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The microbiological and chemical characteristics of mozzarella cheese supplemented with different level of kefir

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Abstract. The effect of kefir supplementation on the characteristic of mozzarella cheese was investigated. Kefir is a fermented milk product that contains lactic acid bacteria, acetic bacteria, and yeast. The quality of mozzarella cheese has strongly influenced the level of kefir. The characteristics of mozzarella cheese with the addition of kefir were studied by measuring pH value, moisture content, protein content, fat content, total viable count, and total lactic acid bacterial count. The experimental design was a completely randomized design with four treatments and five replications. The treatments were performed by kefir supplementation levels, i.e., 0%, 4%, 8%, and 12%. The higher level of kefir supplementation influenced the microbiological and chemical characteristics of mozzarella cheese. The optimal characteristics of mozzarella cheese were 12% kefir with a pH of 4.8 ± 0.13 , a fat of $11.97 \pm 0.12\%$, protein of $23.05 \pm 0.22\%$, moisture of $47.61 \pm 0.72\%$. The mesophilic viable count was 4.7 ± 0.2 log CFU/g, and the lactic acid bacteria was 9.02 ± 0.2 log CFU/g. It is concluded that the best mozzarella cheese by the supplementation of 12% kefir is based on microbiological and chemical characteristics.

1. Introduction

Cheese producing are consists of filtration of whey and curd and handling into a solid form. Some nutrient was cheese former such as protein, fat, salt, moisture, and other milk components. Mozzarella is a kind of unripened cheese, with white and soft cheese whose dilution properties are suitable for adding several products [1]. The expansion of fast-food chains affected the demand for mozzarella cheese as a popular food around the world. In Indonesia, the manufacture of cheese source from cow, goat, and buffalo milk. Especially for cow milk in Indonesia still for small scale industries. Therefore, it is necessary to improve the quality of local cheese by optimizing the process of cheese constituents material. In many countries, the source of the best raw material for producing mozzarella cheese in many variations of food is buffalo milk. Nowadays, however, the excellent quality of milk and another additional component must be developed to having diversification of mozzarella cheese. The insurance quality and preparation of mozzarella cheese is very fundamental to the industries and dairy farmers. According to [2], several species of lactic acid bacteria (LAB) were derived from other raw milk (cow, goat, and buffalo milk) that have very possibilities for development to become probiotics.

Several yeasts and lactic acid bacteria have been recognized source of kefir and kefir grains, including *Kluyveromyces lactis*, *Pichia fermentans*, *Kluyveromyces marxianus*, *Lactobacillus Kefir*, *Leuconostoc mesenteroides*, *Lactobacillus brevis*, and *Lactobacillus helveticus*. The microbial that represent the kefir grains have an excellent activity to produce organic acid consist of acetic acid, lactic

acid, and succinic acid. The growth of pathogenic microorganisms and spoilage in kefir can be inhibited by metabolites component of lactic acid bacteria and yeast such as bacteriocins and some antibiotics. In connection with the kefir mineral value, calcium and magnesium are some minerals can finding in kefir [3]. The history from the Caucasus mountains shown of kefir is a kind of fermented dairy product. In Central Asia, kefir has been consumed for thousands of years [4]. Kefir grains look like cauliflower, irregularly, small size, containing a mixture of more than 300 species of both lactic acid bacteria and acetic acid bacteria (such as *leuconostocs*, *lactobacilli*, *acetobacter*, and *lactococci*) and yeasts who are fermenting and non-fermenting lactose in fermented milk product. Previous research on kefir has reported anti-tumor, immunological, and antimicrobial effects [5].

According to [6], the kefir has a chemical composition ranging from 0.7–1.1% for ash, 2.9–6.4% for crude protein, 10.6% to 14.9% for total solids, and 3.8–4.7% for carbohydrate. In another study, [7] reported that kefir grains have 4.5% protein, 86.3% moisture, 1.2% ash, and 0.03% fat. [8] found that Brazilian kefir contained 9.62% dry matter, 2.34% fat, and 3.91% protein after 24 h of fermentation. This study, therefore, aimed to be conducted on supplementation of different levels of kefir on mozzarella cheese. The objectives of the research help the farmers or industries to prepare mozzarella cheese using different levels of kefir.

