

Microbiological Characteristic and Nutrition Quality of Goat Milk Kefir Based on Vitamin D₃ Fortification Time

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Abstract. Goat milk kefir fortified with vitamin D₃ is expected to benefit individual with insulin resistance. Different vitamin D₃ fortification time allegedly effect microbiological characteristic and nutrition quality of goat milk kefir due to its microbial growth curve, thus this study aimed to analyze those parameters. This study was an experimental research. This study contains five treatments (vitamin D₃ fortification at 0, 6, 12, 18, or 24 hours of fermentation) and a group of control. Total lactic acid bacteria, vitamin D₃, protein level, fat content, crude fiber, viscosity, and pH was analyzed by Total Plate Count, spectrophotometry, Bradford method, Babcock method, gravimetric analysis, Ostwald method, and pH meter respectively. Time of vitamin D₃ fortification significantly effect vitamin D₃ content (p=0,021), fat content (p=0,001), crude fiber (p=0,0001), viscosity (p=0,010), and total lactic acid bacteria (p=0,048). The highest vitamin D₃ content was found on the group fortified at 6 hours of fermentation. All treatment groups has lower fat content and crude fiber content than control group. Total LAB in all group meet the Codex standard ($\geq 10^7$ CFU/ml). Control group and fortification group at 24 hours of fermentation have higher viscosity than other groups. There was no significant difference found in goat milk kefir protein level (p=0,262) and pH (p=0,056) despite the difference of fortification time. Vitamin D₃ fortification time effect vitamin D₃ content, fat content, crude fiber, viscosity, and total lactic acid bacteria of goat milk kefir, but did not effect protein content and pH of goat milk kefir.

Keywords : goat milk kefir, fortification time, vitamin D₃, lactic acid bacteria, nutrition quality.

1. Introduction

Indonesia as developing country with a tropical climate, is now facing new health problems which is shifting from infectious disease to degenerative disease and malignancy[1]. Diabetes melitus is a kind of degenerative disease occurring in Indonesia. A study stated that the prevalence of diabetes in urban Indonesia was 5,7%, consisting of 1,5% diagnosed diabetes mellitus, 4,2% undiagnosed diabetes mellitus, and 10,2% encounter impaired glucose tolerance[2]. *Riset Kesehatan Dasar* 2013 stated that there was 6,9% of population (age of ≥ 15 years) in Indonesia whose diabetes mellitus, which 90% of the case classified as diabetes mellitus type 2[3]. Individual with obesity and insulin resistance will partially develop diabetes mellitus type 2[4]. Insulin resistance is a condition where the lack of cell insulin receptor led to cell inability to uptake glucose[5]. Vitamin D deficiency apparently play role in pathogenesis of Diabetes Mellitus type 2. Vitamin D help stimulates the expression of the insulin



receptor in peripheral tissue to increase glucose uptake[6]. In addition, a good status of vitamin D may suppress chronic inflammation, thus insulin sensitivity will increased[7][8]. Human body could synthesize vitamin D with the help of the sun UV irradiation of 7-dehydrocholesterol on skin[9]. Unfortunately, vitamin D synthesis in human body limited to clothes they are wearing and depends on other factors like the intensity of ultraviolet, race, and age[10]. Sunny country in tropical and subtropical region was not an exception to suffer from vitamin D deficiency[11]. Vitamin D deficiency in Southeast Asia varied from 6-70%[12]. Indonesia, a tropical country with plentiful sunshine, also suffered a high number of vitamin D deficiency. There are only 5% children with adequate 25(OH)D in Indonesia[13]. Vitamin D deficiency itself could ensue all population groups due to its various risk factors[14]. Therefore, diabetes melitus type 2 and vitamin D deficiency are both important concerns in Indonesia.

Goat milk, a kind of milk which can be found in Indonesia, has multiple benefit. Goat milk contains high level of MCT and short chain fatty acids, which can easily digested and used for metabolism. In addition, goat milk contains higher protein, vitamin A, thiamin, riboflavin, niacin, panthotenat, calcium, and phospor compared to cow milk. But, both goat milk and cow milk are deficient to vitamin B6, vitamin C, and vitamin D[15].

