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# Incorporation of oat milk with probiotic *Lacticaseibacillus casei* AP improves the quality of kefir produced from goat milk

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**Abstract**

In this study, we evaluated the quality of kefir with combined additions of oat milk (8, 12, and 16% w/v) and *Lacticaseibacillus casei* AP (2 and 4% v/v), and observed the products' physicochemical characteristics (nutrient content, pH and acidity, viscosity and syneresis, ethanol concentration, and fatty acid profiles), microbiological characteristics (total lactic acid bacteria, total plate count, total probiotic, and total yeast), and sensory characteristics. The result showed that an increasing level of oat milk addition decreased water content and improved viscosity. A combination of 16% oat milk and 4% (v/v) *L. casei* AP increased the viscosity and water content, and resulted in the highest favorability and acceptability of kefir products. However, the increased concentration of *L. casei* AP inoculum and oat milk quantity did not affect microbiological qualities. It can be concluded that incorporating 16% oat milk and 4% *L. casei* AP improves the physical quality and sensory characteristics of kefir products.

**Keywords:** goat milk kefir; oat milk; *Lacticaseibacillus casei* AP; kefir quality.

**Practical Application:** Improving the physicochemical and sensory characteristics of kefir products.

## 1 Introduction

Kefir is a fermented drink that tastes sour and has a cream-like consistency (thick and soft); it is characterized by a natural carbonation and contains a small concentration of ethanol (Zamberi et al., 2016; Garofalo et al., 2020). Kefir can be produced using commercial starter cultures in various forms, such as freeze dried, kefir grain (traditional), and kefir product itself (Bensmira et al., 2010). Compared to commercial kefir, traditional kefir is more enriched in variety of yeast, probiotic, and functional role (Goktas et al., 2021). Bacterial content in traditional kefir drink mostly consists of *Firmicutes* phylum (93.66%-99.98%), *Streptococcaceae* family (89.12-99.83%), *Lactobacillaceae* (36.68%) dan *Streptococcaceae* genus (36.68%) (Biçer et al., 2021). Syneresis, which produces whey, is a physical weakness of kefir as it causes its pH to reach the isoelectric point for casein. Syneresis is the process through which water escapes from the gel matrix where curd is separated from the kefir. Excessive syneresis should be avoided when it comes to the nutritional and sensory characteristics of fermented milk (Barukčić et al., 2017). An alternative solution to address this issue is the incorporation of additional dietary fiber to increase viscosity.

The benefits of incorporating dietary fiber into food products include: 1) the reduction of syneresis, 2) improvement of sensory characteristics that are acceptable for consumers, and 3) extension of the products' shelf-life (Ramirez-Santiago et al., 2010). Dietary fiber plays a vital role in human health due to its prebiotic function. Oat is a small grain plant that contains sufficient dietary fiber and is a source of protein, fat, minerals, and vitamins, as well as soluble fiber and β-Glucan, which promote a healthy heart in both humans and animals (Zhang et al., 2012). Furthermore, soluble fiber and β-Glucan in oats is associated with a positive impact on health (Bernat et al., 2015). Previous findings have shown that oats are a suitable substrate for several species of lactic acid bacteria (LAB) (Bernat et al., 2015; Dinkçi et al., 2015; Demïr et al., 2021). Oat milk is a water-soluble extract and it is considered as a lactose- and gluten-free product (Deora & Deswal, 2018).

Incorporating LAB species that produce a high level of exopolysaccharide (EPS) is associated with high viscosity in fermented milk products (Han et al., 2016). The probiotic *Lacticaseibacillus casei* strain AP (Widodo et al., 2012a; Widodo et al., 2012b; Widodo et al., 2014) can produce EPS (Widodo et al., 2019), and it was shown to function as a starter culture for milk fermentation (Widodo et al., 2017). In vivo experiments on mice fed with milk fermented using *L. casei* AP showed a decreased level of blood glucose and low density lipoprotein, and an increased level of high density lipoprotein (Widodo et al., 2019). As *L. casei* AP can synthesize EPS, its incorporation for kefir fermentation will theoretically decrease syneresis and subsequently increase the products' viscosity. The probiotic function can be enhanced by adapting fermentation technology to produce high-probiotic content in supplement or fermented food (Champagne et al., 2018). Probiotic consists of three main classes: true probiotic (TP) is the viable, active probiotics; pseudo prebiotic (PP) is the viable, non-active probiotics in form

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of vegetative or spores; and ghost probiotic (GP) is dead/non-living cells either intact or broken. Each of this class is classified into two groups based on the location, i.e., in vivo or in vitro (Zendeboodi et al., 2020). Incorporating *Lactobacillus rhamnosus* GG may render potential probiotic carrier into kefir product with theurapetic effect (Mitra & Ghosh, 2020) and improve the status from fermented by-product to functional food (Santos et al., 2019). This study investigated whether the incorporation of oat milk and *L. casei* AP can increase the quality of kefir produced from goat milk. It is expected that this treatment would improve the viscosity of kefir without causing negative effects on its physicochemical, microbiological, and sensory characteristics.

## 2 Materials and methods

### *2.1 Materials*

Fresh goat milk was obtained from the dairy goat farm "Susu Poang" (Yogyakarta, Indonesia) and kefir grain from "Kefira" (Yogyakarta, Indonesia). For supplementation, 800 g of oat milk (Quaker Oats, Indonesia) was used, which contained 5% total fat, 0% cholesterol, 3% saturated acid, 8% protein, 7% total carbohydrate, 11% dietary fiber, and 0% salt. The bacterial starter used in this study was *Lacticaseibacillus casei* AP (Widodo et al., 2012a; Widodo et al., 2012b; Widodo et al., 2014).

### *2.2 Methods*

The experiment was conducted using a 3 × 2 factorial completely randomized design. The first factor was the level of oat addition (8, 12, and 16%) and the second was the concentration of *L. casei* AP inoculum (2 and 4%), with four replicates each.

*L. casei AP culture preparation*

A total of 100 mL of skim milk solution (18%, w/v) was sterilized at 110 °C and 13 Psi for 10 min. The sterilized skim milk was inoculated with 1% (v/v) of *L. casei* AP and was then incubated at 37 °C for 12-18 h until the formation of curd. This product was used as mother starter, 3% (v/v) of which was inoculated into sterilized goat milk (18%, w/v), and was subsequently incubated for 12-18 h to produce the bulk starter. This was ready to be used as starter for kefir fermentation or was stored at 10 °C for later use.

*Oat milk preparation*

The oat milk to be incorporated into the kefir fermentation process was prepared by dissolving oatmeal in aquadest to obtain three specific concentrations, namely 8, 12, and 16% (w/v.) This was conducted by heating 100 mL of aquadest to 90 °C, followed by the dissolution of oat milk for 15 min. The oat milk solution was then homogenized using a blender (LG) for 2 min until it was finely pulverized and no whole oatmeal particles could be detected. The oat milk solution was freshly prepared before kefir production Demïr et al. (2021).

*Kefir grain preparation*

Kefir starter was prepared by heating goat milk at 85 °C for 15 min, letting it cool to room temperature, and then inoculating it with 3% (v/v) of kefir grain. The inoculated goat milk was incubated at room temperature for 18 h, and then the kefir grains were sieved to separate them from the fermented product. The obtained grains were then ready for kefir fermentation.

*Kefir fermentation and analysis of its quality*

Goat milk was added to the oat milk solution at a ratio of 75: 25 (75% goat milk: 25% oat milk) and was pasteurized at 85 °C for 15 min. After cooling, the mixture was inoculated with 3% (w/w) kefir grains and the fermentation process was allowed to occur for 6 h at room temperature to produce ethanol. The bulk starter of culture *L. casei* AP fermented milk was added either at 2 or 4% (v/v), and was followed by 12 h of incubation at room temperature to produce lactic acid (Kwak et al., 1996; Widodo et al., 2019). After this fermentation period, the product was harvested and analyzed with respect to the microbiological and physicochemical qualities, fatty acids profile, and organoleptic characteristics.

Microbiological analysis

The microbiological quality of kefir was analyzed based on total LAB, total plate count (TPC), total probiotic, and total yeast. Total LAB analysis was conducted on modified de Man Rogosa and Sharpe (MRS) Agar (Merck), and samples were incubated at 37 °C for 48 h in microaerobic conditions, while TPC was calculated on plate count agar (Merck), and samples were incubated at 37 °C for 24 h in aerobic conditions. Total probiotic was measured by plating samples on modified MRS supplemented with bile salt (1.5%; w/w) and samples were incubated at 37 °C for 48 h. Total yeast was counted on malt extract agar (Merck), and samples were incubated at 37 °C for 48 h. Upon incubation, colonies were counted and calculated based on the dilution rates of samples (Nurliyani et al., 2014).