2. Materials and methods

Cow milk (20 L) is derived from Lassy Dairy Farm, West Sumatera, Indonesia. Rennet enzyme 0.25%, citric acid 2 g/1000 ml cow milk, salt 2%. The design of this research used was a Completely Randomized Design 4 treatments (Supplementation kefir 0, 4, 8, and 12% kefir) with five replications. Data were analyzed by One Way ANOVA [9].

2.1. Kefir preparation

The technique of directly adding kefir grains in kefir manufacturing. Before inoculated 10% kefir grain, the raw milk must be pasteurized at 65°C for 30 min and cooled to 20-25°C. After fermentation 24 hours at 20-25oC, the grains are parted from the milk by filtration. Kefir as a filtrate kept at a cold temperature [10], for being used in mozzarella preparation.

2.2. Mozzarella manufacture and sampling

The manufacturing of mozzarella cheese consists of five replication and four different levels of kefir. Mozzarella cheese was produced using cow's milk. The filtered milk was heated to 65°C for 30 min and cooled to 20-25oC and inoculated with (0, 4, 8, and 12%) kefir and (0.25%) of rennet enzyme and added with 0,002% of citric acid. The combination was let for 30-60 minutes until the curd parted from the whey. After about 20 minutes, the curd was congregating, then cut with a small size. Then, the hot water at 85°C was added to the curd in a glass bowl, make the uniform paste by mixing the curd. Use the cold Na Cl solution (2%) to immersing the curd in at around 6°C for six hours, and then let to dry for 4 hours. Eventually, packaged the curd (mozzarella cheese) in sterilized plastic bags and kept it at (4°C) for the next study.

2.3. Microbiological analysis

The colony enumeration of total mesophilic viable count (TMVC) using Plate Count Agar (PCA) (Merck) at 37°C for 24 h incubation. Further, the colony enumeration of lactic acid bacteria using MRS agar (Merck) at 37C for 48 h after incubation [11].

2.4. Chemical analysis

In connection with the pH value of mozzarella samples, cheese was determined using a digital pH meter. Calibrated the pH meter use buffers of pH 4 and 7. The pH value of homogenized cheese samples was recorded according to AOAC for [12]. The cheese moisture content was analyzed by AOAC [12]. Use the soxhlet method for measuring the fat content of cheese samples and the Kjeldahl method for determined their total protein contents [12].

3. Results and discussion

3.1. Total mesophilic viable count (TMVC) and lactic acid bacteria

The statistical analysis result indicated a decrease in TMVC according to the increased level of kefir supplementation. It means the metabolites of acetic acid bacteria, lactic acid bacteria, and yeast non-pathogen on kefir caused stunts growth of aerobic mesophilic bacteria on mozzarella cheese. Table 1 shows the means of the total mesophilic viable count (TMVC) and the total count of lactic acid bacteria (TCLAB) of mozzarella cheese in each treatment.

Table 1. Total mesophilic viable count (TMVC) and lactic acid bacteria in mozzarella cheese with different kefir supplementation levels.

Treatments	TMVC (Log CFU/g)	TCLAB (Log CFU/g)
A (0%)	5.25 ^a	8.56 ^a
B (4%)	5.13 ^a	8.65 ^a
C (8%)	5.05 ^a	8.73 ^b
D (12%)	4.70 ^b	9.02 ^b

Note: Means with different superscript represented by small letter are significantly different ($P<0.05$)

The observed changes in TMVC depend on the processing of mozzarella cheese. The TMVC value shows a significant reduction in observed, inversely proportional to the levels of kefir supplemented compared with the control ($P < 0.05$), from 5.25 ± 0.3 log CFU/g to 4.7 ± 0.2 log CFU/g. The different levels of kefir supplementation on mozzarella cheese was significantly affecting for TMVC value. This study shows that kefir has activity inhibiting the growth of pathogenic bacteria. Several studies have demonstrated that many gram-negative and positive bacteria as food-borne pathogens can be prevented with kefir activities [13]. [14] informed that kefir prevented *Salmonella Enteritidis*, *Salmonella aureus*, *E. coli*, *E. faecalis*, and *Bacillus subtilis*, but did not inhibit *Candida albicans* or *P. aeruginosa*. [15] and [16] informed that kefir could impede *L. monocytogenes*. Considering that kefir, with many species of bacterial and yeast with contains several metabolites and inhibitory ingredients like ethyl alcohol, organic acids, hydrogen peroxides, peptidesbacteriocins, and diacetyl, it might affect each other to attack their antimicrobial effects [17]. In the present study, there was little difference with [18], who reported the mozzarella cheese TMV about 4.29 ± 0.3 log CFU/g. For instance, organic acids or enzymatic degradation were sources of antimicrobial activities of several bacteriocins [19].

