer lipid content. Table 5 shows the presence and increase of antibacterial activity against *S. aureus*. Lactic acid acts as antibacterial agent on kefir. Antibacterial agents during kefir fermentation include organic acids (lactic and acetic acid), carbon dioxide, hydrogen peroxide, ethanol diacetyl, and peptide that hinder the growth of pathogenic and decomposing bacteria (Farnworth and Edward, 2005). Liu *et al*. (2005) have investigated the effects of bacteriostatic on *S. aureus*. The bacteriostatic has greater effect on positive gram bacteria than negative gram bacteria.

Chemical quality

Value of acidity. Table 6 shows the increase on value of acidity, which increases along with the extended storage period. The higher value of acidity is caused by the more bacteria present in the kefir and their greater capability in producing lactic acid. The value of acidity also increases along with the angkak extract concentration supplemented on kefir, namely at level of 4 and 6%. According to Codex standard no 243 (Codex Alimentarius Commission, 2003), the minimum value of acidity titration on kefir fermentation is 0.6%. According to Winarno (2007), the value of acidity of kefir ranges from 0.85 to 1%.

According to average on this study, i.e. 0.79; 0.97; 1.06; and 0.73%, the higher angkak extract supplemented on kefir reduces the value of acidity. This finding is aligned with Rojsuntornkitti *et al.* (2010) who reported that the TPC value on angkak-supplemented sausage decreases along with the length of storage period. *Monascuss spp* in angkak can yield antibacterial compound of citrinin (Monascidin A) thus suppresses the bacteria growth rate.

pH value. Table 7 shows that the pH value of angkak-supplemented kefir decreases along with the length of storage period. The decreases were observed at level of 4 and 6% supplementation. The pH alteration is caused by the increasing lactic acid content

Table 6. Average of acidity value (%) of angkak-supplemented kefir during storage

Angkak concentration (%)	Storage period (days)			
	0	7	14	Average
0	0.61±0.08	0.83±0.03	0.95±0.45	0.79±0.14^{a}
2	0.90±0.04	0.93±0.01	1.07±0.01	0.97±0.81^{b}
4	0.90±0.05	1.05±0.03	1.24±0.01	1.06±0.14^{c}
6	0.79±0.02	0.85±0.03	0.55±0.54	0.85±0.30^{a}
Average	0.80±0.12^{a}	0.91±0.93^{b}	0.10±0.35^{b}	

^{a,b} different superscripts on the same row indicates significant difference (P<0.05).

Table 7. Average of pH value of angkak-supplemented kefir during storage

Angkak concentration (%)	Storage period (days)			
	0	7	14	Average
0	4.83±0.45	4.22±0.21	4.09±0.49	4.38±0.49[b]
2	4.44±0.10	4.07±0.26	3.87±0.40	4.13±0.35[a]
4	4.33±0.13	3.92±0.10	3.74±0.21	3.99±0.29[a]
6	4.50±0.21	4.24±0.27	3.88±0.34	4.21±0.36[ab]
Average	4.52±0.29[b]	4.11±0.23[a]	3.89±0.35[b]	

[a,b] different superscripts on the same row indicates significant difference (P<0.05).

Table 8. Average of alcohol content (%) of angkak-supplemented kefir during storage

Angkak concentration (%)	Storage period (days)			
	0	7	14	Average
0	0.29±0.04[c]	0.29±0.05[c]	0.19±0.02[a]	0.26±0.04[p]
2	0.30±0.05[d]	0.31±0.07[d]	0.20±0.02[ab]	0.27±0.05[q]
4	0.31±0.01[d]	0.31±0.05[d]	0.21±0.05[b]	0.28±0.05[r]
6	0.33±0.03[e]	0.33±0.01[e]	0.20±0.01[ab]	0.29±0.06[s]
Average	0.31±0.01[x]	0.31±0.01[x]	0.20±0.09[y]	

[a,b,c,d] different superscripts on the same row indicates significant difference (P<0.05).

and CO_2 decomposition. One of bacteria on kefir is lactic acid bacteria that produce lactic acid as final product of sugar (carbohydrate) metabolism. Lactic acid lower the pH value and gives sour taste. Lactic acid bacteria are able to oxidize alcohol and other carbohydrate into acetic acid (Soeparno *et al.*, 2001).

