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Microbiological, chemical, fatty acid and antioxidant characteristics of goat milk kefir enriched with *Moringa oleifera* leaf powder during storage

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Abstract

Fortification of compounds that contain functional components can improve product quality without affecting the product characteristics in the storage. This study evaluated the effect of supplementing Moringa leaf powder (0; 0.5; 1.0; 1.5; 2.0% w/w) and different storage periods (0, 7, and 14 days) on the quality of goat milk kefir. The observed parameters were microbiological characteristics (total lactic acid bacteria (LAB), total plate count or TPC, total yeast) chemical characteristics (total solids, acidity, pH, alcohol level, free fatty acid (FFA)), fatty acid profiles, and antioxidant properties (total phenol and DPPH). The result showed that Moringa leaf powder supplemented into kefir increased acidity, total phenol, and DPPH and decreased the alcohol level. Storage time decreased TPC, total yeast, and total solids but increased alcohol, total phenol, and DPPH. We also identified 31 fatty acids. Supplementing 2% Moringa leaf powder combined with 14-day storage can produce ± twice as much total phenol and DPPH as those in the control group and does not negatively affect the quality of the kefir product.

Keywords: antioxidant; LAB; functional food; TPC; yeast.

Practical Application: Product quality improvement using organic additives.

1 Introduction

Goat milk has abundant medicinal values. It provides an antioxidant source (Alyaqoubi et al., 2014), prevents malabsorption syndrome and intestinal inflammation (Zenebe et al., 2014), improves glucose homeostasis (Liu et al., 2021), and contains more digestible protein than that in cow milk (Yangilar, 2013). Goat milk has been made into many products, including kefir, which gains popularity as a functional food. Kefir is a fermented product that involves yeast and different LAB, such as *Lactobacillus kefiranofaciens* and *Lactobacillus parakefiri* (Leite et al., 2012). Consumers are currently keen on goat milk kefir for its probiotic bacteria that help reduce glucose level (Ostadrahimi et al., 2015). Goat milk also contains antioxidant and antidiabetes (Muntafiah et al., 2018; Nurliyani et al., 2015; Yilmaz-Ersan et al., 2018), anti carcinogenic (Hatmal et al., 2018), and anti inflammatory properties (Seo et al., 2018).

Fortification of substances that are perceived to have functional components is an of the effort to improve the nutritional values and health benefits of food products (Aiello et al., 2020; Atik et al., 2021; Yépez et al., 2019). *Moringa oleifera* is a herbal plant with high antioxidant that attributes to its flavonoid content (Wu et al., 2020; Xu et al., 2019) and antibacterial properties (Abalaka et al., 2012; Delelegn et al., 2018). Previous studies have attempted to incorporate *Moringa oleifera* leaf into yogurt and reported no negative effect on the growth of yoghurt bacteria.

However, to the best of our knowledge, there are limited studies on the effect of supplementing *Moringa oleifera* leaf powder into goat milk kefir during shelf life. It is vital to investigate whether antioxidant properties in *Moringa oleifera* leaf can improve the antioxidant characteristics in kefir or whether the antibacterial properties can inhibit the bacterial growth in kefir manufacture. This fortification is expected to have no negative effect on the characteristics of the fermented product during storage. Therefore, this study aimed to evaluate the effect of supplementing *Moringa oleifera* leaf powder and storage period on the quality of goat milk kefir.

2 Materials and methods

2.1 Materials

Fresh goat milk was obtained from goat farmer associations in Pakem area (Yogyakarta, Indonesia). We used "Kefira" kefir grain manufactured by Adhigana Multi Makmur Ltd. (Yogyakarta, Indonesia) and *Moringa oleifera* leaf powder by Nasuha Herb Laboratory (Jakarta, Indonesia).

2.2 Kefir preparation

We manufactured kéfir by adopting the method from the previous study (Nurliyani et al., 2015). The goat milk was mixed with different concentrations of *Moringa oleifera* leaf powder (0, 0.5, 1.0, 1.5, and 2% w/w) and pasteurized at 85 °C for 15 minutes. The milk was inoculated with 3% grain kefir at room

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temperature then incubated for 18 hours. The fermented product was collected as a sample in a sterile container and stored at 4 °C to analyze on days 0, 7, 14 of storage. The measured parameters were total LAB, TPC, and total yeast, total solids, acidity, pH value, alcohol level, free fatty acid (FFA), fatty acid profile, total phenol, and DPPH.

2.3 Microbial analysis

Microbiological properties were tested using a method performed in a previous study (Nurliyani et al., 2014). The kefir sample was diluted to 10^{-5} and 10^{-6} by incorporating 1.0 mL kefir into 9 mL NaCl (phys). Exactly 0.1 mL of sample in 10^{-5} and 10^{-6} dilution was poured into a petri dish of each medium, i.e., Plate Count Agar (PCA) (Merck) for TPC, modified deMan, Rogosa and Sharpe (MRS) Agar (Merck) for LAB, and Ekstrak Malt Agar (MEA) (Oksoid) for yeast. The samples were incubated at 37 °C for 24 and 48 hours to count TPC and LAB, respectively.

2.4 Chemical analysis

The analysis of total solid parameter was conducted using an oven at 105 °C for ± 18 hour or until it reached the constant weight. The chemical characteristic (acidity) was determined using NaOH titration with phenolphtalin indicator and the result was expressed as lactic acid content. The pH value was measured using a pH meter and calibrated with buffer solution (pH 4). The alcohol content was determined using the Conway microdiffusion method modified by Nurliyani et al. (2015) using ethanol standard, saturared solution of potassium carbonate, and saturated solution of potassium bichromate sulfuric acid. The samples were measured at 480 nm using the spectrophotometer (Spektronik 200, Termo Ilmiah). The FFA titration was conducted by incorporating the samples with alcohol and PP indicator then titrated with 0.1N NaOH.

2.5 Analysis of fatty acid profile

The fatty acid profile of kefir was analyzed using the gas chromatography following a method by Vieira et al. (2015). The liquid phase was separated from the organic phase and injected into the gas chromatography which met the required 1:80 ratio split and 1 µL volume with a linear speed of 23.6 cm/s. We compared the retention time and peak of each component to gain information on the type of component.

2.6 Analysis of antioxidant characteristics

We determined the total phenol with a method by Biesaga & Pyrzyńska (2013) using a spectrophotometer and Folin-ciocalteu calorimetric with the standard curve of galactic acid. The effect of kefir on the level of 2.2-Diphenyl-pirrylhydrazyl (DPPH) radicals was measured in a procedure by Boligon et al. (2014), and the sample was diluted with methanol and addition of DPPH.

2.7 Statistical analysis

The results were subjected to the multivariate analysis of variance and Duncan's New Multiple Range Test ($\alpha = 0.05$) using SPSS 16.0.

