

Article

Quality Assessment of Organic Kefirs Made with Kefir Grains and Freeze-Dried Starter Cultures

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Abstract: Organic dairy products, including fermented milk, are gaining popularity among consumers. Traditionally, for kefir production, kefir grains are used; however, in the modern industry, freeze-dried cultures are commonly employed. This study aimed to analyze the quality of kefir produced from organic cow milk with two fermentation times (12 and 24 h) with the use of kefir grains or freeze-dried culture. During a 3-week storage period, physicochemical properties, color, syneresis index, texture, sensory, and microbiological quality were evaluated. The results proved that organic cow milk was suitable for kefir production both with the use of kefir grains and freeze-dried culture. Using freeze-dried culture and a 24 h fermentation period resulted in kefir with the lowest syneresis tendency (3.65–9.62%), along with the best textural properties (the highest cohesiveness and viscosity index), and desired sensory characteristics. Kefir grains had a better acidification ability, and the resulting products had a higher count of yeasts, lactobacilli, and lactococci compared with kefir obtained with freeze-dried culture. However, both the type of starter culture and the fermentation time influenced the product's quality. The longer fermentation time of 24 h was more appropriate for the kefir production. Kefir grains, as a traditional form of kefir culture, may be preferred in organic kefir production.

Keywords: kefir; kefir grains; freeze-dried kefir culture; organic milk



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1. Introduction

Organic food, otherwise known as BIO food, refers to products derived from organic raw materials. Organic dairy products are made from milk sourced from certified organic farms that adhere to strict requirements regarding the nutrition, maintenance, and treatment of dairy animals. Organic milk processing involves the highest standards of quality and safety [1]. European Union regulations, mainly Regulation (EU) 2018/848 of the European Parliament and of the Council [2], define the rules for the production, labeling, and inspection of organic products. Organic dairy products are gaining popularity among consumers. This trend is driven by the fact that, when making a purchasing decision, consumers are increasingly guided by environmental considerations (such as sustainability in farming) and animal welfare [3,4]. Another factor influencing the choice of organic dairy products is the assurance that they are free from residues of artificial fertilizers and pesticides, antibiotics, and hormones, which are prohibited in organic production. Consumers are also guided by the conviction that organic milk is superior in many ways to conventional milk. Those are the reasons why consumers usually choose the more expensive organic products [4–6].

Due to differences in organic and conventional milk processing, the resulting products may differ in terms of quality characteristics. These variations are a result of different feeding and animal housing conditions on organic farms compared with a conventional one [5–8].

Organic milk is characterized by a more favorable fatty acid profile than conventional milk. It has a higher concentration of beneficial fatty acids (FA) including n-3 polyunsaturated fatty acids (n-3 PUFA) [9–11], conjugated linoleic acid (CLA) [12–15], and α -linolenic acid [13,16]. Other studies have demonstrated higher concentrations of fat-soluble vitamins tocopherol and β -carotene in organic than conventional milk [12,14,17]. Moreover, organic milk has a higher concentration of such bioactive compounds as lactoferrin [18,19], lysozyme [18,19], and phospholipids [15] than conventional milk. In case of proximate chemical composition of organic milk, many studies indicate a lower protein and casein content compared with conventional milk [11,19–22]. It is likely caused by a lower amount of concentrated feed starch in the nutrition of organic dairy cattle [20]. In the case of fat content, a meta-analysis conducted by Średnicka-Tober et al. [23] found no significant difference between milk from organic and conventional farming. The literature presents contradictory results regarding lactose content, which may be similar [4] or lower [16,20,24] in organic milk compared with conventional milk. The mineral content is lower in organic milk, as conventional farming allows for mineral supplementation in concentrated feed [4,18,25]. However, conventional milk tends to be more contaminated with heavy metals [4,26]. In terms of sensory quality, organic and conventional milk were compared by Brodziak et al. [27]. The study indicated a positive consumer perception and full acceptance of organic milk. Conversely, a study conducted by Bloksma et al. [28] showed that organic milk from grazing cows was creamier and had a stronger grassy flavor compared with conventional milk from cows kept in barns. The differences in the composition of organic and conventional milk, especially the higher concentration of long-chain PUFA and bioactive compounds in organic milk, make it a better medium for lactic acid bacteria (LAB) growth. Thus, organic milk appears to be a better raw material for fermented beverage production such as kefir and yogurt than its conventional counterpart [25].