Cheese microflora is dominated by Lactic Acid Bacteria introduced with a cheese composition. Lactic acid bacteria counts varied little and significant differences between samples. The increasing of kefir supplemented level on mozzarella cheese was directly proportional to the number of lactic acid bacteria compared with the control, from 8.56 ± 0.2 log CFU/g to 9.02 ± 0.2 log CFU/g. The higher LAB count was primarily due to characteristics of kefir who supplemented to mozzarella cheese. Kefir consists of a composite mixture of useful microorganisms such as acetic bacteria, lactic acid bacteria, and yeast non-pathogen. [20]Informed that kefir and kefir grains have much variety of microbial species in where lactic acid (*Lactococci*, *Lactobacilli*, *Streptococci*), acetic bacteria and yeast (*Kazachstania sp.*, *Candida sp.*, *Kluyveromyces sp.*, *Saccharomyces sp.*, *Torulosis Sp.*, *Zygosaccharomyces sp.*). The results of this study is significantly different from [18], who reported the number of LAB on mozzarella cheese about 3.78 ± 0.02 log CFU/g. In particular, control samples presented a lower cell load than the supplemented samples.

3.2. pH

The statistical analysis result showed a was a decrease in pH according to the increased level of kefir supplementation. Table 2 declares the mean pH value on a mozzarella cheese in each treatment.

Table 2. The pH of mozzarella cheese with different kefir supplementation levels

Treatments	pH
A (0%)	6.24±0.18 ^a
B (4%)	5.41±0.12 ^b
C (8%)	5.08±0.26 ^b
D (12%)	4.8±0.13 ^c

Note: Means with different superscript represented by small letter are significantly different ($P<0.05$)

Table 2 shows the mozzarella cheese pH values for a different level of kefir. The pH of Mozzarella cheese with a 12% level of kefir was significantly ($P<0.05$) lower than that of 0%, 4%, and 8% level kefir. The pH of mozzarella cheese with 4% compared to that of an 8% level of kefir was no significant difference. The pH of mozzarella cheese without adding kefir was 6.24 ± 0.18 . This value is higher than that of [21], who informed a cow's milk mozzarella cheese with a pH value of 5.23 ± 0.01 . It showed that the pH of the mozzarella cheese with the addition of kefir supplementation resulted was decreased. The pH value was decreased by supplementation highest level of 12% resulted in the lowest pH value of 4.8 ± 0.13 . It caused due to lactic acid bacteria acts as a source of organic acid that has been formed of hydrogen dissociated (H^+) ions. The main factors that determine the acidification during kefir production are the size of grain inoculation, incubation temperature, and agitation process. The same direction on the result with the prior representation by [22].

3.3. Fat content

The statistical analysis result showed a decrease in fat content according to the increased levels of kefir supplementation. Table 3 reveals the mean the fat content of mozzarella cheese in each treatment.

Table 3. The fat content of mozzarella cheese with different kefir supplementation levels

Treatments	Fat content (%)
A (0%)	21.91±0.01 ^a
B (4%)	18.10±0.49 ^b
C (8%)	13.75±2.85 ^c
D (12%)	11.97±0.12 ^c

Note: Means with different superscript represented by small letter are significantly different ($P<0.05$)

A significant reduction of fat content was observed inversely proportional to the level of kefir supplemented compared with the control ($P < 0.05$). The mozzarella cheese without the kefir addition has a fat content $21.91\pm0.01\%$ lower than the value obtained by [23], who found the that fat content of cow milk mozzarella cheese was $(24.87 \pm 0.01\%)$. The decreasing of fat content in mozzarella cheese with kefir supplementation due to the formation of volatile free fatty acids by the lipase enzyme on the activity of microorganisms during processing. This result found the fat content of mozzarella cheese samples was statistically different. While the kefir addition used was affected the total solids content ($P<0.05$).