Physicochemical analysis

The physicochemical qualities analyzed included water, protein, fat, lactose level, ash content, pH and acidity, syneresis and viscosity, ethanol concentration, and fatty acids profile. Water and protein content were analyzed using the weighing method (Association of Official Analytical Chemists, 1975) and the Kjeldahl's method (Association of Official Analytical Chemists, 1975), respectively, while fat content was analyzed using the titration method. The ash content was also measured using the weighing method (Association of Official Analytical Chemists, 1975). Acidity was determined by NaOH titration with phenolphthalein as indicator (Association of Official Analytical Chemists, 1975), and the results were expressed as lactic acid percentage. The pH value was measured using a pH meter (PT-70, Boeco, Germany), while viscosity was measured using a rotational viscometer. Syneresis analysis was performed by centrifugation methods. Ethanol concentration was measured following the Conway microdiffusion method modified by Nurliyani et al. (2015) using the typical standard curve for ethanol. Samples were measured at 480 nm using a spectrophotometer (Spectronic 200, Termo Scientific).

Fatty acids profile

The fatty acids profile of kefir was analyzed using gas chromatography (Simadzu GC-2010) following a detailed procedure described in the Association of Official Analytical Chemists (1975). In brief, 350 mg of sample was transferred into an Erlenmeyer flask and was added with 6 mL of 0.5 M methanolic NaOH and boiling chips. The cooler was connected and refluxed to let oil lumps out (5-10 min), 7 mL of boron trifluoride ($BF_3$) was added through the cooler, and reflux proceeded for 2 h. Then, heptane (5 mL) was also added through the cooler and was refluxed for 1 min. The heater and cooler were removed, and 15 mL of saturated NaCl was added and mixed for 15 s or until the heptane solution reached the bottle's neck, then 1 mL of heptane was poured into the tube, followed by NaSO. Finally, the solution was filtered and injected into the gas chromatography instrumentation (Shimazu GC-2010). The gas chromatographic conditions were as follows: 1) Rtx-5 column; oven-dried at 180 °C, retained for 2 min, increased to 270 °C pada 10 °C/min, retained for 4 min, with a total duration of 15 min; 2) gas bearing helium was 2.43 mL/m, air flow rate was 190 mL/s, and hydrogen flow was 80 mL/m; 3) injector temperature and flame ionization detector were set at 290 °C; 4) methyl laurate standard (10% in heptane) was injected 0.10 µL; and 5) the peak in chromatogram samples that shared common retention time with the standard retention was the fatty acid peak.

Sensory characteristics

Sensory analysis of the samples was performed by 15 panelists from the Department of Animal Products, Technology Universitas, Gadjah Mada, who were familiar with kefir products. The kefir was analyzed in term of its color, aroma, acidity, alcohol taste, texture, and overall acceptability, and the evaluation used a nine-point hedonic scale (Bodyfelt et al., 1988) described as follows: extremely dislike, dislike very much, moderately dislike, slightly dislike, neither like nor dislike, slightly like, moderately like, like very much, extremely like. The panelists were presented with kefir samples labeled with random three-digit codes and drinking water to wash them down after tasting them.

### *2.3 Statistical analysis*

Data were subjected to multivariate analysis of variance, and Duncan's new multiple range test was applied to test for differences ($\alpha = 0.05$) using SPSS 16.0.

## 3 Results

### *3.1 Quality of raw materials*

The raw materials used for kefir fermentation in this study were goat milk, oat milk (8, 12, and 16% concentration), kefir grain, and *L. casei* AP. The fresh goat milk contained fat, lactose, and protein at concentrations of 5.44, 4.56, and 3,56%, respectively, while the oat milk was lactose-free, as described in Table 1. The higher concentration of oat milk in an oat milk solution had a lower concentration of fat but showed a higher concentration of protein and viscosity (Table 1).

Table 1 shows the raw materials utilized to produce kefir with the supplementation of various concentrations of oat milk solution, combined with kefir grain and *L. casei* AP as culture for fermentation. After 18 h of fermentation, the physicochemical quality of kefir products was evaluated, and the data are presented in Table 2. Table 3 shows the physicochemical properties of kefir produced using different concentrations of *L. casei* AP as starter culture and the incorporation of various oat milk volumes. It was revealed that increasing the quantity of oat milk decreased the water content and enhanced the viscosity of kefir products ($P < 0.005$) (Table 2). At the same time, the concentration of *L. casei* AP used as starter culture and the interaction between oat milk and the bacterium did not affect all the parameters. The addition of 16% (w/v) oat milk combined with *L. casei* AP at a concentration of 2 or 4% (v/v) increased total solids and the viscosity of products, resulting in the kefir with the most favorable characteristics.

### *3.2 Microbiological characteristics*

Table 3 shows that the microbiological characteristics of kefir products (total LAB, TPC, probiotic, and total yeast) were not significantly affected by the combined addition of oat milk and *L. casei* AP, suggesting that their incorporation (alone or combined) did not produce a negative effect on the fermentation of goat milk kefir.

### *3.3 Fatty acids profile*

The fatty acids profile of goat milk products fermented using a combination of kefir grain and *L. casei* AP culture with the supplementation of oat milk was evaluated (Table 4). It was revealed that goat milk kefir fermented with *L. casei* AP

**Table 1**. Quality of raw materials.

| Composition | Fresh goat milk | Oat milk 8% | Oat milk 12% | Oat milk 16% |
|---|---|---|---|---|
| Fat (%) | 5.44 | 3.30 | 2.37 | 1.78 |
| Solid Non-Fat (%) | 8.84 | 4.33 | 8.52 | 12.4 |
| Lactose (%) | 4.56 | - | - | - |
| Protein (%) | 3.56 | 0.7719 | 1.06 | 1.56 |
| Water content (%) | 85.7 | 92.4 | 89.1 | 85.8 |
| Viscosity (mPa's) | 400 | 1,100 | 3,400 | 11,399 |
| pH | 6.6 | 6.6 | 6.6 | 6.6 |

mPa's: millipascal-sekon.

**Table 2**. Physicochemical properties of kefir produced using different concentrations of *Lacticaseibacillus casei* AP as starter culture and incorporation of various oat milk volumes.

| Parameter | *Lacticaseibacillus casei* AP (%) | Oat Milk (%) | | | Mean |
|---|---|---|---|---|---|
| | | 8 | 12 | 16 | |
| Water content (%) | 2 | 87.2 ± 0.26 | 86.6 ± 0.45 | 85.72 ± 0.40 | 86.5 ± 0.73 |
| | 4 | 86.9 ± 0.48 | 86.5 ± 0.76 | 85.86 ± 0.43 | 86.5 ± 0.70 |
| | Mean | 87.1 ± 0.38[c] | 86.6 ± 0.58[b] | 85.8 ± 0.39[a] | 86.5 ± 0.70 |
| Protein (%) | 2 | 3.19 ± 0.09 | 3.10 ± 0.26 | 3.16 ± 0.06 | 3.15 ± 0.16 |
| | 4 | 2.98 ± 0.07 | 3.16 ± 0.12 | 3.10 ± 0.25 | 3.08 ± 0.17 |
| | Mean | 3.09 ± 0.13 | 3.13 ± 0.19 | 3.13 ± 0.18 | 3.12 ± 0.16 |
| Fat (%) | 2 | 4.21 ± 0.33 | 4.82 ± 0.8 | 4.85 ± 0.65 | 4.63 ± 0.66 |
| | 4 | 4.67 ± 0.69 | 4.74 ± 0.29 | 4.47 ± 0.83 | 4.63 ± 0.62 |
| | Mean | 4.44 ± 0.57 | 4.78 ± 0.58 | 4.66 ± 0.74 | 4.63 ± 0.63 |
| Lactose (%) | 2 | 1.75 ± 0.77 | 1.97 ± 0.71 | 2.29 ± 0.56 | 2.00 ± 0.68 |
| | 4 | 1.79 ± 0.54 | 1.62 ± 0.36 | 1.38 ± 0.57 | 1.59 ± 0.49 |
| | Mean | 1.77 ± 0.63 | 1.79 ± 0.57 | 1.84 ± 0.72 | 1.80 ± 0.62 |
| Ash (%) | 2 | 0.47 ± 0.25 | 0.67 ± 0.04 | 0.68 ± 0.04 | 0.61 ± 0.17 |
| | 4 | 0.56 ± 0.32 | 0.68 ± 0.04 | 0.55 ± 0.32 | 0.59 ± 0.25 |
| | Mean | 0.52 ± 0.27 | 0.68 ± 0.04 | 0.62 ± 0.22 | 0.60 ± 0.21 |
| Acidity (%) | 2 | 1.64 ± 0.18 | 1.81 ± 0.45 | 1.47 ± 0.12 | 1.64 ± 0.30 |
| | 4 | 1.62 ± 0.07 | 1.52 ± 0.21 | 4.67 ± 6.22 | 2.6 ± 3.60 |
| | Mean | 1.63 ± 0.13 | 1.66 ± 0.34 | 3.07 ± 4.42 | 2.12 ± 2.54 |
| pH | 2 | 4.15 ± 0.13 | 4.18 ± 0.13 | 4.16 ± 0.05 | 4.16 ± 0.10 |
| | 4 | 4.1 ± 0.08 | 4.25 ± 0.13 | 4.28 ± 0.10 | 4.21 ± 0.12 |
| | Mean | 4.13 ± 0.10 | 4.21 ± 0.12 | 4.22 ± 0.09 | 4.19 ± 0.11 |
| Viscosity (mPa's) | 2 | 1,980 ± 20 | 4,240 ± 160 | 5,119 ± 222 | 3,779 ± 1,409 |
| | 4 | 1,986 ± 80 | 3,713 ± 337 | 5,400 ± 608 | 3,699 ±1,518 |
| | Mean | 1,983 ± 52 | 3,976 ± 372 | 5,259 ± 437 | 3,739 ± 1,422 |
| Syneresis (%) | 2 | 29.8 ± 4.81 | 40.7 ± 36.98 | 33.6 ± 22.66 | 34.7 ± 23.27 |
| | 4 | 24.5 ± 2.91 | 16.5 ± 4.94 | 21.5 ± 24.03 | 20.9 ± 13.36 |
| | Mean | 27.2 ± 4.63 | 28.6 ± 27.63 | 27.5 ± 22.58 | 27.7 ± 19.86 |
| Alcohol level (%) | 2 | 1.45 ± 0.59 | 1.15 ± 0.4 | 1.51 ± 0.43 | 1.46 ± 0.46 |
| | 4 | 1.43 ± 0.33 | 1.47 ± 0.49 | 1.64 ± 0.42 | 1.51 ± 0.4 |
| | Mean | 1.44 ± 0.46 | 1.44 ± 0.43 | 1.58 ± 0.41 | 1.49 ± 0.43 |