This study demonstrates that pH value is inversely proportional to value of acidity (Table 7). Higher value of acidity results in lower pH value due to sugar (carbohydrate) metabolism. Lactic acid bacteria produce greater number of lactic acid. The lactic acid will reduce the pH value (Tamime and Robinson, 2000). Kefir is one of product achieved through fermentation process. Fermentation produces lactic and acetic acids (Atasoglu *et al.*, 2010).

Acids formed during fermentation process shortly reduce the pH value to reach isoelectric point (Winarno, 2007) in which the alteration from soluble to insoluble state occurs through proteolytic steps. High proteolytic activity results in fermentation product with high quality in terms of taste and nutrition value. The acidity value of milk can decline with the presence of lactic acid bacteria in converting lactose into lactic acid until reaching 4.5 of pH value.

Alcohol content. Table 8 indicates interaction between angkak extract concentration and storage period on alcohol content. The alcohol content increases along with the increasing angkak extract concentration. The alcohol content on kefir is influenced by yeast metabolism and heterofermentative bacteria which produces ethanol. The ethanol level on kefir with different level of oligosaccharide addition are obtained during fermentation process.

Based on the average value, angkak extract supplementation has higher alcohol content than goat milk kefir. The longer storage period reduce alcohol content on goat milk kefir. Although the alcohol content on the kefir was altered by angkak supplementation and different length of storage period, the values are still within alcohol level standard. Winarno (2007) stated that the standard of alcohol content on kefir is 0.08 to 2%. Alcohol is produced by homofermentative bacteria, along with CO_2, ethanol, and other volatile compounds production (Garrote *et al.*, 2000). Kefir has 0.5 to 2% alcohol (Liu and Lin, 2000).

Physical quality

Viscosity. On Table 9, it is indicated that both angkak supplementation and storage period increases viscosity of goat milk kefir. The increases are observed at supplementation level of 4 and 6%. This result is caused by protein denaturation. Once protein is denatured, the reactive group on the polypeptide chain is open, allowing the binding to closer reactive group.

Protein denaturant may cause viscosity to increase as the result of the declining pH. Milk viscosity is determined by the presence of casein/micelle and fat globule on lipid. Goat milk has lactic acid bacteria that can increase the kefir viscosity. Farnworth and Edward (2005) reported that exopolysaccharide are produced by lactic acid bacteria, such as *Lactobacillus, Streptococcus, Lactococcus and Leuconostoc* that will interact with milk protein, increasing the kefir viscosity.

Color quality test

Brightness value (L). The interaction between angkak extract supplementation and

Table 9. Average of water content (%) of angkak-supplemented kefir during storage

Treatments	Water content (%)
Goat Milk	85.67±0.02[a]
Kefir+0 % angkak	86.24±0.01[b]
Kefir+2 % angkak	87.58±0.01[c]
Kefir+4 % angkak	88.25±0.02[d]
Kefir+6 % angkak	89.16±0.04[e]

[a,b,c,] different superscripts on the same row indicates significant difference (P<0.05).

Table 10. Average of viscosity value (cP) of angkak-supplemented kefir during storage

Angkak concentration (%)	Storage period (days)			
	0	7	14	Average[ns]
0	158.06±0.61[a]	271.46±0.36[b]	284.90±0.65[bc]	238.14±0.77
2	147.43±0.29[a]	286.76±1.87[bc]	355.73±0.31[c]	263.31±0.94
4	147.40±0.73[a]	264.06±0.45[bc]	420.66±0.30[d]	277.37±0.12
6	155.00±0.77[a]	243.30±0.45[b]	430.56±0.92[e]	263.78±0.10
Average	151.97±0.44[x]	266.40±0.32[y]	372.96±0.35[z]	

[a,b,c] different superscripts on the same row indicates significant difference (P<0.05).
[ns] non significant

storage period is caused by the length of storage period. The longer of storage period, the brightness value of goat milk kefir decreased, from day-0 to day-14. The reduction of brightness value is caused by the fact that as the kefir is stored longer, the angkak will has more solubility in kefir. Angkak extract supplementation at level exceeding 6% and stored for 14 days will reduce the brightness value of goat milk kefir.