3 Result

3.1 Microbiological characteristics

The average total LAB in the kefir samples was 6.17 log CFU (Table 1). The supplementation of *Moringa oleifera* leaf and the storage period significantly decreased the total LAB ($P<0.05$) but did not affect the TPC and yeast ($P>0.05$). TPC and yeast were significantly ($P<0.05$) affected by the storage period. Compared to the control group, 2% w/w *Moringa oleifera* leaf alone into kefir manufacture could decreased the total LAB by 6.73%, while the storage period contributed to the decrease of total LAB by 5.5%, TPC by 3.05%, and yeast by 12.8%.

3.2 Chemical characteristics

The chemical characteristics of kefir include total solids, acidity, pH, alcohol content, and FFA (Table 2). The supplementation of *Moringa oleifera* leaf powder significantly increased acidity

Table 1. Microbiological characteristics of goat milk kefir fortified with *Moringa oleifera* leaf powder during storage.

Parameter	Storage (days)	Level of supplementation of *Moringa oleifera* leaf powder (%)					Mean
		0	0.5	1.0	1.5	2.0	
LAB (log CFU)	0	6.39 ± 0.48	6.55 ± 0.47	6.68 ± 0.62	6.16 ± 0.11	6.02 ± 0.46	6.36 ± 0.43 ^{p}
	7	5.96 ± 0.19	6.10 ± 0.14	6.51 ± 0.54	6.07 ± 0.06	6.01 ± 0.56	6.13 ± 0.30 ^{p}
	14	6.04 ± 0.90	6.06 ± 0.09	6.03 ± 0.10	5.97 ± 0.17	5.95 ± 0.18	6.01 ± 0.14 ^{q}
	Mean	6.133 ± 0.32^{ab}	6.24 ± 0.35 ^{ab}	6.41 ± 0.51 ^{b}	6.07 ± 0.13 ^{a}	5.99 ± 0.10 ^{a}	6.17 ± 0.34
TPC (log CFU)	0	8.04 ± 0.07	7.86 ± 0.12	7.97 ± 0.31	7.74 ± 0.45	7.28 ± 0.66	7.78 ± 0.43 ^{p}
	7	8.20 ± 0.09	8.08 ± 0.1	8.16 ± 0.13	8.29 ± 0.48	8.33 ± 0.46	8.21 ± 0.27 ^{pq}
	14	7.93 ± 1.73	7.34 ± 0.61	7.9 ± 0.98	7.3 ± 1.54	7.29 ± 1.12	7.55 ± 1.11 ^{q}
	Mean	8.05 ± 0.87	7.76 ± 0.45	8.01 ± 0.53	7.78 ± 0.94	7.63 ± 0.86	7.85 ± 0.74
Yeast (log CFU)	0	5.85 ± 0.28	6.54 ± 0.59	6.42 ± 0.79	5.91 ± 1.35	5.92 ± 0.20	6.13 ± 0.5 ^{p}
	7	4.94 ± 0.25	5.42 ± 0.69	5.52 ± 0.55	5.54 ± 0.44	5.27 ± 0.42	5.34 ± 0.47 ^{q}
	14	5.29 ± 0.6	5.59 ± 0.58	5.25 ± 0.51	5.62 ± 0.45	5.29 ± 0.43	5.41 ± 0.47 ^{q}
	Mean	5.36 ± 0.53	5.85 ± 0.75	5.73 ± 0.76	5.69 ± 0.36	5.49 ± 0.45	5.62 ± 0.59

^{a,b}Different superscripts within line indicated $P<0.05$; ^{p,q} Different superscripts within line indicated $P<0.05$.

Table 2. Chemical characteristics of goat milk kefir fortified with *Moringa oleifera* leaf powder during storage.

Parameter	Storage (days)	Level of supplementation of *Moringa oleifera* leaf powder (%)					Mean
		0	0.5	1.0	1.5	2.0	
Total Solids (%)	0	18.55 ± 2.51	17.64 ± 0.89	18.46 ± 2.32	17.39 ± 1.77	18.77 ± 0.925	18.16 ± 1.63^{p}
	7	17.03 ± 1.57	17.10 ± 0.56	17.3 ± 1.04	17.25 ± 1.10	18.41 ± 0.522	17.42 ± 1.02^{pq}
	14	16.13 ± 1.24	16.65 ± 1.59	15.84 ± 0.98	16.36 ± 0.64	18.07 ± 0.87	16.61 ± 1.23^{q}
	Mean	17.23 ± 1.924	17.13 ± 1.04	17.20 ± 1.77	17.00 ± 1.19	18.41 ± 0.73	17.39 ± 1.44
Acidity (%)	0	0.71 ± 0.61	0.84 ± 0.07	1.02 ± 0.02	0.89 ± 0.13	1.11 ± 0.02	0.91 ± 0.15
	7	0.95 ±0.13	0.97 ± 0.24	1.12 ± 0.28	1.09 ± 0.13	1.27 ± 0.37	1.08 ± 0.26
	14	0.97 ± 0.24	0.92 ± 0.36	1.06 ± 0.28	1.08 ± 0.34	1.25 ± 0.34	1.05 ± 0.29
	Mean	0.87 ± 0.19 ^{b}	0.91 ± 0.22 ^{b}	1.06 ± 0.21 ^{ab}	1.02 ± 0.25 ^{ab}	1.21 ± 0.26 ^{a}	1.01 ± 0.25
pH	0	5.54 ± 0.23	5.37 ± 5.02	5.19 ± 0.64	5.3 ± 0.17	5.07 ± 0.17	5.29 ± 0.23
	7	5.11 ± 0.38	5.02 ± 0.48	5.02 ± 0.49	5.1 ± 0.47	4.87 ± 0.54	5.02 ± 0.41
	14	5.04 ± 0.52	5 ± 0.68	4.92 ± 0.63	5.06 ± 0.59	4.75 ± 0.66	4.95 ± 0.53
	Mean	5.23 ± 0.41	5.13 ± 0.47	5.04 ± 0.41	5.15 ± 0.4	4.9 ± 0.45	5.09 ± 0.43
Alcohol level (% wb)	0	0.07 ± 0	0.07 ± 0	0.07 ± 0	0.06 ± 0	0.073 ± 0.03	0.07 ± 0.01 ^{p}
	7	0.17 ± 0.01	0.15 ± 0.01	0.14 ± 0.01	0.1 ±0.01	0.11 ± 0.01	0.14 ± 0.03 ^{q}
	14	0.41 ± 0.04	0.32 ± 0.02	0.22 ± 0.02	0.13 ± 0.03	0.13 ± 0.05	0.24 ± 0.11 ^{r}
	Mean	0.21 ± 0.15 ^{d}	0.18 ± 0.11 ^{c}	0.14 ±0.06 ^{b}	0.09 ± 0.03 ^{a}	0.1 ± 0.04 ^{a}	0.15 ± 0.09
FFA (% wb)	0	0.43 ± 0.01	0.34 ± 0.04	0.26 ± 0.02	0.11 ± 0.01	0.19 ± 0.12	0.26 ± 0.12 ^{p}
	7	0.45 ± 0.01	0.38 ± 0.02	0.31 ± 0.47	0.27 ± 0.02	0.25 ± 0.03	0.33 ± 0.08 ^{q}
	14	0.6 ± 0.03	0.47 ± 0.04	0.86 ± 0.02	0.34 ±0.02	0.46 ± 0.18	0.44 ± 0.12 ^{r}
	Mean	0.49 ± 0.81 ^{a}	0.39 ± 0.07 ^{b}	0.31 ± 0.05 ^{c}	0.24 ± 0.1 ^{d}	0.3 ± 0.16 ^{c}	0.34 ± 0.13

^{a,b,c,d}Different superscripts within line indicated P<0.05; ^{p,q,r}Different superscripts within line indicated P<0.05.

by 39.1% but did not affect total solids. The storage period significantly decreased (P<0.05) total solids by 8.5% but did not affect acidity. The pH value of kefir in this study (5.09) was not affected by the supplementation of *Moringa oleifera* leaf powder nor the storage period (P>0.05).