Kefir is a type of fermented dairy beverage in which LAB conducting lactic acid fermentation as well as yeasts conducting alcoholic fermentation are involved [29]. Kefir is an acidified, slightly carbonated fermented milk containing small amounts of ethyl alcohol with specific flavor typical of yeasts [30,31]. Kefir originates from the Caucasus Mountains, but it is now gaining popularity throughout the world [31,32]. Traditionally, in kefir production, a starter culture in the form of kefir grains (KGs) is used. KGs are conglomerates of symbiotic microorganisms immobilized in a natural matrix of exopolysaccharide kefiran and proteins. The size of kefir grains ranges from 1 to 4 cm, they are irregular in shape, are white to light yellow in color, and resemble small cauliflower florets [32,33]. They are added to milk in a ratio of 2 to 10%, and after fermentation, they are removed from kefir and may be re-used [34,35]. The predominant microbiota of KGs includes homofermentative and heterofermentative LAB belonging to the *Lactobacillus*, *Lactococcus*, *Leuconostoc*, and *Streptococcus* species, as well as acetic acid bacteria (AAB) of the *Acetobacter* genus, and yeasts, primarily *Saccharomyces cerevisiae*, *Candida kefir*, *Kluyveromyces marxianus*, *Torulaspora delbrueckii*, and *Kazachstania unispora* [31,32,36,37].

The wide diversity of microorganisms present in traditional kefir allows it to be considered as a natural probiotic product [35,38]. LAB strains found in traditional kefir produce organic acids, bacteriocins, and hydrogen peroxide which are antimicrobial agents and inhibit the growth of pathogens [38–40]. Kefir also exhibits immune-modulating activity and has anti-cancer, anti-hypertensive, and calcium-binding properties, all of which contribute to its health-promoting and therapeutic effects [29,30,33,41]. Moreover, kefir consumption is effective in preventing and treating obesity and metabolic disorders such as diabetes [42–44]. Its consumption is recommended during periods of convalescence or anemia [31,33]. Additionally, it has a beneficial effect on digestion, improves lactose digestibility, and may influence plasma glucose levels [31,33].

In industrial-scale kefir production, KGs are rarely used due to their complex application and the variability in kefir properties. Instead, commercial freeze-dried cultures, which contain selected species of LAB and yeasts in fixed proportions are often used. This kind of culture is easier to use and provides standardization of kefir quality. How-

ever, it is characterized by lower species diversity resulting in kefir with properties that differ from those produced with KGs, particularly in terms of reduced therapeutic benefits [30,32,38,41]. Traditional processing methods, like the production of kefir with KGs, are typically more time-consuming and labor-intensive than modern methods [1]. However, in organic food processing, traditional production methods are preferred [2], making the use of KGs desirable for organic kefir production.

The aim of this study was to analyze the quality characteristics of kefir obtained from organic cow milk using traditional kefir grains or freeze-dried cultures as a starter. The fermentation of kefir was conducted for a period of 12 or 24 h. The obtained products were refrigerated and stored for a period of 21 days.

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2. Materials and Methods

2.1. Materials

Pasteurized organic cow milk originated from an organic farm located in Eastern Poland and was purchased at a local market in Szczecin, Poland. The milk was obtained during the summer period. Samples of kefir were manufactured under laboratory conditions. Four variants of organic kefir were produced during the study (Table 1).

For kefir fermentation, a traditional culture in the form of kefir grains (KG) (Serowar, Szczecin, Poland) and a freeze-dried (lyophilized) culture, Kefir-31 (Biochem s.r.l., Rome, Italy) intended for direct inoculation of processed milk were used. The freeze-dried culture contained the following microorganisms: *Lactococcus lactis* subsp. *cremoris*, *Lactococcus lactis* subsp. *lactis* biovar *diacetylactis*, *Leuconostoc mesenteroides* subsp. *cremoris*, *Lactobacillus delbrueckii* subsp. *bulgaricus*, and *Saccharomyces cerevisiae*. The culture in the form of KGs was added in the amount of 20 g/1 L of milk [31,45–47], while the freeze-dried culture was added at a rate of 0.1 g per 1 L, according to the manufacturer's recommendations. Kefir fermentation was carried out at 30 °C, which is within the optimum temperature range for the freeze-dried culture and can also be used in the production of kefir using the traditional KGs [33,47]. The kefir was produced using the tank method. The procedure of kefir production is shown in Figure 1.