Angkak supplementation at level of 0 %, 2%, 4% and 6% had significant effects (P<0.05) on brightness value (77.42±1.27, 69.50±0.15, 67.33±0.04, dan 62.93±0.74). The highest L value was observed at level of 0% supplementation for all different storage periods. The kefir receiving 6% angkak supplementation has the lowest L value and significantly different compared to other groups. Angkak extract supplementation as natural coloring agent gives significant effect on goat milk kefir. The more angkak extract added on goat milk kefir, the less brightness value on goat milk kefir – thus it has negative correlation. Higher angkak supplementation results in lower L value (Eman and Abbady, 2014).

Red value (a). Table 11 indicates the highest value on day- 0, 7, and 14 was observed on kefir supplemented with angkak at level of 6%. Compared to other groups, kefir receiving no angkak supplementation has the lowest a value. Angak extract supplementation on goat milk kefir has significant effects on a value. As the supplementation level increases, the a value of products increases. Presumably, it might be cause by monaskorubin or pigment in yeast. The red pigment causes the goat milk kefir to have reddish color (Kasim *et al.*, 2005). The pigment has high solubility, stable color, high digestibility, as well as non-carcinogenic property. The red pigment of angkak are widely used to color food products such as fish paste, pickle, and other foods (Chairote *et al.*, 2007).

Table 11 shows that on day- 0 and 7, the red value were observed to be higher as angkak has high solubility in kefir. The a value declined on day-14. Jenie *et al*. (1997) stated that the declining color intensity is a result of the damage on chromophore of the pigment which indicated by the declining absorbance spectrum. Temperature, storage period, and heating are several factors causing the color damage by producing kinetic energy.

Yellow value (b). Table 12 indicates that the b value ranges from 6.81 to 9.30, with the highest value of kefir stored for 0, 7, and 14 days was observed on group receiving 6% of angkak supplementation. The 0% group has lowest b value and significantly different compared to others. Angkak supplementation is associated with sample color. The higher angkak supplemented on product, the b value gets higher. Angkak contains monaskoflavin pigment, thus increases the b value (Wild *et al.*, 2002). As the goat milk kefir are stored longer, the b value decreases, i.e. day-0 (7.83), day-7 (8.24) and day-14 (8.26).

The water content was significantly affected (P<0.05). the average water content of goat milk and its kefir are 86.04±0.13% and 96.93±0.04%. The average water content of kefir receiving angkak extract at level of 2, 4 dan 6% are 87.16±0.01%; 87.44±0.02%; and 88.14±0.04%. Based on those values, the water content increases along with the increasing concentration of angak supplemented on goat

Table 11. Average of brightness value of angkak-supplemented kefir during storage

Angkak concentration (%)	Storage period (days)			
	0	7	14	Average
0	79.10± 0.02[k]	76.75±0.14[j]	76.41±0.06[i]	77.42±1.27[s]
2	70.58±0.03[j]	69.91±0.09[g]	69.50±0.51[f]	69.50±0.15[r]
4	67.55±0.01[e]	67.70±0.09[e]	66.75±0.07[d]	67.33±0.04[q]
6	62.82±0.09[b]	63.84±0.08[c]	68.70±5.40[e]	62.93±0.74[p]
Average	70.01±6.19[x]	69.55±4.89[y]	68.70±5.40[x]	

[a,b,c,d] different superscripts on the same row indicates significant difference (P<0.05)

Tabel 12. Average of red value of angkak-supplemented kefir during storage

Angkak concentration (%)	Storage period (days)			
	0	7	14	Average
0	3.03± 0.06[ab]	2.92±0.04[a]	3.19±0.05[b]	3.04±0.12[p]
2	8.49±0.06[f]	7.79±0.11[e]	6.06±0.02[c]	7.44±1.08[q]
4	10.74±0.05[h]	9.89±0.22[g]	6.66±0.33[d]	9.09±1.87[r]
6	13.61±0.03[i]	13.46±0.09[i]	13.63±0.05[i]	13.56±0.09[s]
Average	8.96±4.05[z]	8.51±3.98[y]	7.38 ±4.01[x]	

[a,,b,c,d] different superscripts on the same row indicates significant difference (P<0.05).

Table 13. Average of yellow value of angkak-supplemented kefir during storage

Angkak concentration (%)	Storage period (days)			
	0	7	14	Average
0	6.98±0.09[b]	7.16±0.12[c]	6.30±0.10[a]	6.81±0.10[p]
2	7.46±0.06[d]	7.52±0.05[d]	7.40±0.06[d]	7.46±0.06[q]
4	8.18±0.16[e]	9.03±0.05[g]	9.44±0.04[i]	8.88±0.04[r]
6	8.73±0.04[f]	9.27±0.01[h]	9.90±0.05[j]	9.30±1.53[s]
Average	7.83±0.70[x]	8.24±0.95[y]	8.26±1.53[y]	

[a,b,c,d] different superscripts on the same row indicates significant difference (P<0.05).