Different superscript letters within line indicate $P < 0.05$.

**Table 3**. Microbiological characteristics of goat milk kefir with the combined addition of oat milk and *Lacticaseibacillus casei* AP.

| Parameters | *Lacticaseibacillus casei* AP (%) | Oat Milk (%) | | | Mean |
|---|---|---|---|---|---|
| | | 8 | 12 | 16 | |
| Total LAB (log CFU/mL) | 2 | 8.57 ± 1.19 | 8.44 ± 1.36 | 8.08 ± 0.414 | 8.36 ± 0.99 |
| | 4 | 8.23 ± 0.70 | 8.28 ± 0.36 | 8.04 ± 0.61 | 8.18 ± 0.53 |
| | Mean | 8.39 ± 0.93 | 8.36 ± 0.92 | 8.06 ± 0.48 | 8.27 ± 0.78 |
| TPC (log CFU/mL) | 2 | 7.78 ± 1.13 | 6.62 ± 0.78 | 7.5 ± 1.16 | 7.3 ± 1.0 |
| | 4 | 7.1 ± 1.51 | 6.96 ± 0.48 | 6.7 ± 0.46 | 6.92 ± 0.85 |
| | Mean | 7.44 ± 1.25 | 6.79 ± 0.61 | 7.1 ± 0.91 | 7.11 ± 0.94 |
| Probiotic (log CFU/mL) | 2 | 7.34 ± 0.08 | 7.36 ± 0.08 | 7.39 ± 0.06 | 7.36 ± 0.71 |
| | 4 | 7.55 ± 0.47 | 7.53 ± 0.49 | 7.45 ± 0.42 | 7.51 ± 0.42 |
| | Mean | 7.44 ± 0.33 | 7.45 ± 0.34 | 7.42 ± 0.28 | 7.44 ± 0.31 |
| Yeast (log CFU/mL) | 2 | 6.85 ± 0.31 | 6.67 ± 0.42 | 6.47 ± 0.17 | 6.66 ± 0.33 |
| | 4 | 6.51 ± 0.33 | 6.82 ± 0.48 | 6.62 ± 0.37 | 6.65 ± 0.38 |
| | Mean | 6.68 ± 0.35 | 6.75 ± 0.42 | 6.54 ± 0.28 | 6.66 ± 0.35 |

**Table 4**. Fatty acids profile and groups detected in goat milk kefir added with oat milk and *Lacticaseibacillus casei* AP.

| Parameter | *Lacticaseibacillus casei* AP (%) | Oat Milk (%) | | | Mean |
|---|---|---|---|---|---|
| | | 8 | 12 | 16 | |
| **SFA (%)** | | | | | |
| Caproic Acid (C6:0) | 2 | 1.25 ± 0.09 | 1.20 ± 0.04 | 1.28 ± 0.13 | 1.24 ± 0.09 |
| | 4 | 1.20 ± 0.07 | 1.26 ± 0.07 | 1.24 ± 0.11 | 1.23 ± 0.77 |
| | Mean | 1.23 ± 0.08 | 1.23 ± 0.06 | 1.26 ± 0.11 | 1.24 ± 0.08 |
| Caprylic Acid (C8:0) | 2 | 1.94 ± 0.17 | 1.88 ± 0.05 | 2.01 ± 0.21 | 1.94 ± 0.15 |
| | 4 | 1.87 ± 0.08 | 1.95 ± 0.12 | 1.97 ± 0.21 | 1.93 ± 0.13 |
| | Mean | 1.90 ± 0.12 | 1.92 ± 0.09 | 1.99 ± 0.19 | 1.94 ± 0.14 |
| Capric Acid (C10:0) | 2 | 6.58 ± 0.44 | 6.31 ± 0.12 | 6.72 ± 0.59 | 6.54 ± 0.41 |
| | 4 | 6.36 ± 0.08 | 6.59 ± 0.42 | 6.60 ± 0.59 | 6.52 ± 0.39 |
| | Mean | 6.47 ± 0.31 | 6.45 ± 0.32 | 6.66 ± 0.53 | 6.53 ± 0.39 |
| Undecanoic Acid (C11:0) | 2 | 0.15 ± 0.01 | 0.14 ± 0.01 | 0.16 ± 0.02 | 0.15 ± 0.01 |
| | 4 | 0.15 ± 0.01 | 0.15 ± 0.01 | 0.15 ± 0.02 | 0.15 ± 0.01 |
| | Mean | 0.15 ± 0.01 | 0.15 ± 0.01 | 0.16 ± 0.01 | 0.15 ± 0.01 |
| Lauric Acid (C12:0) | 2 | 2.85 ± 0.24 | 2.69 ± 0.04 | 2.92 ± 0.29 | 2.82 ± 0.21 |
| | 4 | 2.73 ± 0.01 | 2.88 ± 0.18 | 2.93 ± 0.29 | 2.84 ± 0.19 |
| | Mean | 2.79 ± 0.17 | 2.78 ± 0.15 | 2.92 ± 0.26 | 2.83 ± 0.19 |
| Myristic Acid (C14:0) | 2 | 6.73 ± 0.15 | 6.49 ± 0.05 | 6.77 ± 0.23 | 6.66 ± 0.19 |
| | 4 | 6.66 ± 0.07 | 6.80 ± 0.15 | 6.66 ± 0.14 | 6.71 ± 0.13 |
| | Mean | 6.69 ± 0.11 | 6.65 ± 0.19 | 6.72 ± 0.18 | 6.69 ± 0.16 |
| Pentadecanoic Acid (C15:0) | 2 | 0.66 ± 0.01 | 0.63 ± 0 | 0.65 ± 0.02 | 0.65 ± 0.02 |
| | 4 | 0.65 ± 0.01 | 0.66 ± 0.02 | 0.65 ± 0.02 | 0.65 ± 0.02 |
| | Mean | 0.65 ± 0.01 | 0.65 ± 0.02 | 0.65 ± 0.01 | 0.65 ± 0.02 |
| Palmitic Acid (C16:0) | 2 | 20.96 ± 0.35 | 20.83 ± 0.18 | 21.16 ± 0.55 | 20.98 ± 0.37 |
| | 4 | 20.85 ± 0.11 | 21.01 ± 0.61 | 21.07 ± 0.58 | 20.98 ± 0.44 |
| | Mean | 20.91 ± 0.24 | 20.92 ± 0.41 | 21.12 ± 0.51 | 20.98 ± 0.39 |
| Heptadecanoic Acid (C17:0) | 2 | 0.14 ± 0.03 | 0.14 ± 0.03 | 0.14 ± 0.05 | 0.14 ± 0.03 |
| | 4 | 0.14 ± 0.02 | 0.15 ± 0.02 | 0.13 ± 0.04 | 0.14 ± 0.03 |
| | Mean | 0.14 ± 0.02 | 0.14 ± 0.02 | 0.14 ± 0.05 | 0.14 ± 0.03 |
| Stearic Acid (C18:0) | 2 | 19.21 ± 1.64 | 18.12 ± 0.93 | 17.99 ± 1.36 | 18.44 ± 1.29 |
| | 4 | 18.89 ± 1.40 | 18.91 ± 0.78 | 18.24 ± 2.30 | 18.68 ± 1.44 |
| | Mean | 19.05 ± 1.38 | 18.52 ± 0.88 | 18.12 ± 1.69 | 18.56 ± 1.34 |
| Arachidic Acid (C20:0) | 2 | 6.91 ± 0.28 | 7.94 ± 0.23 | 7.43 ± 0.28 | 7.43 ± 0.50 |
| | 4 | 7.26 ± 0.49 | 6.54 ± 1.18 | 7.89 ± 0.18 | 7.23 ± 0.87 |
| | Mean | 7.08 ± 0.40 | 7.24 ± 1.08 | 7.66 ± 0.33 | 7.33 ± 0.69 |
| **USFA (%)** | | | | | |
| Cis-10-Pentadecenoic Acid Methyl Acid (C15:1) | 2 | 0.21 ± 0.02 | 0.21 ± 0.02 | 0.20 ± 0.02 | 0.21 ± 0.02 |
| | 4 | 0.21 ± 0.02 | 0.21 ± 0.01 | 0.19 ± 0.02 | 0.21 ± 0.02 |
| | Mean | 0.21 ± 0.01 | 0.21 ± 0.02 | 0.19 ± 0.02 | 0.21 ± 0.02 |
| Palmitoleic Acid (C16:1) | 2 | 1.16 ± 0.04 | 1.18 ± 0.09 | 1.20 ± 0.15 | 1.18 ± 0.91 |
| | 4 | 1.19 ± 0.10 | 1.30 ± 0.15 | 1.17 ± 0.10 | 1.22 ± 0.12 |
| | Mean | 1.18 ± 0.07 | 1.24 ± 0.13 | 1.19 ± 0.11 | 1.20 ± 0.11 |
| Cis-1-Heptadecanoic Acid (C17:1) | 2 | 0.17 ± 0.06 | 0.16 ± 0.05 | 0.19 ± 0.06 | 0.17 ± 0.5 |
| | 4 | 0.16 ± 0.03 | 0.17 ± 0.02 | 0.19 ± 0.07 | 0.17 ± 0.4 |
| | Mean | 0.16 ± 0.05 | 0.17 ± 0.03 | 0.19 ± 0.06 | 0.17 ± 0.5 |
| y-Linolenic Acid (C18:3n6) | 2 | 0.22 ± 0.04 | 0.21 ± 0.03 | 0.21 ± 0.03 | 0.21 ± 0.03 |
| | 4 | 0.22 ± 0.02 | 0.22 ± 0.02 | 0.20 ± 0.04 | 0.22 ± 0.24 |
| | Mean | 0.22 ± 0.02 | 0.22 ± 0.02 | 0.21 ± 0.03 | 0.21 ± 0.02 |
| Linoleate Acid (C18:2) + Linolelaidate (C18:2n9t) | 2 | 6.91 ± 0.28 | 7.94 ± 0.23 | 7.43 ± 0.27 | 7.43 ± 0.50 |
| | 4 | 7.26 ± 0.49 | 6.54 ± 1.19 | 7.89 ± 0.18 | 7.23 ± 0.87 |
| | Mean | 7.08 ± 0.40 | 7.24 ± 1.08 | 7.66 ± 0.33 | 7.33 ± 0.69 |