Contrastive effects of *Moringa oleifera* leaf powder and storage duration were observed from the alcohol content and FFA of kefir goat milk. The alcohol content was decreased (52.38%) by *Moringa oleifera* leaf powder but increased (70.38%) when stored for 14 days (P<0.05). The FFA significantly decreased by 38.77% (P<0.05) due to 2% supplementation of *Moringa oleifera* leaf powder but increased (40.1%) by storage time.

3.3 Fatty acid profile

Table 3 shows that goat milk kefir fortified by *Moringa oleifera* leaf powder and stored for 14 days contained 31 fatty acids. The highest average of fatty acids in this study was Palmitoleic Acid (C16:1), Cis-9-Octadecenoate (Trans-9-Octadecenoate) Acid Methyl Ester, Capric Acid (C10:0), and Palmitic Acid (C16:0). Supplementing *Moringa oleifera* leaf powder when manufacturing kefir did not significantly affect all fatty acids, but it decreased the concentration of Cis-5,8,11,14,17-Eicosapentaenoic Acid (C20:5n3). The storage period affected almost all components of fatty acid. In particular, Oleic Acid (C18:1n9c), Lenolelaidic Acid Methyl Ester (18:2), and Linolenic Acid (C18:3n3) were not detected on days 7 and 14 of storage. However, the storage period increased Palmitoleic Acid (C16:1) and Cis-9-Octadecenoate Acid Methyl Ester (C18:1).

The average fatty acids of the short-chain (C2-C6), medium-chain (C7-C11), and long-chain (>C12) in this study were 3.89%, 12.76%, and 83.35%, respectively. Our data showed that most (64.11%) of these fatty acids were Unsaturated Fatty Acid (USFA) and the rest 35.89% were the Saturated Fatty Acids (SFA). The dominant component in USFA is Capric Acid (C10:0) (8.87%) and in SFA is Palmitoleic Acid (C16:1) (20.18%). The Monounsaturated Fatty Acid (MUFA) surpassed the Polyunsaturated fatty Acid (PUFA) with an average of 45.42% and 18.69%, respectively, of the total USFA. About 44.42% of the MUFA are derived from Palmitoleic acid (C16:1), while PUFA is composed of relatively similar quantities across components.

3.4 Antioxidant characteristics

The antioxidant properties of the goat milk kefir fortified with *Moringa oleifera* leaf powder are expressed in total phenol and DPPH. The antioxidant mechanism can be measured through DPPH radical-scavenging mechanism using the free radical protons to evaluate the radical scavenging activity. The result of supplementing goat milk kefir with *Moringa oleifera* leaf powder on the total phenol and DPPH is presented in Table 4. We observed an interaction between the fortification and storage period with the total phenol (P<0.05); the higher the supplementation and the longer the storage period, the higher the total phenol in the kefir. Likewise, *Moringa oleifera* leaf powder combined with the storage period would significantly (P<0.05) increase the DPPH – the higher the supplementation and the longer the storage period, the higher the DPPH activity. This study showed that incorporating up to 2% of *Moringa oleifera* leaf powder could increase the total phenol (46.44%) and DPPH (59.65%) compared to those of the control. Additionally, storing the kefir in a refrigerator for 14 days increased the total phenolic

Table 3. Fatty acid profile of goat milk kefir fortified with *Moringa oleifera* leaf powder during storage period.