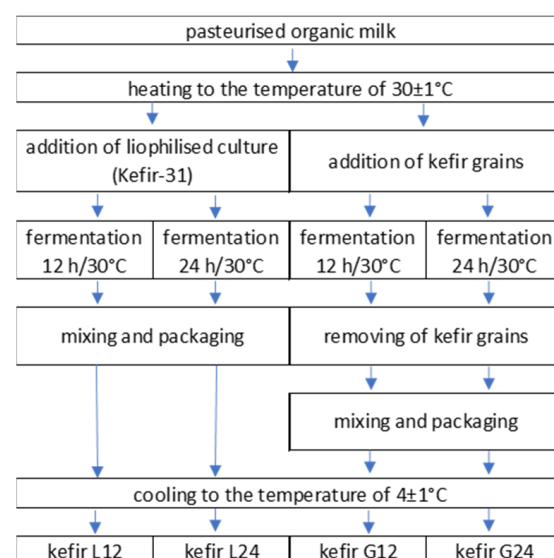


Figure 1. Scheme of experimental kefir production.

Table 1. Variants of experimental organic kefir.

Symbol	Kind of Starter Culture	Time and Temperature of Fermentation
L12	Freeze-dried culture kefir-31	12 h/30 °C
L24	Freeze-dried culture kefir-31	24 h/30 °C
G12	Kefir grains	12 h/30 °C
G24	Kefir grains	24 h/30 °C

Each variant of kefir was produced in a quantity of 3 L and bottled in 30 disposable plastic cups with lids of 100 mL each. The kefir was stored at 4 ± 1 °C for a period of three weeks. The kefir was analyzed after 1, 7, 14, and 21 days of refrigerated storage (4 ± 1 °C).

2.2. Methods

2.2.1. Organic Milk Assessment

In organic milk, the following parameters were tested: pH using a pH-meter (Elmetron, Zabrze, Poland) calibrated with 4.01 and 7.01 buffers, titratable acidity, density, total solids content, fat content with Gerber's method, and total protein content with Kjeldahl's method [48]. Additionally, color parameters were assessed with a colorimeter model FRU[®] WR-18 with an 8 mm aperture (Shenzhen Wave Optoelectronics Technology Co., Ltd., Shenzhen, China), which was calibrated with the standard white plate. Color was measured in the CIELab system and L , a^* , and b^* parameters were determined. The parameter L represents lightness and its value oscillates on a scale from 0—pure black to 100—pure white. Parameter a^* indicates the proportion of green or red color, and parameter b^* reflects the proportion of blue or yellow in the analyzed color. Color was analyzed in a 2 cm bromosilicon cuvette for spectroscopy. Additionally, the whiteness index (WI), which combines lightness and yellow–blue into a single value, was measured using the following equation [49]:

$$WI = 100 - \sqrt{(100 - L)^2 + a^2 + b^2} \quad (1)$$

2.2.2. Physicochemical Assessment

In organic kefir, the following parameters were estimated: pH (pH-meter Elmetron, Zabrze, Poland), titratable acidity (expressed as % of lactic acid), total solids content with the drying method [48], ethanol content, index of syneresis, texture parameters, and color and sensory assessment. Color was assessed with the same method as in milk. Ethanol, as the main volatile aroma compound in kefir, was measured according to the method described by Sulmiyati et al. [47] through the titration of a 10 g sample with 0.1 M NaOH using phenolphthalein as the indicator. The syneresis index (SI) was determined using the centrifuge method [50] with modifications. A 20 g sample of kefir was weighed into 50 mL centrifuge tubes. Care was taken to disturb the curd as little as possible. Subsequently, the tubes were centrifuged at 3000 rpm for 20 min. The whey, separated above the curd surface, was gently transferred to a beaker and weighed. The syneresis index (SI) was calculated using the following formula:

$$SI(\%) = \frac{\text{mass of whey, g}}{\text{mass of kefir, g}} \times 100 \quad (2)$$