Kefir, a kind of fermented milk, can be made by inoculating kefir grains into milk. Kefir grains contain microorganisms such as *Lactobacillus* spp., *Lactococcus* spp., *Streptococcus* spp., *Enterococci* spp., *Leuconostoc* spp, *Acetobacter* spp., *Bacillus* spp., *Kluyveromyces marxianus*, *Saccharomyces* sp., *Torulaspora delbrueckii*, *Brettanomyces anomalus*, and *Issatchenkia occidentalis*[16][17]. Kefir grains itself is a potential probiotic and antioxidant, which may benefit individual who have insulin resistance[18]. Probiotic drinks could lower fasting glucose and HbA1C in patient with diabetes mellitus type 2[19]. Furthermore, kefiran inside kefir could activate PI 3-kinase, thus help insulin signaling[20].

Kefir contains lactic acid bacteria and yeast. The minimum amount of lactic acid bacteria and yeast in kefir is 10^7 CFU/ml and 10^4 CFU/g, respectively[21][22][23]. Chemical composition in milk could contribute to yeast growth. Yeast existence can help provide nutrition that are important for lactic acid bacteria growth, such as vitamin and amino acid[24]. Yeast has proteolytic and lypolytic activity[25][26]. Lactic acid bacteria also has proteolytic[27] and lypolytic activity[28]. Thus, activity of yeast and lactic acid bacteria could affect kefir composition and characteristics. Kefiran, an exopolisaccharide inside kefir, is suitable as viscofying, stabilizing, gelling, and emulsifying factor[29]. Bacteria activity that produce kefiran will later effect the viscosity of the product[30]. Acidity (pH) also affected by lactic acid bacteria and yeast contain[31]. Acidity (pH) of kefir ranged from 4,2 to 4,6[32].

Fortification aimed to increase nutrition value of food and to offer health benefits for consuments[10]. Milk and its products usually be used as media for vitamin D₃ fortification[10][33]. Vitamin D₂ dan vitamin D₃ are commonly used as food fortificant[10][34]. Vitamin D₂ retention in fortified milk is 76,96%[35], whereas vitamin D₃ retention in milk products such as cheese, yoghurt, and ice cream have higher retention (95-97%, 96,6-97,8%, and 99,8-99,3%, respectively)[33].

Microorganisms in kefir have different growth curve. At 0 hour of fermentation, those microorganisms just started to began the fermentation. At 6 hours of fermentation, lactic acid bactria, acetic acid bacteria, and yeast start to increasing. At 12 hours of fermentation, lactic acid bacteria and acetic acid bacteria increased significantly. Yeast keep growing untill 12 hours of fermentation, meanwhile for the rest fermentation it shows no significance growth. At 18 hours of fermentation, lactic acid bacteria population reach its peak, meanwhile acetic acid bacteria shows a little increase. At 24 hours of fermentation, acetic acid bacteria reach its optimum number[36]. There was no previous research that study about the effect of different time of vitamin D₃ fortification on microbiological characteristic and nutrition quality of goat milk kefir. Different vitamin D₃ fortification time allegedly effect microbiological characteristic and nutrition quality of goat milk kefir. Goat milk kefir fortified

with vitamin D₃ produced in this research is expected to have good microbiological characteristic and nutrition quality which could benefit individual with insulin resistance.

2. Method

The making of goat milk kefir, vitamin D₃ fortification, microbiological (lactic acid bacteria) analysis, nutrition quality (vitamin D₃, protein, fat, and crude fiber) analysis, viscosity analysis, and acidity (pH) measurement was conducted in Laboratorium Terpadu Universitas Diponegoro. Preliminary study was conducted to analyze microbiological characteristic and nutrition quality of goat milk used for this research. Goat milk for this research was obtained from Oemah Kefir. Kefir grains used from this research was first obtained from Oemah Kefir, and then reared by the researcher. Vitamin D₃ was sourced from *Healthy Care* Australia.