Different superscript letters within line and column indicate $P < 0.05$. [1]Caproic Acid (C6:0); [2]Caprilic Acid (C8:0), Capric Acid (C10:0), Undecanoic Acid (C11:0); [3]Lauric Acid (C12:0), Myristic Acid (C14:0), Pentadecanoic Acid (C15:0), Palmitic Acid (C16:0), Heptadecanoic Acid (C17:0), Stearic Acid (C18:0), Arachidic Acid (C20:0), Cis-10-Pentadecenoic Acid Methyl Acid (C15:1), Palmitoleic Acid (C16:1), Cis-1-Heptadecanoic Acid (C17:1), y-Linolenic Acid (C18:3n6), Linoleate Acid (C18:2) + Linolelaidate (C18:2n9t), Cis-9-Oleat Acid (18:1) + trans 9 elaidat acid (C19:1n9c), Linolenic Acid (C18:3), Cis-11-Eicosenoic Acid (C20:1), Cis-11-14-17-eicosatrienoat (C20:3n6), Erucat Acid (C22:1n9), Cis-13-16-Docosadienoic Acid Methyl Ester (C22:2), Nervonate Acid (C24:1); [4]Lauric Acid (C12:0), Myristic Acid (C14:0), Pentadecanoic Acid (C15:0), Palmitic Acid (C16:0), Heptadecanoic Acid (C17:0), Stearic Acid (C18:0), Arachidic Acid (C20:0); [5]Cis-10-Pentadecenoic Acid Methyl Acid (C15:1), Palmitoleic Acid (C16:1), Cis-1-Heptadecanoic Acid (C17:1), y-Linolenic Acid (C18:3n6), Linoleate Acid (C18:2) + Linolelaidate (C18:2n9t), Cis-9-Oleat Acid (18:1) + trans 9 elaidat acid. (C19:1n9c), Linolenic Acid (C18:3), Cis-11-Eicosenoic Acid (C20:1), Cis-11-14-17-eicosatrienoat (C20:3n6), Erucat Acid (C22:1n9), Cis-13-16-Docosadienoic Acid Methyl Ester (C22:2), Nervonate Acid (C24:1); [6]Cis-10-Pentadecenoic Acid Methyl Acid (C15:1), Palmitoleic Acid (C16:1), Cis-1-Heptadecanoic Acid (C17:1), Cis-9-Oleat Acid (18:1) + trans 9 elaidat acid (C19:1n9c), Cis-11-Eicosenoic Acid (C20:1), Erucat Acid (C22:1n9), Nervonate Acid (C24:1); [7]y-Linolenic Acid (C18:3n6), Linoleate Acid (C18:2) + Linolelaidate (C18:2n9t), Linolenic Acid (C18:3), Cis-11-14-17-eicosatrienoat (C20:3n6), Cis-13-16-Docosadienoic Acid Methyl Ester (C22:2).