Components	Storage period	Level of supplementation of *Moringa oleifera* leaf powder (%)					
		0	0.5	1.0	1.5	2.0	Mean
Saturated Fatty Acid (SFA)							
Butirat Acid (C4:0)	1	0.75 ± 0.11	0.75 ± 0.26	1,01 ± 0.28	1.26 ± 0.78	1.03 ± 0.25	0.99 ± 0.38^{q}
	7	1.42 ± 0.49	1.42 ± 0.85	2.02 ± 0.23	1.58 ± 0.02	1.54 ± 0.15	1.66 ± 0.44^{p}
	14	1.57 ± 0.83	1.57 ± 0.83	1.13 ± 0.36	0.98 ± 0.59	1.08 ± 0.05	1.13 ± 0.48^{q}
	Mean	1.18 ± 0.51	1.24 ± 0.72	1.39 ± 0.55	1.27 ± 0.55	1.21 ± 0.28	1.26 ± 0.52
Caproic Acid (C6:0)	1	0.91 ± 0.11	0.75 ± 0.90	1.01 ± 0.28	1.26 ± 0.78	1.03 ± 0.26	1.98 ± 0.46^{q}
	7	1.75 ± 0.5	1.42 ± 0.90	2.03 ± 0.23	1.58 ± 0.02	1.54 ± 0.15	3.33 ± 0.36^{p}
	14	0.90 ± 0.22	1.58 ± 0.76	1.13 ± 0.33	0.98 ± 0.60	1.08 ± 0.05	2.57 ± 0.39^{q}
	Mean	1.19 ± 0.51	1.25 ± 0.72	1.39 ± 0.54	1.27 ± 0.56	1.22 ± 0.29	2.63 ± 0.97
Caprilic Acid (C8:0)	1	3.3 ± 0.66	3.15 ± 0.8	2.77 ± 0.5	3.84 ± 0.91	3.23 ± 0.85	3.26 ± 0.71^{q}
	7	4.2 ± 0.76	4.75 ±0.31	4.73 ± 0.22	4.16 ± 0.72	4.25 ± 0.58	4.42 ± 0.55^{p}
	14	3.37 ± 0.36	4.34 ± 2.06	3.2 ± 0.38	3.12 ± 0.26	3.39 ± 0.19	3.48 ± 0.93^{q}
	Mean	3.62 ± 0.69	4.08 ± 1.32	3.56 ± 0.92	3.71 ± 0.74	3.62 ± 0.71	3.72 ± 0.88
Capric Acid (C10:0)	1	6.19 ± 5.64	5.11 ± 4.42	5.21 ± 4.51	6.91 ± 6.55	5.14 ± 4.46	5.71 ± 4.45^{q}
	7	11.99 ± 2.34	13.48 ± 0.38	13.56 ± 0.46	12.33 ± 1.49	12.61 ± 1.48	12.8 ± 1.37^{p}
	14	5.82 ± 5.07	5.95 ± 5.18	10.32 ± 2.05	9.05 ± 1.31	9.47 ± 0.56	8.12 ± 3.48^{q}
	Mean	8 ± 4.98	8.17 ± 5.25	9.69 ± 4.42	9.43 ± 4.16	9.08 ± 4.01	8.87 ± 4.43
Undecanoic Acid (C11:0)	1	0.9 ± 0.15	0.12 ± 0.21	0.08 ± 0.13	0.14 ± 0.24	0.24 ± 0.42	0.13 ± 0.22^{q}
	7	0.4 ± 0.07	0.04 ± 0.69	0.04 ± 0.69	0 ± 0	0 ± 0	0.02 ± 0.05^{q}
	14	0.28 ± 0.2	0.42 ± 0.16	0.42 ± 0.16	0.34 ± 0.72	0.44 ± 0.14	0.35 ± 0.11^{p}
	Mean	0.13 ± 0.14	0.19 ± 0.22	0.19 ± 0.22	0.16 ± 0.19	0.23 ± 0.29	0.17 ± 0.2
Lauric Acid (C12:0)	1	2.39 ± 2.17	1.97 ± 1.71	2.03 ± 1.76	2.71 ± 2.49	2.1 ± 1.64	2.24 ± 1.69^{q}
	7	4.59 ± 0,91	5.04 ± 0.16	5.18 ± 0.14	4.76 ± 0.39	3.18 ± 2.65	4.55 ± 1.31^{p}
	14	2.19 ± 1.91	2.37 ± 2.06	2.48 ± 2.21	1.41 ± 2.36	1.22 ± 1.97	1.93 ± 1.86^{q}
	Mean	3.06 ± 1.91	3.13 ± 1.97	3.23 ± 2.04	2.96 ± 2.26	2.17 ± 2.04	2.91 ± 1.99
Tridecanoic Acid (C13:0)	1	0.86 ± 0.08	0.07 ± 0.06	0.4 ± 0.06	0.05 ± 0.8	0 ± 0	0.04 ± 0.06
	7	0.04 ± 0.06	0.03 ± 0.06	0.03 ± 0.05	0 ± 0	0.09 ± 0.08	0.04 ± 0.06
	14	0 ± 0	0.07 ± 0.13	0 ± 0	0.09 ± 0.08	0.04 ± 0.07	0.04 ± 0.07
	Mean	0.41 ± 0.06	0.06 ± 0.08	0.02 ± 0.05	0.02 ± 0.05	0.04 ± 0.06	0.04 ± 0.06
Myristic Acid (C14:0)	1	5.76 ± 5.11	4.92 ± 4.26	4.65 ± 3.93	6.11 ± 5.69	4.68 ± 3.93	5.23 ± 3.97^{p}
	7	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0^{q}
	14	2.27 ± 3.84	0.13 ± 0.14	0.08 ± 0.07	0.08 ± 0.08	2.47 ± 4.17	1.01 ± 2.43^{q}
	Mean	2.67 ±4.06	1.68 ± 3.23	1.58 ± 3.03	2.06 ± 4.16	2.38 ± 3.51	2.07 ± 3.48
Pentadecanoic Acid (C15:0)	1	0.7 ± 0.19	0.62 ± 0.09	0.43 ± 0.37	0.53 ± 0.47	0.6 ± 0.05	0.57 ± 0.26^{q}
	7	0.87 ± 0.22	0.96 ± 0.01	0.94 ± 0.02	0.97 ± 0.05	±1.02 0.04	0.95 ± 0.1^{p}
	14	0.24 ± 0.06	0.29 ± 0.09	0.32 ± 0.07	0.33 ± 0.15	0.41 ± 0.21	0.32 ± 0.12^{r}
	Mean	0.61 ± 0.32	0.62 ± 0.29	0.56 ±0.34	0.61 ± 0.38	0.67 ± 0.29	0.62 ± 0.32
Palmitic Acid (C16:0)	1	17.62 ± 15.71	14.46 ± 12.24	16.6 ± 12.43	18.31 ± 16.66	14.86 ± 12.7	15.97 ± 12.0^{p}
	7	17.16 ± 16.03	20.50 ± 17.76	10.12 ± 17.52	0 ± 0	0 ± 0	9.55± 14.24^{pq}
	14	0.24 ± 0.07	0.29 ± 0.16	0.23 ± 0.06	0.2 ± 0.35	0.21 ± 0.09	0.24 ± 0.08^{q}
	Mean	11.67 ± 14.12	11.75 ± 14.03	8.32 ± 12.49	6.17 ± 12.34	5.02 ± 9.73	8.59 ± 12.37
Heptadecanoic Acid (C17:0)	1	0.91 ± 0.78	0.73 ± 0.94	0.26 ± 0.23	0.84 ± 0.98	0.52 ± 0.58	0.65 ± 0.68^{q}
	7	0.46 ± 0.13	0.51 ± 0.02	0.52 ± 0.04	0.57 ± 0.1	0.39 ± 0.34	0.49 ± 0.15^{q}
	14	1.23 ± 0.47	1.66 ± 0.94	1.35 ± 0.24	1.17 ± 0.25	0.98 ± 0.06	1.28 ± 0.48^{p}
	Mean	0.86 ± 0.57	0.97 ± 0.85	0.71 ± 0.52	0.86 ± 0.57	0.63 ± 0.43	0.81± 0.59
Stearic Acid (C180)	1	5.17 ± 8.96	9.92 ± 8.59	10 ± 8.68	11.85 ± 10.9	9.88 ± 8.46	9.36 ±8.08^{p}
	7	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0^{q}
	14	0.15 ± 0.16	0.07 ± 0.06	0.09 ± 0.08	0.1 ± 0.09	0.08 ± 0.07	0.10 ± 0.92^{q}
	Mean	1.77 ± 5.15	3.33 ± 6.6	3.36 ± 6.6	3.98 ± 8.03	3.32 ±6.49	3.15 ± 6.36
Arachidic Acid (20:0)	1	0.95 ± 1.28	0.78 ± 0.84	0.68 ± 0.95	0.69 ± 1.01	0.73 ± 0.54	0.76 ± 0.81^{q}
	7	0.06 ± 0.11	0.19 ± 0.01	0.16 ± 0.04	0.11 ± 0.1	0.11 ± 0.09	0.13 ± 0.08^{r}
	14	1.62 ± 0.61	2.11 ± 1.28	2.29 ± 1.08	1.49 ± 0.41	0.9 ± 0.64	1.68 ± 0.89^{p}
	Mean	0.88 ± 0.98	1.02 ± 1.14	1.04 ± 1.2	0.76 ± 0.81	0.58 ± 0.55	0.86 ± 0.94

^{a,b}Different superscripts within line indicated P<0.05; ^{p,q,r}Different superscripts within line indicated P<0.05.

Table 3. Continued...