The mozzarella cheese with 12% kefir has a fat content $11.97\pm0.12\%$ lower than the value obtained by [24], who reported an amount of $16.59\pm 0.11\%$ who found the fat content of mozzarella cheese with 2% strain of *Lactobacillus kefirancifaciens* ZW3. The fat content of mozzarella cheese with adding 12% kefir was significantly lower than other.

3.4. Protein content

The statistical analysis result showed an increase in protein content according to the increased level of kefir supplementation. Table 4 declares the mean protein content of mozzarella cheese in each treatment.

Table 4. The protein content of mozzarella cheese with different kefir supplementation levels

Treatments	Protein content (%)
A (0%)	15.59±2.11 ^a
B (4%)	17.35±0.78 ^a
C (8%)	18.09±2.30 ^b
D (12%)	23.05±0.22 ^c

Note: Means with different superscript represented by small letter are significantly different (P<0.05)

The protein content of mozzarella cheese without adding kefir (Table 4) was $15.59\pm2.11\%$, which is higher than the value $14.78 \pm 0.78\%$ and lower than the values 23.33 ± 2.12 and $22.1 \pm 0.1\%$ obtained by [21] and [25], respectively. The kefir supplementation until 12% has the most increase in protein content found in mozzarella cheese produced. The protein content of cheese was depended on endogenous proteinase, residual coagulant, and microbial proteinases from starter and non-starter component. During the milk fermentation, the amino acid profile changes that kefir was found to contain the essential amino acid higher levels than milk. Kefir also contains other amino acids, such as methionine, valine, isoleucine, lysine, phenylalanine, and tryptophan [26]; [27]. The essential amino acid contents in kefir are (mg/100 g) valine, 220; isoleucine, 262; methionine, 137; lysine, 376; threonine, 183; phenylalanine, 231; and tryptophan, 70 [7].

The protein content of mozzarella cheese with 12% kefir level was $23.05\pm0.22\%$, higher than the value $22.29 \pm 0.08\%$ determined by [24], who found the mozzarella cheese using 2% strain of *Lactobacillus kefirnafaciens ZW3* in protein content. The mozzarella cheese with a 12% level of kefir has a protein content of was significantly higher protein content than that of 0%, 4%, and 8% level of kefir.

3.5. Moisture content

The statistical analysis result showed an increased level of kefir supplementation directly proportional decrease in moisture content. Table 5 declares the mean moisture content of mozzarella cheese in each treatment.

Table 5. The moisture content of mozzarella cheese with different kefir supplementation levels

Treatments	Moisture content (%)
A (0%)	58.57±1.58 ^a
B (4%)	56.91±1.72 ^a
C (8%)	50.88±1.19 ^b
D (12%)	47.61±0.72 ^c

Note: Means with different superscript represented by small letter are significantly different (P<0.05)

Table (5) shows the moisture content for mozzarella cheese with different level of kefir supplementation. The mozzarella cheese has a moisture content without kefir was $58.57\pm1.58\%$, which higher than the value $52.49 \pm 0.49\%$ obtained by [21] and the value $45.48 \pm 2.85\%$ obtained by [25]. The mozzarella cheese with 0% compared to that of 4% kefir was no significant difference in moisture content. The moisture content of Mozzarella cheese with 12% level of kefir ($47.61\pm0.72\%$) was significantly lower than that of 0%, 4%, and 8% level of kefir and lower than the value $54.02\pm0.19\%$ obtained by [24], who found the moisture content of mozzarella cheese with 2% strain of *Lactobacillus kefirnafaciens ZW3*.

4. Conclusion

According to our results, the use of the different levels of kefir as supplementation had no adverse effects on mozzarella cheese with the microbiological and chemical properties. Indeed, cheese produced using kefir was exhibited good quality characteristics, in terms of total mesophilic viable count, total lactic acid bacteria, pH, protein content, fat content, and moisture content. Thus, it may be concluded that mozzarella cheese with kefir supplementation has a good quality product.

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