**Table 4**. Continued...

| Parameter | *Lacticaseibacillus casei* AP (%) | Oat Milk (%) | | | Mean |
|---|---|---|---|---|---|
| | | 8 | 12 | 16 | |
| Cis-9-Oleat Acid (18:1) + trans 9 elaidic acid (C19:1n9c) | 2 | 19.21 ± 1.64 | 18.12 ± 0.93 | 17.00 ± 1.36 | 18.44 ± 1.29 |
| | 4 | 18.89 ± 1.40 | 18.91 ± 0.78 | 18.24 ± 2.30 | 18.68 ± 1.44 |
| | Mean | 19.05 ± 1.37 | 18.51 ± 0.87 | 18.12 ± 1.69 | 18.56 ± 1.34 |
| Linolenic Acid (C18:3) | 2 | 0.82 ± 0.07 | 0.81 ± 0.06 | 0.83 ± 0.03 | 0.82 ± 0.05 |
| | 4 | 0.79 ± 0.09 | 0.73 ± 0.20 | 0.86 ± 0.03 | 0.79 ± 0.13 |
| | Mean | 0.81 ± 0.07 | 0.77 ± 0.14 | 0.85 ± | 0.81 ± 0.09 |
| Cis-11-Eicosenoic Acid (C20:1) | 2 | 0.27 ± 0.05 | 0.33 ± 0.08 | 0.32 ± 0.04 | 0.31 ± 0.06 |
| | 4 | 0.31 ± 0.05 | 0.36 ± 0.15 | 0.29 ± 0.03 | 0.32 ± 0.08 |
| | Mean | 0.29 ± 0.05 | 0.35 ± 0.11 | 0.31 ± 0.34 | 0.32 ± 0.07 |
| Cis-11-14-17-eicosatrienoat (C20:3n6) | 2 | 0.19 ± 0.02 | 0.18 ± 0.02 | 0.18 ± 0.02 | 0.18 ± 0.02 |
| | 4 | 0.17 ± 0.04 | 0.17 ± 0.04 | 0.19 ± 0.01 | 0.18 ± 0.03 |
| | Mean | 0.18 ± 0.03 | 0.18 ± 0.03 | 0.19 ± 0.01 | 0.18 ± 0.02 |
| Erucic Acid (C22:1n9) | 2 | 0.26 ± 0.08 | 0.25 ± 0.06 | 0.29 ± 0.09 | 0.27 ± 0.07 |
| | 4 | 0.24 ± 0.05 | 0.24 ± 0.02 | 0.29 ± 0.11 | 0.26 ± 0.07 |
| | Mean | 0.26 ± 0.06 | 0.24 ± 0.04 | 0.29 ± 0.09 | 0.27 ± 0.07 |
| Cis-13-16-Docosadienoic Acid Methyl Ester (C22:2) | 2 | 0.14 ± 0.4 | 0.14 ± 0.3 | 0.14 ± 0.05 | 0.14 ± 0.04 |
| | 4 | 0.14 ± 0.3 | 0.15 ± 0.2 | 0.13 ± 0.04 | 0.14 ± 0.03 |
| | Mean | 0.14 ± 0.3 | 0.14 ± 0.2 | 0.14 ± 0.04 | 0.14 ± 0.03 |
| Nervonic Acid (C24:1) | 2 | 0.17 ± 0.06 | 0.16 ± 0.05 | 0.19 ± 0.06 | 0.17 ± 0.52 |
| | 4 | 0.16 ± 0.04 | 0.17 ± 0.02 | 0.19 ± 0.07 | 0.17 ± 0.04 |
| | Mean | 0.16 ± 0.05 | 0.17 ± 0.03 | 0.19 ± 0.06 | 0.17 ± 0.05 |
| **Grouping (%)** | | | | | |
| SCFA[1] | 2 | 1.19 ± 0 | 1.19 ± 0.03 | 1.23 ± 0.10 | 1.21 ± 0.06 |
| | 4 | 1.25 ± 0.12 | 1.27 ± 0.06 | 1.28 ± 0.14 | 1.27 ± 0.09 |
| | Mean | 1.23 ± 0.09 | 1.23 ± 0.06 | 1.25 ± 0.11 | 1.24 ± 0.08 |
| MCFA[2] | 2 | 8.37 ± 0.42 | 8.35 ± 0.19 | 8.69 ± 0.76 | 8.48 ± 0.48 |
| | 4 | 8.64 ± 0.58 | 8.63 ± 0.57 | 8.79 ± 0.74 | 8.72 ± 0.61 |
| | Mean | 8.53 ± 0.48 | 8.49 ± 0.42 | 8.79 ± 0.74 | 8.61 ± 0.55 |
| LCFA[3] | 2 | 90.45 ± 0.42 | 90.46 ± 0.20 | 90.10 ± 0.81 | 90.32 ± 0.51 |
| | 4 | 90.11 ± 0.69 | 90.09 ± 0.64 | 89.86 ± 0.97 | 90.02 ± 0.69 |
| | Mean | 90.25 ± 0.56 | 90.28 ± 0.47 | 89.98 ± 0.97 | 90.17 ± 0.61 |
| SFA[4] | 2 | 69.47 ± 0.28 | 70.24 ± 1.10 | 70.58 ± 1.35 | 70.18 ± 0.88 |
| | 4 | 70.60 ± 1.27 | 70.97 ± 1.28 | 70.48 ± 2.17 | 70.69 ± 1.43 |
| | Mean | 70.15 ± 1.10 | 70.61 ± 0.93 | 70.53 ± 1.61 | 70.45 ± 1.19 |
| USFA[5] | 2 | 30.53 ± 0.28 | 29.76 ± 0.34 | 29.45 ± 1.30 | 29.83 ± 0.86 |
| | 4 | 29.40 ± 1.27 | 29.03 ± 1.28 | 29.55 ± 2.14 | 29.32 ± 1.42 |
| | Mean | 29.85 ± 1.10 | 29.39 ± 0.93 | 29.49 ± 1.58 | 29.55 ± 1.18 |
| MUFA[6] | 2 | 22.32 ± 0.18 | 20.38 ± 0.45 | 20.44 ± 1.03 | 20.88 ± 1.07 |
| | 4 | 20.82 ± 1.47 | 20.82 ± 1.21 | 20.36 ± 2.13 | 20.67 ± 1.45 |
| | Mean | 21.42 ± 1.33 | 20.60 ± 0.85 | 20.39 ± 1.49 | 20.77 ± 1.25 |
| PUFA[7] | 2 | 8.08 ± 0.09 | 9.22 ± 0.15 | 8.84 ± 0.33 | 8.79 ± 0.51 |
| | 4 | 8.41 ± 0.26 | 8.02 ± 1.69 | 9.00 ± 0.25 | 8.48 ± 0.96 |
| | Mean | 8.27 ± 0.27 | 8.62 ± 1.26 | 8.92 ± 0.27 | 8.62 ± 0.78 |

Different superscript letters within line and column indicate $P < 0.05$. [1]Caproic Acid (C6:0); [2]Caprilic Acid (C8:0), Capric Acid (C10:0), Undecanoic Acid (C11:0); [3]Lauric Acid (C12:0), Myristic Acid (C14:0), Pentadecanoic Acid (C15:0), Palmitic Acid (C16:0), Heptadecanoic Acid (C17:0), Stearic Acid (C18:0), Arachidic Acid (C20:0), Cis-10-Pentadecenoic Acid Methyl Acid (C15:1), Palmitoleic Acid (C16:1), Cis-1-Heptadecanoic Acid (C17:1), y-Linolenic Acid (C18:3n6), Linoleate Acid (C18:2) + Linolelaidate (C18:2n9t), Cis-9-Oleat Acid (18:1) + trans 9 elaidat acid (C19:1n9c), Linolenic Acid (C18:3), Cis-11-Eicosenoic Acid (C20:1), Cis-11-14-17-eicosatrienoat (C20:3n6), Erucat Acid (C22:1n9), Cis-13-16-Docosadienoic Acid Methyl Ester (C22:2), Nervonate Acid (C24:1); [4]Lauric Acid (C12:0), Myristic Acid (C14:0), Pentadecanoic Acid (C15:0), Palmitic Acid (C16:0), Heptadecanoic Acid (C17:0), Stearic Acid (C18:0), Arachidic Acid (C20:0); [5]Cis-10-Pentadecenoic Acid Methyl Acid (C15:1), Palmitoleic Acid (C16:1), Cis-1-Heptadecanoic Acid (C17:1), y-Linolenic Acid (C18:3n6), Linoleate Acid (C18:2) + Linolelaidate (C18:2n9t), Cis-9-Oleat Acid (18:1) + trans 9 elaidat acid. (C19:1n9c), Linolenic Acid (C18:3), Cis-11-Eicosenoic Acid (C20:1), Cis-11-14-17-eicosatrienoat (C20:3n6), Erucat Acid (C22:1n9), Cis-13-16-Docosadienoic Acid Methyl Ester (C22:2), Nervonate Acid (C24:1); [6]Cis-10-Pentadecenoic Acid Methyl Acid (C15:1), Palmitoleic Acid (C16:1), Cis-1-Heptadecanoic Acid (C17:1), Cis-9-Oleat Acid (18:1) + trans 9 elaidat acid (C19:1n9c), Cis-11-Eicosenoic Acid (C20:1), Erucat Acid (C22:1n9), Nervonate Acid (C24:1); [7]y-Linolenic Acid (C18:3n6), Linoleate Acid (C18:2) + Linolelaidate (C18:2n9t), Linolenic Acid (C18:3), Cis-11-14-17-eicosatrienoat (C20:3n6), Cis-13-16-Docosadienoic Acid Methyl Ester (C22:2).

culture added with oat milk resulted in 23 types of fatty acids (Table 4). The data show that the mean values of short-chain fatty acids (SCFA), medium-chain fatty acids (MCFA), and long chain fatty acids (LCFA) in kefir products were 1.24, 8.61, and 90.17%, respectively. The SCFA group has been known to have more than 12 fatty acids, namely C2-C6, MCFA C7-C11, and LCFA. Our data showed that saturated fatty acids (SFA) outnumbered the unsaturated fatty acids (USFA), with values of 70.45 and 29.55%, respectively. The most dominant SFA were the palmitic (20.91%) and stearic (19.05%) acids, while those

classified as USFA were the cis-9-oleic and trans-9-elaidic acids (19.21%). The USFA were divided into monounsaturated fatty acids (MUFA) and polyunsaturated fatty acid (PUFA) with mean values of 20.77 and 8.62%, respectively (Table 4).

### *3.4 Sensory characteristics*

The sensory characteristics of kefir were assessed in terms of color, aroma, acidity, alcoholic taste, texture, and acceptance level, and the obtained data are presented in Table 5. The combined addition of oat milk and *L. casei* AP culture significantly affected color, acidity, alcoholic taste, texture, and acceptance level ($P < 0.05$), but did not significantly affect the aroma. In addition, the sensory score of all parameters increased with the amount of added oat milk and *L. casei* AP (Table 5). It was found that the supplementation of 16% oat milk in combination with the inoculation of 4% *L. casei* AP produced the most favorable kefir product, as observed by the panelists.

## 4 Discussion

### *4.1 Physicochemical quality of kefir*

Physicochemical analysis showed that the higher the concentration of oat milk was, the lower the water content in kefir products, regardless the concentration of *L. casei* AP added as starter culture. Lower water content was observed in kefir that contained a high concentration of oat milk. It was positively correlated with data in Table 2, where higher oat milk concentrations resulted in lower water contents. The mean water content value obtained in the present study was 86.48%, which is not significantly different from the previously reported values of 82.61% (Wulansari et al., 2021), 87.91% (Cais-Sokolińska et al., 2015), 87.33% (Nurliyani et al., 2015), and 87.63 (Satir & Guzel-Seydim, 2016). A high total solid content is expected to reduce syneresis, which negatively affects nutrition and sensory qualities (Barukčić et al., 2017). The levels of protein, fat, lactose, and ash in kefir products in this study were not affected by the combined addition of oat milk and *L. casei* AP. Different compositions of protein, fat, and lactose level in raw materials did not affect the kefir product. In this study, the mean values of protein, fat, lactose level, and ash content in kefir products were 3.12%; 4.63%; 1.80%, and 0.602%, respectively.