Components	Storage period	Level of supplementation of *Moringa oleifera* leaf powder (%)					
		0	0.5	1.0	1.5	2.0	Mean
Tricosanoic Acid (C23:0)	1	0.18 ± 0.16	0 ± 0	0.09 ± 0.15	0.13 ± 0.22	0.11 ± 0.2	0.1 ± 0.15^{q}
	7	0.26 ± 0.07	0.15 ± 0.13	0.24 ± 0.02	0.21 ± 0.03	0.16 ± 0.15	0.2 ± 0.09^{p}
	14	0.28 ± 0.04	0.31 ± 0.16	0.33 ± 0.12	0.29 ± 0.05	0.08 ± 0.14	0.26 ± 0.14^{p}
	Mean	0.24 ± 0.1	0.15 ± 0.17	0.22 ± 0.15	0.21 ± 0.14	0.12 ± 0.15	0.19 ± 0.14
Unsaturated Fatty Acid (USFA)							
Myristoleic Acid (C14:1)	1	3.39 ± 5.64	3.56 ± 6.17	2.77 ± 4.72	3.78 ± 6.54	3.08 ± 5.18	3.32 ± 4.82^{q}
	7	0.16 ± 0.06	0.11 ± 0.09	0.11 ± 0.19	0.11 ± 0.98	0.09 ± 0.08	0.12 ± 0.1^{r}
	14	6.23 ± 5.71	11.77 ± 6.64	9.3 ± 1.68	8.37 ± 1.87	5.39 ± 4.61	8.21 ± 4.52^{p}
	Mean	3.26 ± 4.8	5.15 ± 6.89	4.06 ± 4.8	4.09 ± 4.94	2.85 ± 4.16	3.88 ± 5.02
Cis-10-pentadecenoic Acid Metyl Ester (C15:1)	1	0.14 ± 0.13	0.39 ± 0.53	0,33 ± 0.43	0 ± 0	0.36 ± 0.45	0.24 ± 0.35^{q}
	7	0 ± 0	0 ± 0	0.08 ± 0.14	0.08 ± 0.14	0.09 ± 0.16	0.05 ± 0.11^{q}
	14	0.42 ± 0.36	0.86 ± 0.93	0.53 ± 0.48	0.55 ± 0.49	0.51 ± 0.44	0.57 ± 0.51^{p}
	Mean	0.18 ± 0.26	0.41 ± 0.65	0.31 ± 0.38	0.21 ± 0.35	0.32 ± 0.37	0.29 ± 0.42
Palmitoleic Acid (C16:1)	1	12.34 ± 18.87	12.04 ± 18.69	10 ± 14.95	13.32 ± 19.94	10.24 ± 15.79	11.58 ± 15.1^{q}
	7	11.87 ± 17.78	12.95 ± 18.36	23.42 ± 18.44	34.63 ± 1.18	35.27 ± 1.5	23.63±16.68^{pq}
	14	26.5 ± 8.5	23.48 ± 1.04	27.85 ± 5.15	25.97 ± 5.33	23.94 ± 1.67	25.70 ± 4.77^{p}
	Mean	16.9 ± 15.42	15.24 ± 14.98	20.42 ± 14.58	24.64 ± 13.89	23.14 ± 13.47	20.18 ± 14.24
cis-10-Heptadecanoic Acid (C17:1)	1	0.27 ± 0.22	0.36 ± 0.63	0.2 ± 0.25	0.76 ± 0.13	0.12 ± 0.21	0.14 ± 0.18^{q}
	7	0.13 ± 0.11	0.19 ± 0	0.19 ± 0.15	0.26 ± 0.11	0.19 ± 0.01	0.19 ± 0.07^{q}
	14	0.38 ± 0.2	0.48 ± 0.25	0.4 ± 0.13	0.33 ± 0.06	0.33 ± 0.06	0.38 ± 0.14^{p}
	Mean	0.26 ± 0.19	0.23 ± 0.23	0.26 ± 0.17	0.22 ± 0.14	0.21 ± 0.14	0.24 ± 0.17
Oleic Acid (C18:1n9c)	1	16.11 ± 13.98	16.41 ±14.22	16.77 ± 14.54	7.97 ± 13.81	16.61 ± 14.39	14,77 ± 12.5^{p}
	7	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0^{q}
	14	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0^{q}
	Mean	5.37 ± 10.66	5.47 ± 10.86	5.59 ± 11.10	2.65 ± 7.97	5.53 ± 10.98	4.92 ± 9.96
Lenolelaidic Acid Methyl Ester (18:2)	1	2.03 ± 3.52	3.97 ± 3.44	4.08 ± 3.55	2.05 ± 3.56	±1.97 3.41	2.82 ± 3.13^{p}
	7	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0^{q}
	14	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0^{q}
	Mean	0.67 ± 2.03	1.32 ± 2.62	1.36 ± 2.71	0.68 ± 2.05	0.65 ± 1.97	0.94 ± 2.21
y-Linolenic Acid (C18:3n6)	1	0.23 ± 0.04	2.66 ± 4.61	0.07 ± 0.13	2.99 ± 4.87	0.12 ± 0.11	1.22 ± 2.88^{q}
	7	0.25 ± 0.07	0.09 ± 0.17	0 ± 0	0.08 ± 0.14	0.08 ± 0.13	0.1 ± 0.13^{q}
	14	4.39 ± 3.59	4.3 ± 3.48	2.33 ± 3.57	11.34 ± 15.41	4.1 ± 3.44	5.29 ± 7.17^{p}
	Mean	1.62 ± 2.74	2.35 ± 3.42	0.8 ± 2.12	4.81 ± 9.53	1.43 ± 2.64	2.2 ± 4.91
Cis-11-Eicosenoic Acid (C20:1)	1	0.04 ± 0.08	0.1 ± 0.09	0.06 ± 0.1	0.05 ± 0.09	0.18 ± 0.18	0.08 ± 0.11^{q}
	7	0.12 ± 0.11	0.17 ± 0.01	0.12 ± 0.1	0.2 ± 0,03	0.12 ± 0.1	0.15 ±0.08^{q}
	14	0.19 ± 0.17	0.44 ± 0.31	0.34 ± 0.39	0.28 ± 0.14	0.33 ± 0.05	0.32 ± 0.22^{p}
	Mean	0.12 ± 0.13	0.24 ± 0.22	0.17 ± 0.24	0.18 ± 0.13	0.21 ± 0.14	0.18 ± 0.18
Linolenic Acid (C18:3n3)	1	0.45 ± 0.78	1.03 ± 0.9	1.38 ± 1.2	1.03 ± 1.79	1.94 ± 2.73	1.17 ± 1.48^{p}
	7	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0^{q}
	14	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0^{q}
	Mean	0.15 ± 0.45	0.34 ± 0.68	0.46 ± 0.91	0.34 ± 1.03	0.64 ± 1.67	0.39 ± 1
Cis-11,14-Eicosedienoic Acid (C20:2)	1	0.73 ± 0.48	0.68 ± 0.43	0.6 ± 0.52	0.37 ± 0.44	0.78 ± 0.28	0.63 ± 0.4
	7	0.38 ± 0.2	0.55 ± 0.13	0.3 ± 0.23	0.27 ± 0.27	0.13 ± 0.11	0.13 ± 0.11
	14	0.28 ± 0.26	0.37 ± 0.38	0.42 ± 0.28	0.93 ± 1.03	0.36 ± 0.11	0.36 ± 0.11
	Mean	0.46 ± 0.35	0.53 ± 0.32	0.44 ± 0.34	0.52 ± 0.65	0.42 ± 0.33	0.42 ± 0.32
Cis-5,8,11,14,17-Eicosapentaenoic Acid (C20:5n3)	1	0 ± 0	0.07 ± 0.13	0.07 ± 0.12	0.08 ± 0.13	0 ± 0	0.04 ± 0.09
	7	0.22 ± 0.02	0.23 ± 0.01	0.22 ± 0.02	0.21 ± 0.1	0.07 ± 0.13	0.19 ± 0.07
	14	0.25 ± 0.04	0.29 ± 0.18	0.31 ± 0.17	0.03 ± 0.06	0.14 ± 0.12	0.2 ± 0.15
	Mean	0.15 ± 0.12^{ab}	0.2 ± 0.15^{b}	0.19 ± 0.14^{b}	0.11 ± 0.11^{ab}	0.07 ± 0.11^{a}	0.14 ± 0.13
Cis-13-16-Docosadienoic Acid Methyl Ester (C22:2) (Cis13,16)	1	3.49 ± 2.62	3.18 ± 2.44	1.84 ± 2.75	2.2 ± 1.86	3.69 ± 2.48	2.88 ± 2.2
	7	13.54 ± 20.39	3.21 ± 0.99	1.78 ± 1.51	1.66 ± 1.71	0.72 ± 0.45	4.18 ± 9.19
	14	1.2 ± 0.64	0.4 ± 0.36	0.35 ± 0.31	3.9 ± 6.34	0.77 ± 0.43	1.32 ± 2.78
	Mean	6.07 ± 11.75	2.27 ± 1.93	1.32 ± 1.74	2.59 ± 3.56	1.73 ± 1.95	2.79 ± 5.68