This research was experimental, completely-randomized designed. This research were performed in five group of different fortification time, namely group of fortification on 0, 6, 12, 18, and 24 hours fermentation and a group of control. Group of control was goat milk kefir without any treatment. Vitamin D₃ was added 42 IU/100 ml kefir[37]. Vitamin D₃ used for this research was in form of emulsified oil. All of treatments was replicated three times. All control group and treatment groups was fermented for 24 hours[38].

The making of goat milk kefir was begun with milk pasteurization on 72°C for 15 second, then was cooled until 25°C[38]. Milk was divided into 5 groups of treatment and a group of control, then was inoculated with 5% of kefir grains. All of samples were fermented for 24 hours. During fermentation, vitamin D₃ fortification was held based on each group (0, 6, 12, 18, and 24 hours of fermentation). All samples were homogenized every 6 hours, and then filtered after the fermentation finished.

Vitamin D₃ was analyzed by spectrophotometry (Shimadzu). Samples was dissolved in a solution mixture of kloroform:methanol = 1:9. Afterwards, the mixture was read by spectrophotometer at 264 nm[39]. Fat content was analyzed by Babcock method, which samples were dissolved with hexane. Crude fiber was analyzed by gravimetric analysis. Viscosity was analyzed by Ostwald method. Acidity (pH) was measured with pH meter (LaMotte). Total lactic acid bacteria was analyzed with *Total Plate Count* (TPC) method, which the bacteria was inoculated on M.R.S Agar (Oxoid).

Data obtained from this study were analyzed by statistic software. Data distribution were examined by *Saphiro Wilk*. Effect of vitamin D₃ fortification time on vitamin D₃ content, protein content, fat content, crude fiber, and total lactic acid bacteria was examined by ANOVA *one way*. Meanwhile, effect of vitamin D₃ fortification time on viscosity and pH was examined by *Kruskall-Wallis*.

3. Results

Table 1. shows the results of preliminary study on goat milk used in this research. This data was used for better understanding of the characteristic of goat milk used by this study. Table 2. Shows nutrition quality of goat milk kefir based on various fortification time and nutrition quality of the control group.

Table 1. Goat Milk Characteristics

Sample	Vitamin D ₃ (IU)	Vitamin B ₁₂ (µg)	Protein (%)	Fat (%)	Crude Fiber (%)	Total LAB (10 ⁷)	Viscosity (cm/s ²)	pH
A1	69,7	735,3	1,4	14,8	3,2	4,2	0,0134	6,5
A2	72,5	805,0	2,4	14,6	3,4	3,4	0,0134	6,6
A3	70,4	918,7	2,6	14,6	2,4	3,9	0,0139	6,6
Mean	70,9±1,4	819,7 ± 92,6	2,2 ± 0,6	4,7 ± 0,1	3 ± 0,53	3,83 ± 0,43	0,0136± 0,0002	6,6 ± 0,008

There was significant different found on vitamin D₃ content in goat milk kefir due to its fortification time ($p=0,021$). The highest concentration of vitamin D₃ was $34,65 \pm 5,63$ IU, which was found on group of fortification at 6 hours of fermentation (**Table 2.**).

Protein content does not show significant different due to its fortification time ($p=0,262$). Fat content show significant different ($p=0,001$) due to its fortification time. After *post hoc* test, it was found that group of control had significantly higher fat content than all treatment groups (**Table 2.**).

Table 2. Nutrition Quality of Goat Milk Kefir Based on Fortification Time

Fortification Time	Mean Vitamin D ₃ Concentration		Mean Protein Content		Mean Fat Content		Mean Crude Fiber Content	
	IU	<i>p</i> value	%	<i>p</i> value	%	<i>p</i> value	%	<i>p</i> value
Control	$22,87 \pm 0,57^b$	0,021*	$0,62 \pm 0,07$	0,262*	$8,47 \pm 0,39^a$	0,001*	$23,27 \pm 1,504^a$	0,000*
0 hour of fermentation	$28,19 \pm 5,34^{ab}$		$0,93 \pm 0,29$		$5,93 \pm 0,73^b$		$3,93 \pm 1,83^b$	
6 hours of fermentation	$34,65 \pm 5,63^a$		$0,63 \pm 0,18$		$6,23 \pm 0,59^b$		$2,77 \pm 0,50^b$	
12 hours of fermentation	$26,55 \pm 1,47^{ab}$		$0,82 \pm 0,09$		$6,67 \pm 0,54^b$		$3,90 \pm 1,31^b$	
18 hours of fermentation	$23,54 \pm 3,29^b$		$0,78 \pm 0,14$		$6,44 \pm 0,52^b$		$3,80 \pm 0,95^b$	
24 hours of fermentation	$25,59 \pm 2,58^{ab}$		$0,81 \pm 0,13$		$5,92 \pm 0,38^b$		$6,47 \pm 2,44^b$	