2.2.3. Texture Analysis

Parameters of texture were measured using a TA-XT Plus texture analyzer (Stable Micro Systems, Surrey, UK). To analyze the texture profile of the samples, a backward extrusion test was performed using a 25 mm diameter aluminum cylindrical probe. The test parameters were as follows: test speed of 1 mm/s; force of 10.0 g; immersion depth of 20 mm; time (test duration) of 5.0 s. The following texture parameters were recorded: firmness, defined as the maximum compressive force exerted on the sample (g); consistency, represented by the area under the curve during extrusion (g·s); cohesiveness, defined as

the maximum compressive force during the withdrawal of the probe from the sample (g); and the viscosity index, represented by the area in the negative region of the curve during probe withdrawal (g·s). The values of individual texture parameters were calculated using Exponent software (version 6.1.1.0) [51], with modifications.

2.2.4. Sensory Assessment

The sensory evaluation of organic kefir was conducted by six panelists trained in the analysis of fermented dairy products. The test assessed the appearance, taste, odor, consistency, and texture using a 1–5 point scale, where 1 point represented the very bad quality, 2 points the bad, 3 points the neutral; 4 points the good, and 5 points the very good quality of a characteristic. The analysis was carried out in a room free from foreign odors. Each evaluator was provided with a separate station and water to rinse the palate between evaluated samples. The samples were coded with two-digit random numbers [47,52].

2.2.5. Weight Gain of Kefir Grains

During the traditional production of kefir using the KGs, the weight gain of grains was determined [47]. For this purpose, kefir grains were weighed before being added to the milk. After the fermentation process was completed, the kefir grains were filtered through a sieve and weighed again. The weight gain of kefir grains, expressed as a percentage, was calculated using the formula:

$$WG(\%) = \frac{F - A}{A} \times 100 \quad (3)$$

where *WG* is the weight gain of kefir grains; *A* is the weight of kefir grains before fermentation; *F* is the weight of kefir grains after fermentation.

2.2.6. The Microbiological Analysis

The analysis was performed on the starters used in the study (KGs and freeze-dried culture) and fermented milk. The study was conducted during the fermentation of kefir (directly after the inoculation of the milk with the starter culture and after 12 h and 24 h of fermentation) and after 7, 14, and 21 days of refrigerated storage. The analyses were performed in three independent replicates. In order to determine the number of microorganisms, expressed as cfu/g of the test sample, the procedure followed the standard [53] using a serial dilution technique and culture methods. Lactic acid bacteria, including lactobacilli and lactococci, were determined according to standard [54], using deMan–Rogosa–Sharpe agar (MRS, OXOID, Basingstoke, UK) and M-17 agar (BTL, Łódź, Poland), respectively. The determination of the total non-lactic acid bacterial count (THC) followed the standard [55] using an agar medium (BTL, Łódź, Poland), while the total fungal count (TYMC), including yeasts and molds, was determined according to the standard [56].

2.2.7. Statistical Analysis

The obtained results were statistically analyzed using Statistica 13.1 software (StatSoft Inc., Tulsa, OK, USA), with a significance level of $p = 0.05$. Mean values and standard deviations were calculated. The Shapiro–Wilk test confirmed that all factors had a normal distribution of variables. A two-way ANOVA followed by Tukey’s post hoc test was used to indicate the differences among the analyzed samples. Analyses were conducted in 3 to 6 repetitions, depending on the methodology.