^{a,b}Different superscripts within line indicated P<0.05; ^{p,q,r}Different superscripts within line indicated P<0.05.

Table 3. Continued...

Components	Storage period	Level of supplementation of *Moringa oleifera* leaf powder (%)					
		0	0.5	1.0	1.5	2.0	Mean
Cis-9-Octadecenoate Acid Methyl Ester (C18:1)	1	0 ± 0	0 ± 0	9.99 ± 17.3	5.34 ± 9.24	7.79 ± 16.96	5.03 ± 10.8^{q}
	7	17.39 ± 5.34	19.26 ± 1.63	19.48 ± 0.93	22. 19 ± 5.05	20.72 ± 0.55	19.81 ± 3.32^{p}
	14	8.74 ± 7.57	14 83 ± 14.72	8.86 ± 15.35	4.3 ± 6.65	18.17 ± 7.94	10.98±10.67^{q}
	Mean	8.71 ± 8.85	11.36 ± 11.45	11.36 ± 11.45	10.61 ± 10.7	16.22 ± 10.59	11.94 ± 10.71
Linolelaidic Acid Methyl Ester + Methyl Linoleate (C18:2)	1	13.52 ± 23.41	11.28 ± 19.54	8.08 ± 14	4.87 ± 8.44	5.36 ± 9.28	8.62 ± 13.97^{q}
	7	0 ± 0	0 ± 0	0 ± 0	0.10 ± 0.17	2.75 ± 4.77	0.57 ± 2.12^{p}
	14	29.3 ± 6.08	30.9 ± 18.9	24.11 ± 9.02	22.65 ± 5.32	22.19 ±6.31	25 .83 ± 9.58^{q}
	Mean	14.27 ± 17.53	14.96 ± 19.21	10.73 ± 13.5	9.21 ± 11.44	10.1 ± 10.98	11.67 ± 14.36
Methyl Linolenic (C18:3)	1	0 ± 0	0.09 ± 0.17	0.07 ± 0.13	0.1 ± 0.17	0.07 ± 0.11	0.07 ± 0.12^{q}
	7	1.2 ± 0.25	1.58 ± 0.12	1.71 ± 0.05	1.67 ± 1.56	2.01 ± 0.36	1.63 ± 0.67^{p}
	14	0.6 ± 0.56	0.33 ± 0.13	0.34 ± 0.08	0.13 ± 0.12	0.17 ± 0.01	0.32 ± 0.29^{q}
	Mean	0.6 ± 0.6	0.67 ± 0.7	0,.71 ± 0.76	0.63 ± 1.11	0.75 ± 0.97	0.67 ± 0.81
Methyl Myristoleic (C14:1)	1	0.04 ± 0.08	0.56 ± 0.98	0.04 ± 0.07	0.06 ± 0.1	0 ± 0	0.04 ± 0.07^{q}
	7	9.75 ± 2.29	10. 69 ± 0.18	11.14 ± 0.45	10.69 ± 0.09	11.06 ± 0.75	10.67 ± 1.06^{p}
	14	0.05 ± 0.08	0.03 ± 0.06	0.04 ± 0.08	0.03 ± 0.06	0.03 ± 0.06	0.39 ± 0.06^{q}
	Mean	3.28 ± 4.98	3.59 ± 5.32	3.74 ± 5.55	3.59 ± 5.32	3.7 ± 5.54	3.58 ± 5.1
Cis-10-Pentadecenoic Acid Methyl Ester (C15:1)	1	0.34 ± 0.59	0.33 ± 0.57	0,28 ± 0.48	0.36 ± 0.62	0 ± 0	0.26 ± 0.45
	7	0.23 ± 0.05	0.27 ± 0.03	0,18 ± 0.16	0.06 ± 0.11	0.18 ± 0.16	0.18 ± 0.12
	14	0.35 ± 0.61	0.23 ± 0.38	0.31 ± 0.53	0.2 ± 0.35	0.23 ± 0.39	0.26 ± 0.4
	Mean	0.31 ± 0.43	0.28 ± 0.35	0.26 ± 0.37	0.21 ± 0.38	0.13 ± 0.23	0.23 ± 0.34

^{a,b}Different superscripts within line indicated P<0.05; ^{p,q,r}Different superscripts within line indicated P<0.05.

Table 4. Antioxidant properties goat milk kefir fortified with *Moringa oleifera* leaf powder selama penyimpanan.