* Tested with *one way* ANOVA

There was significant effect of vitamin D₃ fortification time on crude fiber content ($p=0,000$). Control group has significantly higher crude fiber content than all treatment groups. Group of fortification at 24 hour of fermentation has slightly higher crude fiber content than the other treatment groups, but the difference was not significant (**Table 2.**).

Table 3. Total Lactic Acid Bacteria, Viscosity, and Acidity (pH) of Goat Milk Kefir Based on Fortification Time

Fortification Time	Mean Total LAB		Median Viscosity		Median pH	
	$\times 10^9$ CFU/mL	<i>p</i> value	cm ² /s	<i>p</i> value		<i>p</i> value
Control	$13,4 \pm 6,54^b$	0,048*	$0,1384^b$	0,010**	4,70	0,056**
0 hour of fermentation	$70,0 \pm 27,07^a$		$0,0563^d$		4,45	
6 hour of fermentation	$27,0 \pm 21,23^{ab}$		$0,0532^d$		4,55	
12 hour of fermentation	$17,9 \pm 15,47^{ab}$		$0,0710^c$		4,55	
18 hour of fermentation	$37,1 \pm 29,85^{ab}$		$0,0576^d$		4,55	
24 hour of fermentation	$17,5 \pm 13,81^{ab}$		$0,1652^a$		4,45	

* Tested with *one way* ANOVA

*Tested with *Kruskall Wallis*

Table 3. shows results of total lactic acid bacteria (LAB), viscosity, and pH of goat milk kefir based on fortification time. Viscosity and pH was measured to make sure kefir made in this study met the characteristic of kefir. All of kefir samples made in this study had met the Codex standards for lactic acid bacteria ($\geq 10^7$ CFU/ml). There was significant difference of total LAB due to vitamin D₃ fortification time ($p=0,048$). The highest number of total LAB was found on group of fortification at 0 hour of fermentation. Based on the *post hoc* test, significant different was found between control group with group of fortification at 0 hour of fermentation, meanwhile the other differences between group was not significant.

Fortification time of vitamin D₃ also effect the viscosity ($p=0,010$). Control group and group of fortification at 24 hours of fermentation have higher viscosity than the other groups. Acidity of goat milk kefir does not differ by the vitamin D₃ fortification time($p=0,056$), although there was tendency that all treatment groups had lower pH than control group.

4. Discussion

4.1 Vitamin D₃

The vitamin D₃ content in milk used for this study was $70,9 \pm 1,4$ IU, meanwhile vitamin D₃ content in control kefir group was $22,87 \pm 0,57$ IU. Vitamin D₃ is a fat soluble vitamin, but there was lypolitic activity during kefir fermentation[24][30]. This study performed vitamin D₃ fortification on various time during goat milk kefir fermentation in order to increase vitamin D₃ content in goat milk kefir final product. It was obtained that group of vitamin D₃ fortification at 6 hours of fermentation had the highest vitamin D₃ content (34,65 IU) in final product of goat milk kefir compared to the other group fortification time. This found was different from the other study. Preceding study was found that vitamin D₃ retention in yoghurt could reach 97,8%[33]. The result could be different due to vitamin D sensitivity of light, heat, and oxidation[33][40]. Acidity could also effect vitamin D₃ stability[33][41].