The growth of LAB is known to produce organic acids and increase the acidity during kefir fermentation (Lengkey & Balia, 2014), which eventually decreases the pH level. The kefir in this study had pH of 4.19, which was not significantly different from the pH value of 4.31 recorded for kefir made using a combination of cow's and oat milk (Dinkçi et al., 2015) and kefir added with inconventional sugar was 4.4 (Larosa et al., 2021a). The kefir's acidity level in the present study was 2.12%, which is higher than the value of 0.87% previously (Wulansari et al., 2021), but conforms to the standard acidity indicated in Dinkçi et al. (2015), which is a minimum of 0.60%. The pH value and acidity level measured in this study showed that the combined addition of oat milk and *L. casei* AP did not affect the acidity level of the kefir product.

The results also showed that the oat milk concentration increased the viscosity of the kefir products. Specifically, incorporating 16% oat milk led to a 2.5-fold increase in viscosity compared to the values observed with the addition of 8% oat milk. This is in line with previous findings indicating that viscosity is affected by the concentration of oat milk added into the kefir (Dinkçi et al., 2015). In contrast, there was no evidence of an increase in viscosity from the incorporation of up to 4% *L. casei* AP culture during kefir production.

### *4.2 Microbiological characteristics of the products*

The microbiological characteristics of kefir products were analyzed to evaluate the concentration of LAB, yeast, probiotic, and total aerobic microbes (TPC). The data show that the mean value of total LAB in this study was 8.27 log CFU/mL, which is higher than the Codex standard No. 234 of 7 log CFU/mL. The total LAB concentration measured in this study was not significantly different from the values of 7.27 and 7.20 log CFU/mL previously reported in Nurliyani et al. (2014) and Setyawardani et al. (2020), respectively. Kefir is a popular source of probiotics and, in particular, studies have shown that it is a complex probiotic characterized by a combination of bacteria and yeast. Kefir exhibits functional properties that include antimicrobial, anticancer, cholesterol-lowering agent, lactose-intolerance free, and probiotic and prebiotic (John & Deeseenthum, 2015). *L. casei* AP is a probiotic, EPS-producing LAB that improves viscosity. The mean value of probiotics in this study was 7.44 log CFU/mL. It was found that incorporating 2 and 4% (v/v) *L. casei* AP did not affect the total probiotic value in the final kefir products. The total probiotics across treatments have met the minimum requirement, which is 7 log CFU/mL. Total probiotic at 7.44 log CFU/mL in this study is expected to

**Table 5**. Sensory characteristics of kefir fermented using *Lacticaseibacillus casei* AP and oat milk supplementation.

| Oat Milk (%) | *Lacticaseibacillus casei* AP (%) | Parameter | | | | | |
|---|---|---|---|---|---|---|---|
| | | Color | Aroma | Acidity | Alcoholic taste | Texture | Acceptance level |
| 8 | 2 | $6.13 \pm 1.3^{b}$ | $5.53 \pm 2.2$ | $3.47 \pm 1.9^{d}$ | $4.07 \pm 1.5^{c}$ | $4.60 \pm 2.3^{c}$ | $3.27 \pm 2.2^{d}$ |
| | 4 | $5.67 \pm 1.5^{c}$ | $6.60 \pm 1.1$ | $5.4 \pm 1.9^{c}$ | $6 \pm 1.3^{a}$ | $6.67 \pm 1.9^{a}$ | $5.67 \pm 2.2^{a}$ |
| 12 | 2 | $5.73 \pm 1.9^{c}$ | $6.87 \pm 1.1$ | $4.6 \pm 2.1^{b}$ | $4.47 \pm 1.4^{c}$ | $6.27 \pm 1.5^{b}$ | $4.53 \pm 1.9^{b}$ |
| | 4 | $7.2 \pm 0.8^{a}$ | $6.27 \pm 1.2$ | $5.47 \pm 2.2^{c}$ | $5.67 \pm 1.9^{b}$ | $6.07 \pm 1.7^{b}$ | $5.73 \pm 1.8^{c}$ |
| 16 | 2 | $6.53 \pm 1.4^{b}$ | $6.40 \pm 1.7$ | $5.73 \pm 1.8^{a}$ | $5.6 \pm 1.4^{b}$ | $7.07 \pm 1.5^{a}$ | $5.47 \pm 1.9^{c}$ |
| | 4 | $6.67 \pm 1.3^{b}$ | $7.07 \pm 1.5$ | $6.53 \pm 1.2^{a}$ | $6.07 \pm 1.3^{a}$ | $7.27 \pm 1.7^{a}$ | $6.73 \pm 1.4^{a}$ |

Different superscript letters within line indicate $P < 0.05$.

be actively function as probiotics and passing through digestive tracts. Previous finding reported that incorporating probiotic when making yogurt would maintain the probiotic effect up to 45 days of shelf life (Lucatto et al., 2020).

The microbiological characteristics observed in the examined kefir products generally met the standard minimum requirement. The mean value of TPC in this study was 7.11 log CFU/mL, which is not significantly different from the values reported in other studies, specifically 7.85 log CFU/mL (Wulansari et al., 2021), 8.89 log CFU/mL (Nurliyani et al., 2014), and 7,85 log CFU/mL (HadiNezhad et al., 2013). The mean TPC in this study conformed to the minimum established by the Codex standard No. 234, which is 7 log CFU/mL. The total yeast value reported in this study was 6.6 log CFU/mL, also meeting the above-mentioned standard, in this case of 4 log CFU/mL. The total yeast value obtained (6.6 log CFU/mL) is within the range of the expected amount, and not significantly different from the previously reported values of 5.62 log CFU/mL (Wulansari et al., 2021), 6.76 log CFU/mL (Setyawardani & Sumarmono, 2015), and 5.36 log CFU/mL (Dinkçi et al., 2015). Yeast plays a vital role in kefir fermentation, and its ability to produce ethanol and carbon dioxide generates the unique taste of kefir drinks (Kesenkaş, 2011).

### *4.3 Fatty acids profile*

Developing kefir products depends on essential factors, such as the chemical composition of milk, type and amount of starter culture, temperature, and time of fermentation (Boycheva et al., 2012). This study reported the formation of volatile compounds in goat milk kefir fermented with the combined addition of oat milk and *L. casei* AP culture. (Sumarmono et al., 2015) previously reported that the production of volatile compounds might change the fatty acids profile. However, it was here found that the combined addition of oat milk and *L. casei* AP did not affect it. This result confirms a previous study indicating that the fatty acids profile of goat milk kefir was not affected by the supplementation of *Moringa oleifera* at a concentration of up to 2.5% (w/v) (Wulansari et al., 2021).

The mean value of unsaturated fatty acids (USFA) reported in this study was 29.55%. Boycheva et al. (2012) previously proposed that USFA in food minimize the risk of coronary diseases. At the same time, the mean value of SFA in this study (70.45%) was higher than that of USFA (Table 4). Although SFA are easily oxidized, which results in the production of free radicals, their negative effects can be neutralized by the presence of USFA in fermented goat milk (Kustyawati & Tobing, 2012). The sensory characteristics of milk are affected by the concentration of free fatty acids. While some findings claim that the fatty acids profile generates the aroma of fermented milk, others draw a correlation with the incubation process, which may increase volatile compounds. It has been proposed that the main components of fermented milk's aroma are 2,3-butanediol, ethanol, formic acid, acetic acid, propanoic acid, propionic acid, butyric acid, and carbonyl groups (acetone and acetoin). The favorable aroma of fermented milk is not solely dependent on chemical compounds, but it is also affected by their relative proportion (Boycheva et al., 2012).