Table 14. Average of antioxidant activity (% DPPH) of angkak-supplemented kefir during storage

Angkak concentration (%)	Storage period (days)			
	0	7	14	Average
0	3.95±0.40[b]	4.07±0.14[b]	3.09±0.17[a]	3.70±0.51[p]
2	24.59±0.23[c]	38.63±0.46[d]	41.37±0.73[e]	34.86±7.80[q]
4	43,52±0.14[f]	59.66±0.22[g]	62.00±1.01[h]	55.06±8.72[r]
6	64.06±0.52[i]	71.35±0.30[j]	82.82±0.19[k]	72.74±8.09[s]
Average	34.03±23.27[x]	43.42±26.70[y]	47.32±30.75[z]	

[a,b,c,] different superscripts on the same row indicates significant difference (P<0.05).

milk kefir. It is caused by the fact that the extract angkak was made using sterilized distillated water on 1:2 ratio.

Antioxidant activity

Table 13 indicates the average of angkak extract at level of 0 %, 2%, 4%, and 6% are 3.70±0.51; 34.86±7.80; 55.06±8.72; and 72.74±8.19%. the dimuremic acid contained in angkak can inhibit the release of ROS caused by oxidative stress (Hilario *et al.*, 2010). Antioxidant reacts with free radical to stabilize it, thus preventing the cell damage. Liu *et al.* (2005) stated that goat milk kefir can donate more proton than unfermented milk, so that goat milk can act as protector against free radical.

Organoleptic evaluation

Odor. The statistical analysis confirmed that angkak supplementation on kefir at level of 0, 2, 4, and 6% influenced odor significantly (P<0.05). Odor parameter was on score of 3.60 (kefir), while kefir receiving 2, 4, and 6% of angkak were 3.33 (slightly like kefir), 3.3. (slightly like kefir), and 3.28 (unlike kefir). Kefir has distinctive odor. The higher angkak supplementation produced different odors caused by the unique odor of angkak itself.

Acidity. The statistical analysis revealed that angkak supplementation had no significant effect (P>0.05) on sour taste of kefir. The taste was evaluated on score of 4.20 (slightly sour), while the acidity score of kefir supplemented with angkak extract at level of 2%, 4%, and 6% are 4.13 (slightly sour), 4.06 (not sour), and 4.06 (not sour). Lactic acid bacteria produce lactic acid as final product form sugar (carbohydrate metabolism). The lactic acid will decrease the pH value and give sour taste. Therefore, the sugar content on raw goat milk determines the sour taste on kefir.

Alcoholic. The statistical analysis on this study confirmed that angkak extract supplementation on kefir at level of 0, 2, 4, and 6% did not alter the alcoholic value (P0.05). The organoleptic value obtained on this study is 2.86±0.83 (non-alcoholic). Meanwhile, the values for kefir receiving angkak extract supplementation at level of 2, 4, and 6% are 3.26 (slightly alcoholic), 3.26 (non-alcoholic), and 3.60 (slightly alcoholic). Kefir is foamy and has alcoholic characteristics, with 1% lactic acid and 0.5-1% alcohol. The alcohol content on kefir is influenced by yeast metabolism and ethanol-producing heterofermentative bacteria.

Acceptability. The angkak supplementation on kefir had significant effects (P<0.05) on kefir acceptability among consumers. The value was evaluated on score 2.93 (slightly favorable), while the kefir receiving 2, 4, and 6% of angkak supplementation have values of 2.46 (slightly favorable), 2.40 (slightly favorable), and 2.00 (unfavorable). Panelists added that it was their first time in tasting angkak-supplemented kefir and are not used to yet with the taste. Therefore, the products have not acquired favorability.

Conclusions

Angkak supplementation on kefir at level of 6% and stored for 14 days can improve the lactic acid bacteria, viscosity, alcohol content, antioxidant activity, acceptability for its alcoholic taste, as well as inhibit the growth of pathogenic bacteria (*E. coli* and *S. aureus*). The products of angkak-supplemented kefir have met standard according to Codex no. 243 year 2003, in which the fermented milk (kefir) has minimal value of 10% acidity, 0.5-1% alcohol value, 10^7 CFU/g total microbes, and 10^4 total yeast.