As stated before, the highest vitamin D₃ content in this study was found on the group fortification at 6 hour of fermentation. This result could be caused by the presence of β -laktoglobulin A (β -LG A) and β -kasein (β -CN), proteins that could stabilized vitamin D₃. In dairy products, if vitamin D₃ was not protected by its fat matrix, it will be stabilized by β -laktoglobulin A (β -LG A) and β -kasein (β -CN)[42][43]. The presence of β -LG A dan β -CN could effect stability and availability of vitamin D₃ in the products[42]. During kefir fermentation, there was proteolysis activity[24][30]. In fermented dairy products, vitamin D₃ is bound tightly to β -LG A[43]. During fermentation, β -LG is was not easily hydrolyzed by lactic acid bacteria, meanwhile β -CN was hydrolyzed significantly. After 6 hours of fermentation, β -CN was hydrolyzed about 35%, and the hydrolyzation was increasing untill incubation finished[25]. As more of β -CN hydrolyzed after 6 hours of fermentation, vitamin D₃ could not be stabilized by β -CN.

The increasing of lypolysis and proteolysis activities allegedly contribute to the loss of protein and fat that stabilized vitamin D₃. Lypolysis and proteolysis that happened during kefir fermentation affected by lactic acid bacteria and yeast[24][30]. Fortification at 12 and 18 hours of fermentation concures with the growth peak of Lactic acid bacteria and yeast. Total lactic acid bacteria in kefir fermentation reached its peak at 12 hours of fermentation (*Lactococcus* spp.) and 18 hours of fermentation (*Lactobacillus* spp.), meanwhile yeast reached its peak at 12 hours of fermentation[36]. Addition of vitamin would effects the growth of kefir grains[44], thereby fortification at a certain time would be used by microorganisms for growth. At 12, 18, and 24 hours of fermentation, lypolitic and proteolytic activities continue, thereby it would effect the presence of vitamin D₃ in goat milk kefir.

Yeast in kefir could produce vitamin D₂ from ergosterol with the help of UV light[45]. Vitamin D₃ was analyzed by spectrophotometer at 264 nm[39]. Four metabolic form of vitamin D, namely vitamin D₃, vitamin D₂, 25-hidroxyvitamin D₃ dan 25-hidroxyvitamin D₂, could be analyzed with the same wavelength (254 nm)[46]. The small difference of the wavelength could cause shifting peak, thus vitamin D₂ could unintentionally read by the spectrophotometer.

4.2 Protein

Lactic acid bacteria (BAL) inside kefir could degradate milk protein into peptide and amino acids during fermentation. Free amino acids and small peptides used by lactic acid bacteria as nutritional source for growth[27]. Yeast inside kefir also has proteolytic activities[26].

Casein was build from some fractions, namely α -, β -, dan κ -kasein, meanwhile whey was formed by fractions of α -laktalbumin dan β -laktoglobulin[25]. The presence of β -LG A dan β -CN effect the stability and availability of vitamin D₃ in the product[42]. Fortification of vitamin D₃ at various time resulting that the highest vitamin D₃ content was found if vitamin D₃ fortified at 6 hours of fermentation. A research stated that beta-casein had been hydrolyzed about 35% at 6 hours of

fermentation, and then keep increasing. At 24 hours of fermentation, it already hydrolyzed about 85%. Meanwhile, β -laktoglobulin don't significantly decreased during fermentation[25]. The more protein hydrolyzed may cause vitamin D₃ could not be stabilized by protein. Based on results in this study, it shows that on group fortification at 6 hours of fermentation vitamin D₃ was the highest, but it also shows that protein contents seems pretty lower than the other treatment group although the difference was not significant. This can be explained because the binding of vitamin D₃ with β -laktoglobulin A or β -kasein was stronger when affinity within protein and other protein is lower. Low molecular affinity may cause the presence of monomer in the solvent[42]. Meanwhile, the method being used, Bradford, has characteristic which could not detect the monomer in the solvent[47].

Protein content in this study was not significantly different in all groups. Protein content affected by the kind of the milk that being used in the experiment. The protein content could be affected by the feeding method and the breeding of the goat[48]. Beside, kefir grains and pH of fermentation may contribute to the protein content of the kefir[38]. In this study, the same amount of kefir grain, the same source of milk, and the same pH fermentation was used for every groups, thereby it explains the insignificant difference on the protein content of all goat milk kefir samples.