### *4.4 Sensory characteristics*

Based on the sensory analysis results, the highest score in color (7.2) was observed in kefir fermented using a combination of 12% oat milk and 4% *L. casei* AP (Table 5). In contrast, a lower score was obtained for kefir fermented using a combination of 16% oat milk and 4% of *L. casei* AP. In this study, incorporating 16% oat milk decreased the color preference of the panelists. Food color affects consumers' impressions (Rumeen et al., 2017). Sensory analysis also showed that the score for the aroma parameter ranged between 5.53 and 7.07 (Table 5). Probiotic is likely to improve sensoric properties of goat milk-based product (Ranadheera et al., 2019). The combination of oat milk (8, 12, and 16%) and *L. casei* AP (at concentrations of 2 and 4%) did not affect the aroma. The level of acidity that was favorable to the panelists was obtained from goat milk kefir produced with a combination of 16% oat milk and either 2 or 4% of *L. casei* AP, showing that the addition of oat milk affected the level of acidity. At the same time, the alcoholic taste, which is the typical flavor of kefir, was stronger in kefir added with *L. casei* AP. The sensory characteristics is related to a study by Mituniewicz-Małek et al. (2019) that sample was affected by the population of probiotic strain up to the last day of 14-day shelf life. A high potential of fermentation is indicative of biochemical activities of the strains during fermentation. The most favorable alcoholic taste was observed in kefir obtained with the combined addition of 16% oat milk and 4% *L. casei* AP (Table 5), which also had the most favorable texture with a score 7+ (slightly like). This finding was in line with a previous study suggesting that oat milk is a vital component for kefir's texture (Dinkçi et al., 2015). In this study, it was shown that the addition of oat milk increased the total solids of kefir products (Table 2), confirming a previous finding by Brückner-Gühmann et al. (2019) who drew a strong correlation between products' texture and increased total solids. The acceptance level is the most important evaluation of fermented food products. The acceptance of this sensory characteristics can be enhanced with emotion evaluations that are evoked by the product as a tools to gain additional information to optimize the products and market strategy by milk industry (Larosa et al., 2021b). The parameter of product development is whether the product has a high acceptance level among consumers (Piggott, 2011). The highest acceptance level in this study was obtained in kefir products prepared using a combination of 16% oat milk and 4% *L. casei* AP (Table 5). Our findings suggests that acidity, alcoholic taste, and texture are the main parameters that determine the general acceptance level of a product. This study is in line with previous findings that organoleptic evaluation did not show significant difference in taste, body, texture, and appearance of yogurt fortified with resin extract of *Pistacia atlantica* and the combined resin extract of *Pistacia atlantica* and *Saccharomyces boulardii* (Hadjimbei et al., 2020).

## 5 Conclusion

The data presented in this study show that the combined addition of oat milk and *L. casei* AP increase the viscosity of goat milk kefir and reduce the unwanted syneresis process. This combination did not affect the physicochemical and microbiological characteristics of the products, but it affected the sensory ones.

However, a combination of 16% (w/v) oat milk and 4% (v/v) *L. casei* AP increased the quality and sensory characteristics without producing a negative effect on the microbiological characteristics. This finding shows that a potential development of goat milk kefir based on the combination of oat milk and *L. casei* AP is feasible.

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## References

Association of Official Analytical Chemists – AOAC. (1975). *Official methods of analysis of the Association of Official Analytical Chemists* (12th ed.). Washington: Benjamin Franklin Station.

Barukčić, I., Gracin, L., Jambrak, A. R., & Božanić, R. (2017). Comparison of chemical, rheological and sensory properties of kefir produced by kefir grains and commercial kefir starter. *Mljekarstvo: časopis za unaprjeđenje proizvodnje i prerade mlijeka*, 67(3), 169-176. http://dx.doi.org/10.15567/mljekarstvo.2017.0301.

Bensmira, M., Nsabimana, C., & Jiang, B. (2010). Effects of fermentation conditions and homogenization pressure on the rheological properties of kefir. *Lebensmittel-Wissenschaft + Technologie*, 43(8), 1180-1184. http://dx.doi.org/10.1016/j.lwt.2010.04.005.

Bernat, N., Cháfer, M., González-Martínez, C., Rodríguez-García, J., & Chiralt, A. (2015). Optimisation of oat milk formulation to obtain fermented derivatives by using probiotic Lactobacillus reuteri microorganisms. *Food Science & Technology International*, 21(2), 145-157. http://dx.doi.org/10.1177/1082013213518936. PMid:24464238.

Biçer, Y., Telli, A. E., Sönmez, G., Turkal, G., Telli, N., & Uçar, G. (2021). Comparison of commercial and traditional kefir microbiota using metagenomic analysis. *International Journal of Dairy Technology*, 74(3), 528-534. http://dx.doi.org/10.1111/1471-0307.12789.

Bodyfelt, F. W., Tobias, J., & Trout, G. M. (1988). *The sensory evaluation of dairy products*. New York: Van Nostrand Reinhold.

Boycheva, S., Mihaylova, G., Naydenova, N., & Dimitrov, T. (2012). Amino acid and fatty acid content of yogurt supplemented with walnut and hazelnut pieces. *Trakia Journal of Sciences*, 10(2), 17-25.

Brückner-Gühmann, M., Benthin, A., & Drusch, S. (2019). Enrichment of yoghurt with oat protein fractions: structure formation, textural properties and sensory evaluation. *Food Hydrocolloids*, 86, 146-153. http://dx.doi.org/10.1016/j.foodhyd.2018.03.019.

Cais-Sokolińska, D., Wojtowski, J., Pikul, J., Danków, R., Majcher, M., Teichert, J., & Bagnicka, E. (2015). Formation of volatile compounds in kefir made of goat and sheep milk with high polyunsaturated fatty acid content. *Journal of Dairy Science*, 98(10), 6692-6705. http://dx.doi.org/10.3168/jds.2015-9441. PMid:26277315.

Champagne, C. P., Cruz, A. G., & Daga, M. (2018). Strategies to improve the functionality of probiotics in supplements and foods. *Current Opinion in Food Science*, 22, 160-166. http://dx.doi.org/10.1016/j.cofs.2018.04.008.

Demïr, H., Simsek, M., & Yıldırım, G. (2021). Effect of oat milk pasteurization type on the characteristics of yogurt. *LWT*, 135, 110271. http://dx.doi.org/10.1016/j.lwt.2020.110271.

Deora, N., & Deswal, A. (2018). Non-dairy based beverages: an insight. *Journal of Nutrition, Food Research and Technology*, 1(1), 1-4. http://dx.doi.org/10.30881/jnfrt.00002.

Dinkçi, N., Kesenkaş, H., Korel, F., & Kınık, Ö. (2015). An innovative approach: cow/oat milk based kefir. *Mljekarstvo: časopis za unaprjeđenje proizvodnje i prerade mlijeka*, 65(3), 177-186. http://dx.doi.org/10.15567/mljekarstvo.2015.0304.

Garofalo, C., Ferrocino, I., Reale, A., Sabbatini, R., Milanović, V., Alkić-Subašić, M., Boscaino, F., Aquilanti, L., Pasquini, M., Trombetta, M. F., Tavoletti, S., Coppola, R., Cocolin, L., Blesić, M., Sarić, Z., Clementi, F., & Osimani, A. (2020). Study of kefir drinks produced by backslopping method using kefir grains from Bosnia and Herzegovina: microbial dynamics and volatilome profile. *Food Research International*, 137, 109369. http://dx.doi.org/10.1016/j.foodres.2020.109369. PMid:33233071.

Goktas, H., Dikmen, H., Demirbas, F., Sagdic, O., & Dertli, E. (2021). Characterisation of probiotic properties of yeast strains isolated from kefir samples. *International Journal of Dairy Technology*, 74(4), 715-722. http://dx.doi.org/10.1111/1471-0307.12802.

HadiNezhad, M., Duc, C., Han, N., & Hosseinian, F. (2013). Flaxseed soluble dietary fibre enhances lactic acid bacterial survival and growth in kefir and possesses high antioxidant capacity. *Journal of Food Research*, 2(5), 152-163. http://dx.doi.org/10.5539/jfr.v2n5p152.

Hadjimbei, E., Botsaris, G., Goulas, V., Alexandri, E., Gekas, V., & Gerothanassis, I. P. (2020). Functional stability of goats' milk yoghurt supplemented with Pistacia atlantica resin extracts and Saccharomyces boulardii. *International Journal of Dairy Technology*, 73(1), 134-143. http://dx.doi.org/10.1111/1471-0307.12629.

Han, X., Yang, Z., Jing, X., Yu, P., Zhang, Y., Yi, H., & Zhang, L. (2016). Improvement of the texture of yogurt by use of exopolysaccharide producing lactic acid bacteria. *BioMed Research International*, 2016, 7945675. http://dx.doi.org/10.1155/2016/7945675. PMid:27294135.

John, S. M., & Deeseenthum, S. (2015). Properties and benefits of kefir-a review. *Songklanakarin Journal of Science and Technology*, 37(3), 275-282.

Kesenkaş, H. (2011). Antioxidant properties of kefir produced from different cow and soy milk mixtures. *Journal of Agricultural Sciences*, 17(3), 253-259.

Kustyawati, M. E., & Tobing, D. (2012). Profil asam lemak dan asam amino susu kambing segar dan terfermentasi [Fatty acid and amino acid profile of fresh and fermented goat milk]. *Jurnal Teknologi dan Industri Pangan*, 23(1), 47.

Kwak, H., Park, S., & Kim, D. (1996). Biostabilization of kefir with a nonlactose-fermenting yeast. *Journal of Dairy Science*, 79(6), 937-942. http://dx.doi.org/10.3168/jds.S0022-0302(96)76444-5.