References

Almaas, H., E. Eriksen, C. Sekse, I. Comi, R. Flengsrud, H. Holm, H. Jensen, M. Jacobsen, J. Langsrud, and G. E. Vegarud. 2011. Antibacterial peptides derived from caprine whey proteins by digestion with human gastrointestinal juice. J. Nutr. 106: 896 – 905.

Atasoglu, C., H. I. Akbag, C. Tolu, G. Dos, T. Savas, and I. Y. Yurtman. 2010. Effect of Kefir as a Probiotic Source on The

Performance of Goat Kids. South African J. Anim. Sci.40: 4.

Chairote, E., G. Chairote, and S. Wongpornchai. 2007. Preparation of Red Yeast Using Various Thai Glutinous Rice and Monascus purpureus CM 001 Isolated From Commercial Chinese Red Sampel. 2: 28-37.

Chandan RC. 2006. Milk composition, physical and processing characteristics. In RC Chandan, White CH, Kilara A. and Hui YH (Eds.), Manufacturing Yogurt and Fermented Milks. Oxford, UK: Blackwell Publishing, pp. 3-129.

Codex Alimentarius Commission. 2003. Codex Standard for Fermented Milk: Codex STAN 243. FAO/WHO Food Standards, Rome.

Dare, K., J. Shepered, H. Roy, S. Seveu, M. Ibba. 2014. LysPGS formation in Listeria monocytogenes has broad roles in maintaining membrane integrity beyond antimicrobial peptide resistance. Virulance. 5: 534-546. Doi: 10.4161/viru.28359.

Eman, M. M., and M. S. Abbady. 2014. Secondary Metabolite and Bioactivity of the Monascus Pigment. Reviews article. Global J. Biotechem 9: 01-13.

Farnworth and Edward. 2005. Kefir- A complex Probiotic. Food Science and Technology Bulletin: J. Funct. Food 1: 1-17.

Garrote, G., L. A. G. Abraham, Graciela, and L. Antoni. 2000. Inhibitory Power of Kefir: the Role of Organic Acids. J. Food Prot. 63: 364-369.

Henry, F., Mamocud, and I. Dizon. 2014. Potential of Biopigments from Monascus purpureus Went as Natural Food Colorant for Philippine Native Sausage (Longganisa). Internasional Conference on Nutrition and Food Sciences. PPCBEE. 71.15.

Herreros, M. A., H. Sandoval, L. González, J.M. Castro, J.M. Fresno and M.E. Tornadijo. 2005. Antimicrobial activity and antibiotic resistance of lactic acid bacteria isolated from Armada cheese (a Spanish goats' milk cheese). Food Microbiology. 22: 455-459. DOI: 10.1016/j.fm.2004.11.007.

Hilario, M. C., D. C. Puga, and N. A. Ocana. 2010. Antioxidant Activity, Bioactive Polyphenols in Mexian Goat's Milk Cheese on Summer Grazing. J. Dairy Rest. 77: 20-26.

Indrawati, T., D. Tisnadjaja, and Ismawatie. 2010. Pengaruh suhu dan cahaya terhadap stabilitas angkak hasil fermentasi Monascus purpureus 3090 pada beras. J. Farm. Indones. 5: 85–92.

Indriati, N and F. Andayani. 2012. Pemanfaatan angkak sebagai pewarna alami pada terasi udang. Jurnal Pascapanen dan Bioteknologi 7: 11-20.

Ismaiel, A., Ahmed, G. F. Mohammed, and N. E. Ayman. 2011. Some Physicochemical Analyses of Kefir Produced Under Different Fermentation Conditions. J. Sci. Ind. Res. 70: 365-372.

Jenie, B.S.L., K. D. Mitrajanty and Fardiaz, S. 1997. Produksi Konsentrat dan Bubuk Pigmen Angkak dari Monascus purpureus serta Stabilitasnya selama Penyimpanan. Buletin Teknologi dan Industri Pangan 8: 39-46.

Karitas, M.U and Fatchiyah. 2013. Profil Protein 30-60 kDa pada Yogurt hasil Fermentasi Susu Kambing Peranakan Etawa dengan Kultur Tunggal. Jurnal Biotropika. 1: 65-69.