Parameter	Storage (days)	Level of supplementation of *Moringa oleifera* leaf powder (%)					Mean
		0	0.5	1.0	1.5	2.0	
Total phenol (mg as galat/100 gr)	0	35.96 ± 2.02	43.66 ± 3.84	57.82 ± 4.02	66.86 ± 4.71	70.06 ± 7.73	54.87 ± 14.22 ^{q}
	7	76.54 ± 2.12	97.83 ± 5.85	122.33 ± 3.97	1.35.36 ± 4.22	139.90 ± 5.02	115.47 ± 26.43 ^{p}
	14	80.45 ± 6.22	87.74 ± 2.69	102.08 ± 7.27	143.29 ± 10.88	144.97 ± 10.29	111.71 ± 29.14 ^{p}
	Mean	64.32 ± 21.6 ^{d}	76.41 ± 25.22 ^{c}	94.67 ± 28.87 ^{b}	115.8 ± 36.93 ^{a}	120.1 ± 38.39 ^{a}	94.02 ± 36.64
DPPH (% wb)	0	6.67 ± 0.67	18.71 ± 1.66	23. 82 ± 1.21	34.83 ± 1.14	28.96 ± 11.67	22.6 ± 10.91 ^{r}
	7	17.17 ± 0.39	28.27 ± 0.56	31.72 ± 0.66	40.97 ± 0.94	45.31 ± 1.22	32.69 ± 10.26 ^{q}
	14	23.97 ± 2.13	31.23 ± 0.23	35.39 ± 1.64	43.65 ± 1.28	44.17 ± 0.255	35.68 ± 7.99 ^{p}
	Mean	15.93 ± 7.63 ^{d}	26.07 ± 5.73 ^{c}	30.31 ± 5.23 ^{b}	39.82 ± 4.03 ^{a}	39.48 ± 9.84 ^{a}	30.32 ± 11.12

^{a,b}Different superscripts within line indicated P<0.05. ^{p,q,r}Different superscripts within line indicated P<0.05.

content (52.48%) and DPPH (36.65%) compared to the non-refrigerated kefir.

4 Discussion

4.1 Microbiological characteristics

Moringa oleifera leaf is reported to contain antibacterial properties. Further analysis on the chemical composition reveals that the contributing factors to antibacterial activities are alkaloid, polyphenol, flavonoid, anthraquinone, coumarin, tannin, triterpene, sterols, saponin, and some other secondary metabolites. These properties are considered to help *Moringa oleifera* leaf supplementation to inhibit the fermentation process, thus decreasing the total LAB in kefir (Wang et al., 2016).

However, supplementing up to 2% *Moringa oleifera* leaf powder did not affect the level of TPC and yeast, which is probably due to the low level of antibacterial activities from the Moringa leaf powder. As a result, the TPC and yeast parameters are not affected. This result is in line with Gronnevik et al. (2011) that the storage period could diminish the total LAB of kefir. In contrast, (Gul et al., 2015) reported that the storage period increased the level of yeast.

The modified level of TPC during the storage period is indicative of the ability of microorganisms to grow at refrigerator temperature. Table 1 shows that TPC increases when stored for seven days but decreases on the 14th day. It indicates that the cool storage does not extinguish the microbes but only slowing down the metabolism, thus inhibiting microbial growth (Kimestri, 2018). This study observed that the longer the storage period, the lower the yeast, which contradicts (Gronnevik et al., 2011) reporting an increase in total yeast with storage period that is probably due to the antimicrobial properties of the *Moringa oleifera* leaf powder.

The total LAB in this study was lower than 8.63 log CFU/mL reported by Jascolka et al. (2013). The mean of total LAB in this study is 5.09 log CFU/mL, which is below the standard for fermented milk products according to Codex No. 234, i.e., minimum of 7 log CFU/mL; therefore, the total LAB is substandard (even in kefir without *Moringa oleifera* leaf powder). The average TPC in this study (7.85 log CFU/mL) is similar to 8.89-9.91 log CFU/mL (Nurliyani et al., 2014) and 7.85-9.53 log CFU/mL (HadiNezhad et al., 2013), and the mean total yeast (5.62 log CFU/mL) is almost equal to 5.81 log CFU/mL (Jascolka et al., 2013). Codex No. 234 sets the minimum of TPC and yeast for fermented milk product is 7 log CFU/mL and 4 log CFU/mL, respectively.

4.2 Chemical characteristics

The result of total solids in this study showed that supplementing *Moringa oleifera* leaf powder up to 2% w/w could not improve the total solid of kefir product despite the high level of dry matter (+ 95%) as a result of the pulverized process that decreased the water content. Similarly, a previous study reported that the storage period could decrease the total solids of kefir products (Kök-Taş et al., 2013). We observed the decrease on days 7 and 14 of storage that could be associated with the growing fungi. Compared to previous findings, the mean of total solids in this study was 17.39% higher than 12.09%, 12.37%, and 14.59 from goat milk kefir (Cais-Sokolińska et al., 2015; Satir & Guzel-Seydim, 2016; Setyawardani et al., 2014), 9.49% from ginger-added cow milk kefir (Wulansari & Rahayu, 2019) and 14.84% from cow colostrum kefir (Setyawardani et al., 2020).

The increased acidity, according to Lengkey & Balia (2014) is probably due to the growth of LAB in the kefir. However, we found that the higher the supplementation of *Moringa oleifera* leaf powder resulted in a higher acidity but lower total LAB. The increased acidity can be due to the acidic composition of *Moringa oleifera* leaf powder that subsequently increased the kefir acidity (Mbikay, 2012). The acidity level in this study remains lower than 1.31-1.66% reported by Setyawardani et al. (2020).

The result of pH value in this study confirms (Ismael et al., 2016) that *Moringa oleifera* leaf powder supplemented into whey manufacture did not affect the pH. The excessive supplementation of *Moringa oleifera* leaf powder may have caused the fiber content in Moringa leaf unable to provide energy for LAB to decrease pH. *Moringa oleifera* leaf is a potential source of fiber that contains 6.5% b/b of soluble dietary fiber (Anudeep et al., 2016). Therefore, supplementing *Moringa oleifera* leaf powder up to 2% w/w did not decrease the pH value of the kefir.

Previous studies reported that the pH of fermented milk would decrease with storage period (Dianti et al., 2018; Leite et al., 2013; Putri et al., 2020) because LAB would harness lactose to produce organic acids. However, this study showed that storage period did not affect the pH of kefir. Similarly, Yıldız-Akgul et al. (2018) stated that pH value of kefir was not highly diverse due to the yeast contained in the kefir. LAB grow and produce organic acids more slowly when the yeast presents than in the pure culture. pH value is affected by LAB; the higher the LAB population, the lower the pH. This study showed that storage period has decreased the LAB without affecting the pH value in the kefir product.

Regarding alcohol level, we found that the more *Moringa oleifera* leaf powder incorporated in the kefir manufacture, the lower the alcohol level of the kefir product. In contrast, the alcohol content increased with storage period because we found the alcohol level was higher in kefir stored for 14 days than seven days. In the fermentation process, LAB will convert lactose into lactic acid and other compounds, and lactose fermented with yeast will produce $C0_2$ and small quantity of etanol (Fakruddin et al., 2013). It indicates the ability of *Moringa oleifera* leaf powder to inhibit alcohol production in kefir manufacture. In relation to socio-cultural context, supplementing *Moringa oleifera* leaf powder is considered beneficial because it lowers the alcohol level of a beverage product, thus safe for consumption in an area of Moslem dominant. Alkohol is the product of yeast metabolism that degrades some forms of sugars, but in this study, supplementing *Moringa oleifera* leaf powder did not affect the level of yeast (Table 1), but significantly decreased the alcohol content, thus confirming the results by Leite et al. (2013) and Sunarti et al. (2015).