Larosa, C. P., Balthazar, C. F., Guimarães, J. T., Margalho, L. P., Lemos, F. S., Oliveira, F. L., Abud, Y. K., Sant'Anna, C., Duarte, M. C. K., Granato, D., Raices, R. S. L., Freitas, M. Q., Sant'Ana, A. S., Esmerino, E. A., Pimentel, T. C., Silva, M. C., & Cruz, A. G. (2021a). Can sucrose-substitutes increase the antagonistic activity against foodborne pathogens, and improve the technological and functional properties of sheep milk kefir? *Food Chemistry*, 351, 129290. http://dx.doi.org/10.1016/j.foodchem.2021.129290. PMid:33631613.

Larosa, C. P., Balthazar, C. F., Guimarâes, J. T., Rocha, R. S., Silva, R., Pimentel, T. C., Granato, D., Duarte, M. C. K., Silva, M. C., Freitas, M. Q., Cruz, A. G., & Esmerino, E. A. (2021b). Sheep milk kefir sweetened with different sugars: Sensory acceptance and consumer emotion profiling. *Journal of Dairy Science*, 104(1), 295-300. http://dx.doi.org/10.3168/jds.2020-18702. PMid:33162085.

Lengkey, H., & Balia, R. (2014). The effect of starter dosage and fermentation time on pH and lactic acid production. *Biotechnology in Animal Husbandry*, 30(2), 339-347. http://dx.doi.org/10.2298/BAH1402339L.

Lucatto, J. N., Silva-Buzanello, R. A., Mendonça, S. N. T. G., Lazarotto, T. C., Sanchez, J. L., Bona, E., & Drunkler, D. A. (2020). Performance of different microbial cultures in potentially probiotic and prebiotic yoghurts from cow and goat milks. *International Journal of Dairy Technology*, 73(1), 144-156. http://dx.doi.org/10.1111/1471-0307.12655.

Mitra, S., & Ghosh, B. C. (2020). Quality characteristics of kefir as a carrier for probiotic Lactobacillus rhamnosus GG. *International Journal of Dairy Technology*, 73(2), 384-391. http://dx.doi.org/10.1111/1471-0307.12664.

Mituniewicz-Małek, A., Zielińska, D., & Ziarno, M. (2019). Probiotic monocultures in fermented goat milk beverages–sensory quality of final product. *International Journal of Dairy Technology*, 72(2), 240-247. http://dx.doi.org/10.1111/1471-0307.12576.

Nurliyani, Harmayani, E., & Sunarti (2014). Microbiological quality, fatty acid and amino acid profiles of kefir produced from combination of goat and soy milk. *Pakistan Journal of Nutrition*, 13(2), 107-115. http://dx.doi.org/10.3923/pjn.2014.107.115.

Nurliyani, Sadewa, A. H., & Sunarti (2015). Kefir properties prepared with goat milk and black rice (Oryza sativa L.) extract and its influence on the improvement of pancreatic β-cells in diabetic rats. *Emirates Journal of Food and Agriculture*, 27(10), 727-735. http://dx.doi.org/10.9755/ejfa.2015-04-138.

Piggott, J. (2011). *Alcoholic beverages: sensory evaluation and consumer research*. Cambridge: Woodhead Publishing Limited.

Ramirez-Santiago, C., Ramos-Solis, L., Lobato-Calleros, C., Peña-Valdivia, C., Vernon-Carter, E. J. & Alvarez-Ramírez, J. (2010). Enrichment of stirred yogurt with soluble dietary fiber from Pachyrhizus erosus L. Urban: Effect on syneresis, microstructure and rheological properties. *Journal of Food Engineering,* 101(3): 229-235.

Ranadheera, C. S., Evans, C. A., Baines, S. K., Balthazar, C. F., Cruz, A. G., Esmerino, E. A., Freitas, M. Q., Pimentel, T. C., Wittwer, A. E., Naumovski, N., Graça, J. S., Sant'Ana, A. S., Ajlouni, S., & Vasiljevic, T. (2019). Probiotics in goat milk products: delivery capacity and ability to improve sensory attributes. *Comprehensive Reviews in Food Science and Food Safety*, 18(4), 867-882. http://dx.doi.org/10.1111/1541-4337.12447. PMid:33337004.

Rumeen, S. F., Yelnetty, A., Tamasoleng, M., & Lontaan, N. (2017). Penggunaan level sukrosa terhadap sifat sensoris kefir susu sapi. *Jurnal Zootek*, 38(1), 123-130. http://dx.doi.org/10.35792/zot.38.1.2018.18565.

Santos, W. M., Nobre, M. S. C., Cavalcanti, M. T., Santos, K. M. O., Salles, H. O., & Buriti, F. C. A. (2019). Proteolysis of reconstituted goat whey fermented by Streptococcus thermophilus in co-culture with commercial probiotic Lactobacillus strains. *International Journal of Dairy Technology*, 72(4), 559-568. http://dx.doi.org/10.1111/1471-0307.12621.

Satir, G., & Guzel-Seydim, Z. B. (2016). How kefir fermentation can affect product composition? *Small Ruminant Research*, 134, 1-7. http://dx.doi.org/10.1016/j.smallrumres.2015.10.022.

Setyawardani, T., & Sumarmono, J. (2015). Chemical and microbiological characteristics of goat milk kefir during storage under different temperatures. *Journal of The Indonesian Tropical Animal Agriculture*, 40(3), 183-188. http://dx.doi.org/10.14710/jitaa.40.3.183-188.

Setyawardani, T., Sumarmono, J., Arief, I. I., Rahardjo, A. H. D., Widayaka, K., & Santosa, S. S. (2020). Improving composition and microbiological characteristics of milk kefir using colostrum. *Food Science and Technology*, 40(Suppl. 2), 699-707. http://dx.doi.org/10.1590/fst.31719.

Sumarmono, J., Sulistyowati, M., & Soenarto (2015). Fatty acids profiles of fresh milk, yogurt and concentrated yogurt from Peranakan Etawah goat milk. *Procedia Food Science*, 3, 216-222. http://dx.doi.org/10.1016/j.profoo.2015.01.024.

Widodo, W., Anindita, N. S., Taufiq, T. T., & Wahyuningsih, T. D. (2012a). Identification of Pediococcus strains isolated from feces of Indonesian infants with in vitro capability to consume prebiotic inulin and to adhere on mucus. *Indonesian Journal of Biotechnology*, 17(2), 132-143.

Widodo, W., Anindita, N., Tiyas, T., & Wahyuningsih, T. (2014). Evaluation of two Lactobacillus strains as probiotics with emphasis in utilizing prebiotic inulin as energy source. *International Research Journal of Microbiology*, 5, 33-40.

Widodo, W., Harsita, P. A., Sukarno, A. S., & Nurrochmad, A. (2019). Antidiabetic effect of milk fermented using intestinal probiotics. *Nutrition & Food Science*, 49(6), 1063-1074. http://dx.doi.org/10.1108/NFS-11-2018-0326.

Widodo, W., Taufiq, T. T., Aryati, E., Kurniawati, A., & Asmara, W. (2012b). Human origin Lactobacillus casei isolated from Indonesian infants demonstrating potential characteristics as probiotics in vitro. *Indonesian Journal of Biotechnology*, 17(1), 79-89.

Widodo, T. D., Wahyuningsih, A., Nurrochmad, E., Wahyuni, T. T., Taufiq, N. S., Anindita, S., Lestari, P. A., Harsita, A. S., Sukarno & Handaka, R. (2017). *Bakteri Asam laktat Strain Lokal.* Yogyakarta: Gadjah Mada University Press.

Wulansari, P. D., Nurliyani, Endah, S. R. N., Nofriyaldi, A., & Harmayani, E. (2021). Microbiological, chemical, fatty acid and antioxidant characteristics of goat milk kefir enriched with Moringa oleifera leaf powder during storage. *Food Science and Technology*. Ahead of print.

Zamberi, N. R., Abu, N., Mohamed, N. E., Nordin, N., Keong, Y. S., Beh, B. K., Zakaria, Z. A. B., Rahman, N. M. A. N. A., & Alitheen, N. B. (2016). The antimetastatic and antiangiogenesis effects of kefir water on murine breast cancer cells. *Integrative Cancer Therapies*, 15(4), NP53-NP66. http://dx.doi.org/10.1177/1534735416642862. PMid:27230756.

Zendeboodi, F., Khorshidian, N., Mortazavian, A. M., & Cruz, A. G. (2020). Probiotic: conceptualization from a new approach. *Current Opinion in Food Science*, 32, 103-123. http://dx.doi.org/10.1016/j.cofs.2020.03.009.

Zhang, P.-P., Hu, X.-Z., Zhen, H.-M., Xu, C., & Fan, M.-T. (2012). Oat β-glucan increased ATPases activity and energy charge in small intestine of rats. *Journal of Agricultural and Food Chemistry*, 60(39), 9822-9827. http://dx.doi.org/10.1021/jf3017496. PMid:22970825.