Kasim, E., N. Suharna, and N. Nurhidayat. 2006.Kandungan pigmen dan lovastatin pada angkak beras merah kultivar bah butong dan BP 18041 F9 yang difermentasi dengan Monascus purpureus Jmba. Biodiversitas. 7: 7–9.

Kasim, E., S. Nandang, and N. Nurhidayat. 2005. Kandungan Pigmen dan Lovastatin pada Angkak Beras Merah kultivar bah butong dan BP 1804 IF 9 yang difermentasi dengan *Monascus purpureus* JMBA. Cibinong Science Bogor, Bogor.

Liu, J. R. and C. W. Lin. 2000. Production of Kefir From Soymilk With or Without Added Glucose, Lactose, or Sucrose. J. Food Sci. 65: 716-719.

Liu, J. R., J. M. Chen, and C. W. Lin. 2005. Antimutagenic and Antioxidant Properties of Milk Kefir and Soymilk Kefir. J. Agric Food Chem. 53: 2467-2474.

Miao, Y., X. Han, and L. Zheng. 2016. Fimbrin phosphorylation by metaphase Cdk1 regulates actin cable dynamics in budding yeast. Nat. Commun. 7: 11265. https://doi.org/10.1038/ncomms11265

Milani, X. F. and L. W. Wendorff. 2011. Goat and Sheep Milk Products in The United States (USA). J. Small Ruminant Res. 101: 134 - 139.

Moslehishad, M., M. R. Ehsani M. Salami, S. Mirdamadi, Ezzatpanah, A. N. Naslaji, and A. K. Moosavi-Movaledi, 2013. The comparative assessment of ACE - inhibitory and antioxidant of peptida fraction obtained from fermented camel and bovine milk by *Lactobacillus rhamnous* PTCC 1637. 29: 82 - 87.

Purnomo, H. and L. D. Muslimin. 2012. Chemical charateristics of pasteurisasi goat milk and goat milk kefir of Indonesian kefir and incubation times. J. I. Food. Res. 19: 791 - 794.

Rojsuntornkitti, K., N. Jittrepotch, T. Kongbangkerd, and K. Kraboun. 2010. Substitution of nitrite by Chinese red broken rice powder in Thai traditional fermented pork sausage (Nham). Int. Food Res. J. 17: 153-161.

Shi, Y. C. and T. M. Pan. 2011. Beneficial effects of Monascus purpureus NTU 568-fermented products: a review. Appl. Microbiol. Biotechnol. 90: 1207–1217.

Shiva, D., P. Rezvan, A. Mazaheri, Mahnas, and M. Ali. 2011. Kefir production from soymilk. J. Agr. Food. Chem. 50: 3999 - 4002.

Silmi, N., L. E. Radiati, and Purwadi. 2013. Effect of addition red color ekstrak of (*Monascus Purpureus*) on the quantity of mikrooganism based on the TPC and color analisis of pasteurezed milk. http://fapet.ub.ac.id/penelitian-dan-pengabdian/hasil-penelitian/artikel-skripsi-s1-peternakan/2013-repository-skripsi-fapet-ub/. Universitas Briwijaya, Malang.

Soeparno, R. A. Rihastuti, Indratiningsih, and S. Triatmojo. 2001. Dasar Teknologi Hasil Ternak. Fakultas Peternakan. Gadjah Mada University Press, Yogyakarta.

Sulistyaningtyas, A.R., A.T. Lunggani, E. Kusdiyantini. (2019). Kefir Produced from Red Rice Milk by Lactobacillus bulgaricus and Candida kefir Starter. *IOP Conf. Ser.: Earth Environ. Sci.* 292 012038. doi:10.1088/1755-1315/292/1/012038.

Widodo. 2003. Bioteknologi Industri Susu. Cetakan Ke-1. Lacticia Press, Yogyakarta.

Tamime, A. Y. and R. K. Robinson. 2000. Yoghurt Science Technology. 2nd edn. Woodhead Publishing Limited, England.

Wild, D., G. H. Toth, Ulrich, and Humpf. 2002. New *Monascus* metabolite isolated from red yeast rice (Angkak, Red Keji). J. Agr. Food. Chem. 50: 3999 - 4002.

Winarno. 1997. Kimia Pangan dan Gizi. PT Gramedia Pustaka Utama. Jakarta.