FFA is another important parameter because it represents the other groups of compounds that significantly affect the flavor and aroma of fermented milk products. FFA is derived from degraded milk fat resulting from the microflora activity of the kefir grain. The fermented milk may have 5-10 times as much FFA as milk (Cais-Sokolińska et al., 2008). We found that incorporating up to 2% *Moringa oleifera* leaf powder could decrease kefir grain activity in degrading milk fat into FFA. Therefore, a higher supplementation would produce a lower FFA, but longer storage increased the FFA. Accordingly, the supplementation of *Moringa oleifera* leaf powder can lower the FFA of kefir which then affects the sensory characteristics of the kefir final product.

He chemical properties in this study have generally conformed to those of the standard. The mean total solid in this study (17.39%) conforms to the standard quality set by the National Standardized Agency, i.e., 3% (Badan Standardisasi Nasional, 2009) and the acidity (0.87-1.21%) is up to par with the Standard Codex No. 234 (0.6%). The pH value is not compared against the Codex No. 234 but confirms (Yıldız-Akgul et al., 2018) that the pH value of kefir products is 5-5.5. nearly all pH values in this study have conformed to the standard, with an average of 5.09. Considering the maximum standard level of alcohol is 1%, this study showed that supplementing 2% of *Moringa oleifera* leaf powder has produced kefir with standard alcohol content.

4.3 Fatty acid profile

There are various contributing factors to fatty acid profiles. Different forage feed affects the composition of fatty acid profile in the milk, so modifying the feeding pattern potentially alters the composition of fatty acid profile (Nudda et al., 2014). Even different procedures in product manufacture allow the fatty acid profile to change (Sumarmono et al., 2015). Vieira et al. (2015) reported that storage-induced stress to milk products may lead to the decreased SFA and increased biosynthesis of long-chain

fatty acids by LAB to alter the fatty acid compositions. Therefore, storage can significantly increase desaturase activity (DA) and MUFA but decrease SFA. Florence et al. (2012) on fermented cow milk reported an increased SFA (68.5% vs 69.2%) and MUFA (27.9% vs 28.1%) during the storage period.

In this study, we demonstrate the ability to store fermented milk to modify the composition of the fatty acid profile. In contrast with the previous study, we reported a decreased SFA by 46.99%, 38,14%, and 22.53% when stored at 0, 7, and 14 days, respectively. The most significant decrease was observed in Palmitic Acid (C16:0), i.e., 15.97%, 9.55%, and 0.24%, respectively. Additionally, our findings confirm the increased MUFA reported in the previous study by 35.46%; 54.8%, and 46.81% on day 0, 7, and 14 of storage, respectively, with oleic Acid (C18:1n9c) being the most significantly diminishing component (14.77%; 0%; and 0%). The complex molecular structure of milk protein and Oleic Acid (18:1n9c) may induce death in cancer cells and inhibit the growth of bladder tumors, but the sensitivity is largely affected by the dosage of oleic acid (18:1n9c) may induce death in cancer cells and inhibit the growth of bladder tumors, but its sensitivity is largely affected by the dosage of oleic acid (Xiao et al., 2013). n effective dosage is between 0.15 mg/mL and 0.8 mg/mL, so a higher level of oleic acid 25.0g/100g) potentially increases the antimutagenic and anticarcinogenic of the fermented milk product.

The level lof fatty acids in USFA group was reported to increase on day 0, 7, and 14 of storage, namely 53.01%, 61.86%, and 77.47%, respectively. Boycheva et al. (2012) reported that USFA can help lower cholesterol and minimize the risk of coronary disease. PUFA and MUFA contents also help reduce the formation of ateroschlerotic plaques and decrease the esterified fatty acids oil, cholesterol, and phospholipid (Ramsden et al., 2010). Other fatty acids, such as butyric acid (4:0), palmitic acid (16:0), oleic acid (18:1n9), and conjugated linoleic acid (Conjugated *Linoleic Acid* or CLA) show antimutagenic and/or anticarcinogenic properties that change due to fermentation (Molina et al., 2013; Xiao et al., 2013). LAB can increase the denaturation activity (DA) during fermentation due to stress condition, which can estimate the feed conversion of SFA and USFA (Nantapo et al., 2014). Accordingly, the increased denaturation activity of microorganisms in fermented food is greatly expected and could be investigated in future studies.

4.4 Antioxidant properties

Supplementing *Moringa oleifera* leaf powder into goat milk kefir manufacture significantly increased the total phenol of the kefir due to the total phenol in the leaf powder. Ghafar et al. (2017) reported that *Moringa oleifera* leaf extract contained total phenol up to 2027,67 mg GAE/gram of extract. The storage period also increased the total phenol because the microorganism degraded the components in the Moringa leaf, thus producing phenolic compounds during storage. Similarly, (Shori, 2013) reported the highest phenol level observed in cow-soy milk yogurt stored for 21 days. Also, (Nurliyani et al., 2015) showed that seven-day storage, goat milk kefir had a higher total phenol than that in one-day storage. The increased total phenol during storage is probably due to the proteinase and peptidase activities that produce aromatic amino acid in the peptides that are released and accumulated in the LAB proteolytic system (González-Olivares et al., 2014).

Goat milk kefir fortified with *Moringa oleifera* leaf powder and stored for 14 days produced the highest radical-scavenging activities (Table 4). Kefir plays a protecting role since the milk is fermented with kefir grain and shows the highest radical-scavanging activities than the non-fermented milk. *Moringa oleifera* leaf powder is reported to have a high antioxidant (Gupta et al., 2012) and the extract of *Moringa oleifera* leaf shows a DPPH scavenging activity at IC 50 by 19.12 µg/mL. Therefore, incorporating *Moringa oleifera* leaf powder up to 2% into goat milk kefir manufacture and storing the product for 14 days could significantly improve the antioxidant properties of the goat milk kefir. Antioxidant properties have been reported to improve the functional quality of kefir.

5 Conclusions

Goat milk kefir fortified with *Moringa oleifera* leaf powder is expected to improve the product quality and its functional components without affecting the product characteristics during storage. We found that *Moringa oleifera* leaf powder is the potential additive substance for goat milk kefir. In microbiological parameters, *Moringa oleifera* leaf powder decreased the total LAB while the storage period decreased total BAL, TPC dan yeast. Analysis on chemical substances showed that *Moringa oleifera* leaf powder increased acidity and decreased total alcohol content, while the storage period decreased total solids but increased the FFA and alcohol content. Some compositions of fatty acid profile were affected by the storage period, and a higher level of supplementation (2%) combined with a longer storage would improve the total phenol and DPPH around two-fold of the control treatment, as expected by the study. The characteristics of goat milk kefir supplemented with *Moringa oleifera* leaf powder, except for the LAB, met the Codex and BSN standards.

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