4.3 Fat

The fat content in all treatment groups were lower than the control group. During fermentation, fat was degraded by microorganisms in kefir grains[49]. Vitamin D₃ fortification was performed during fermentation (at 0, 6, 12, 18, or 24 hours of fermentation), it means that vitamin D₃ was added during lactic acid bacteria and yeast growth. The addition of vitamin could effect the growth of kefir grains[44]. Lactic acid bacteria has intracellular and extracellular lipase that cause fat degradation to fatty acids and glycerols[30].

As stated before, this study shown that fat content of treatment group was lower than control group. That results was concurred with the results of total lactic acid bacteria in this study. The number of total lactic acid bacteria on all treatment group was higher than the control group. The increase of total lactic acid bacteria may cause higher lipase produced by them, thus there were more fat being degraded and show a lower fat content as a result[21]. Group fortification at 0 hour of fermentation had the highest number of total lactic acid bacteria, thereby this group also show a low fat content. Yeast in kefir also performed lipolytic activity[26]. *Saccharomyces cerevisiae* has esterase which can hydrolyze short and medium chain fatty acids[50].

Fat content in this study (5,93-8,47%) was higher than other study (5,55%) who used the same concentration of kefir grains and used the same pH[38]. This difference may caused by the kind of milk that being used[48]. The fat content of control group was higher than the treatment groups. This fat content may also effect the viscosity of kefir[51].

4.4 Fiber

Exopolysaccharide (EPS) in kefir, called kefiran, was produced by lactic acid bacteria namely *Lactobacillus*, *Streptococcus*, *Lactococcus*, dan *Leuconostoc*[49]. Kefir grains has α -galaktosidase activity[22]. Lactose in kefir was hydrolyzed, then galactose produced by this process was being used to build kefiran polymer[49]. In this study, all treatment groups has lower crude fiber content than the control group. Kefiran production may affected by culture condition, such as temperature, agitation rate, carbon source, vitamin, and mineral provided in the culture. The addition of vitamin may effect the growth of kefir grains[44]. thus the addition of vitamin at various fortification time may be used by various bacteria or yeast that was being growth at the time. *Lactococcus* spp. reached its growth peak at 12 hours of fermentation, *Lactobacillus* spp. reached its growth peak at 18 hours of fermentation, meanwhile yeast reached its growth peak at 12 hours of fermentation[36].

Yeast contain ergosterol, which are majority located on plasma membrane. Ergosterol played role on yeast mating, through feromon signaling and help plasma membrane fusion[52]. A previous study learned the effect of temperature, pH, and the addition of yeast extract to the growth of EPS-producing bacteria and the production of EPS itself. From that study, it was obtained that higher yeast extract

added may increase the production of EPS by bacteria. However, the higher number of yeast could also increase lactose consumption by yeast, thus there were lower lactose available for biosynthesis of EPS by bacteria[53]. Kefiran content could contribute to the viscosity of kefir[30].

4.5 Total Lactic Acid Bacteria

There were homofermentative lactic acid bacteria (such as *Lactobacillus* spp., *Lactococcus* spp., *Streptococcus thermophilus*) and heterofermentative lactic acid bacteria (such as *L. kefir*, *L. parakefir*, *L. fermentum*, *L. brevis*)[17]. Based on Codex standard of fermented dairy products, kefir has to contain 7 log CFU mL⁻¹ at minimum. All kefir samples made in this study already met the minimum Codex standard of total lactic acid bacteria. Total lactic acid bacteria in group of fortification at 0 hour of fermentation was higher than the other treatment groups and the control group. Nutrition available in the environment would be used by lactic acid bacteria for support their growth[30]. Total lactic acid bacteria on group fortification at 0 hour of fermentation has the highest number. It could be caused because the addition of vitamin D₃ was being used by lactic acid bacteria to growth. A previous study showed that at 0 hour of fermentation, the amount of lactic acid bacteria and acetic acid bacteria was about 6 log unit, it means the amount was higher than yeast[36]. Thus, lactic acid bacteria in group of fortification at 0 hour of fermentation performed high lysoytic activity. On group of fortification at 6, 12, 18, and 24 hours the addition of vitamin may be used by yeast and acetic acid bacteria. It cause the number of lactic acid bacteria on these groups was lower than the group fortification at 0 hour of fermentation and the control group. Microorganisms in kefir performed symbiosis, so it keep the stability of the products[24][54]. A previous study showed that at 6 hours of fermentation, the amount of yeast and *Lactobacillus* spp. increased significantly. At 12 hours of fermentation, lactic acid bacteria and acetic acid bacteria show a significant increase, meanwhile yeast reached its growth peak at this hour and remained stable for the rest of fermentation. At 18 hours of fermentation, *Lactobacillus* spp. reached its growth peak. At 24 hours of fermentation, acetic acid bacteria reached its growth peak[36].