3. Results and Discussion

3.1. Analysis of Organic Milk

Before kefir production, the quality properties of organic milk were assessed. The pH and lactic acid contents were 6.71 ± 0.01 and $0.135 \pm 0.000\%$, respectively. These values are consistent with those of fresh cow milk [52] for kefir production. The contents of fat, total protein, and total solids were $4.0 \pm 0.01\%$, $3.32 \pm 0.01\%$ and $9.1 \pm 0.01\%$,

respectively. These values were higher than in organic milk used for kefir production in the study by Güler et al. [34] (3.15%, 3.12%, and 8.2%, respectively). The high fat content of the organic milk used in our study may have resulted in an increased content of bioactive compounds [9,12]. Linehan et al. [4] presented the following ranges of total protein and fat in organic milk: 3.1–3.26%; 3.7–4.0%. Compared with our results, the content of total protein was higher and the content of fat fell within cited range. According to the *CA Standard for Fermented Milks* [57], kefir should contain at least 2.7% of milk protein and less than 10% of milk fat; thus, the composition of milk was appropriate for kefir production. The color analysis of organic milk revealed the following results: 76.46 ± 0.01 for L, -2.2 ± 0.01 for a^* , 4.35 ± 0.01 for b^* , and 75.96 ± 0.09 for WI. The negative value of the a^* parameter and the positive value of the b^* parameter indicated a green-yellow color of the milk. Higher color values, specifically L (81.0 ± 8.1), a^* (-1.5 ± 3.0), and b^* (7.5 ± 4.4) were recorded by Milovanovic et al. [58] based on the average of color measurements from 41 samples of cow milk. The color of milk, and consequently dairy products, is influenced by many factors, including genetics, feeding, and climatic conditions [59].

3.2. Weight Gain of Kefir Grains

During the fermentation of organic kefir with KGs, an increase in grain weight was observed. For kefir G12 fermented for 12 h, the increase was 4.2%, while for kefir G24 fermented for 24 h, the grain weight increase was more than double, reaching 8.6%. These results indicate that weight gain is proportional to the duration of milk fermentation. The observed increase in the weight of KGs indicated their high activity. The growth of KGs during milk fermentation, as well as the possibility of their recovery and reuse, is an undoubted advantage of this starter in traditional kefir production [34,35]. Similar results regarding KG weight gain (5.1–7.2%) during the production of kefir from goat milk acidified for 12 h at 37 °C were obtained by Sulmiyati et al. [47]. Additionally, Azizi et al. [33] reported that the weight gain of KGs during kefir production ranged from 5 to 7% and was determined by the number of KGs added, the time, and the temperature of fermentation. In another study conducted by Pop et al. [60], which investigated the effect of different conditions on the growth of KGs, the influence of the type of milk—conventional and organic—on kefir quality was assessed. The organic milk composition was characterized by a higher content of protein and lactose, both of which are necessary for the growth of KGs. During the 72 h fermentation, the growth of microbial biomass was found to be higher in the organic milk, indicating the suitability of this raw material for kefir production.

3.3. Analysis of Organic Kefirs During Refrigerated Storage

3.3.1. Physicochemical Parameters

The results of active acidity (pH) and titratable acidity (% of lactic acid) measured over 21 storage days are presented in Table 2. The value of both types of acidity depended on the type of kefir and the storage time (Table A1). Throughout the storage period, kefir G24 had the lowest pH values, followed by G12. Kefirs made with freeze-dried culture (L12 and L24) had significantly higher ($p < 0.05$) pH values, indicating the lower acidification capacity of this culture compared to traditional kefir grains. According to *CA Standard for Fermented Milks* [57], the titratable acidity of kefir cannot be lower than 0.6% of lactic acid, and all the kefir met this standard throughout the storage period. Gul et al. [61] also found lower pH values in cow milk-based kefir made with KGs compared with kefir made with freeze-dried cultures. The acidification ability of the kefir starter culture is determined not only by the type of culture but also by the quantitative proportion of individual microorganisms, the size of the inoculum, as well as the temperature and duration of the process and agitation during fermentation [45,46]. The results for kefir G24 were in agreement with the findings of Yousefvand et al. [62], who reported pH values in the range of 4.3–4.5 for kefir obtained with KG supplementation during 3-week refrigerated storage. Lower values of pH (below

pH 4) in kefir produced with KGs were reported by Bağdat and Kahraman Ilikkan [63], and La Torre et al. [64].

Table 2. Physicochemical properties of organic kefir during refrigerated storage.