Lactic acid bacteria has symbiosis interaction with yeast. *Lactococcus* hydrolyze lactose, then produce lactic acid, thus create a good environment for yeast growth. Bacterial growth would increase, then release organic acids that would be fermented by yeast. Besides that, yeast provide nutrition for the growth of bacteria such as amino acids, vitamins, and other component. Both of the microorganisms may also competing each other in order to get nutrition for supporting their growth, and could also produce metabolite that suppress or stimulate each other's growth. This symbiosis interactions will create the stability of the products[24][54].

4.6 Viscosity

Lactic acid bacteria inside kefir could produce exopolysaccharide (EPS) during fermentation. EPS that was generated would effect rheology of the fermented products[30]. EPS that produced during kefir fermentation is called kefiran[22]. In this study, kefir that fortified at 0, 6, 12, and 18 hours of fermentation has lower viscosity compared to control group and group that was fortified at 24 hours of fermentation. Fortification at 0, 6, 12, or 18 hours of fermentation mean vitamin was added when lactic acid bacteria on growing phase[36]. The decreased of the viscosity could be affected by EPS production of lactic acid bacteria. The amount of EPS that was produced could be affected by temperature, pH and the amount of yeast that was inoculated. The more yeast inoculated may increase the production of EPS by lactic acid bacteria, but may also decrease the lactose available for EPS production[53]. Fortification at 24 hours of fermentation means the growth of lactic acid bacteria reach its optimum[36]. Moreover, the number of fat content may also contribute to the viscosity, whereas in this study the fat content in treatment group is lower than control group[51].

4.7 Acidity (pH)

Acidity (pH) of kefir in this study ranged from 4.45 to 4.7. Kefir has characteristic which has pH 4.2-4.6[32]. Kefir on other study has pH 4.85 after fermentation[36]. Lactic acid bacteria inside kefir

could degrade lactose and yield lactic acid during fermentation. The presence of lactic acid could lower milk pH[31]. A study found that the presence of yeast could reduce the pH lower than single lactic acid bacteria culture[26]. Yeast use milk nutrition, such as protein, fat, lactose, and citrate, then release amino acid, vitamin, and other components to support the growth of lactic acid bacteria[24][26]. Yeast could metabolize lactic acid[26]. The higher kefir grains inoculated, the lower pH would be[49]. In this study, the amounts of kefir grains inoculated was the same (5%), thereby it explain why the results of pH obtained in all groups were unsignificantly different.

5. Conclusion

Vitamin D₃ fortification time on goat milk kefir effects vitamin D₃ content, fat content, and viscosity of goat milk kefir after fermentation. The highest vitamin D₃ content was found on the group fortification after 6 hour of fermentation. In general, fat and crude fiber content on all fortification groups were lower than control group. Meanwhile, viscosity of group fortification at 0, 6, 12, and 18 of fermentation were lower than control group and group of fortification at 24 hour of fermentation. Acidity (pH) of kefir in this study ranged from 4,45 to 4,7. All kefir samples in this study had met Codex Standard of Total Lactic Acid Bacteria in kefir ($\geq 10^7$ CFU/ml). Vitamin D₃ fortification performed at 6 hour of fermentation was recommended for future research. Encapsulation of vitamin D₃ should be considered to increase the availability of vitamin D₃.

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