Day	L12	L24	G12	G24
pH				
1	4.75 ^{c,D} ± 0.01	4.71 ^{b,C} ± 0.01	4.45 ^{b,B} ± 0.01	4.21 ^{a,A} ± 0.01
7	4.70 ^{b,D} ± 0.01	4.65 ^{a,C} ± 0.01	4.41 ^{a,B} ± 0.01	4.25 ^{b,A} ± 0.01
14	4.63 ^{a,C} ± 0.01	4.72 ^{b,D} ± 0.01	4.55 ^{c,B} ± 0.01	4.30 ^{c,A} ± 0.01
21	4.71 ^{b,B} ± 0.01	4.70 ^{b,B} ± 0.02	4.69 ^{d,B} ± 0.00	4.45 ^{d,A} ± 0.01
Titratable acidity, % of lactic acid				
1	0.78 ^{b,B} ± 0.01	0.81 ^{c,C} ± 0.00	0.75 ^{a,b,A} ± 0.01	0.83 ^{b,c,D} ± 0.00
7	0.66 ^{a,A} ± 0.00	0.74 ^{a,B} ± 0.01	0.80 ^{b,C} ± 0.04	0.86 ^{c,D} ± 0.01
14	0.76 ^{b,A,B} ± 0.00	0.78 ^{b,B} ± 0.01	0.70 ^{a,A} ± 0.04	0.82 ^{b,c,D} ± 0.01
21	0.84 ^{c,C} ± 0.01	0.72 ^{a,A,B} ± 0.00	0.68 ^{a,A} ± 0.04	0.77 ^{a,B} ± 0.02
Water content, %				
1	88.86 ^{b,C} ± 0.07	88.62 ^{c,A,B} ± 0.05	88.42 ^A ± 0.05	88.70 ^{b,B,C} ± 0.14
7	88.68 ^{b,A} ± 0.18	88.26 ^{a,b,A} ± 0.02	88.31 ^A ± 0.15	88.59 ^{a,b,A} ± 0.52
14	87.94 ^{a,A} ± 0.14	88.05 ^{a,A} ± 0.08	88.15 ^A ± 0.24	87.96 ^{a,A} ± 0.10
21	88.60 ^{b,A} ± 0.08	88.34 ^{b,c,A} ± 0.20	88.48 ^A ± 0.41	88.32 ^{a,b,A} ± 0.05
Syneresis index, %				
1	57.12 ^{b,C} ± 1.42	3.65 ^A ± 0.38	57.76 ^{a,C} ± 1.15	43.34 ^{a,B} ± 0.44
7	38.34 ^{a,B} ± 2.28	5.13 ^A ± 0.57	61.11 ^{b,D} ± 1.10	47.54 ^{b,C} ± 2.39
14	35.36 ^{a,B} ± 9.06	9.62 ^A ± 3.18	63.87 ^{c,D} ± 0.36	50.31 ^{b,C} ± 0.39
21	23.48 ^{a,B} ± 2.02	8.78 ^A ± 3.40	61.56 ^{b,c,D} ± 0.92	50.62 ^{b,C} ± 0.20
Ethanol content, %				
1	0.35 ^{a,A} ± 0.02	0.40 ^{c,B} ± 0.00	0.37 ^{c,A} ± 0.01	0.42 ^{c,B} ± 0.00
7	0.40 ^{b,c,A} ± 0.00	0.38 ^{b,A} ± 0.00	0.39 ^{d,A} ± 0.00	0.43 ^{d,B} ± 0.00
14	0.41 ^{c,D} ± 0.00	0.37 ^{a,B} ± 0.00	0.33 ^{b,A} ± 0.00	0.40 ^{b,C} ± 0.00
21	0.38 ^{b,C} ± 0.01	0.37 ^{a,b,B,C} ± 0.01	0.31 ^{a,A} ± 0.00	0.37 ^{a,B} ± 0.00

Explanatory notes: The table presents mean values ± standard deviation. L12—kefir fermented with a freeze-dried culture for 12 h; L24—kefir fermented with a freeze-dried culture for 24 h; G12—kefir fermented with kefir grains for 12 h; G24—kefir fermented with kefir grains for 24 h. Different superscript letters (^{a–d}) indicate significant differences in the same kefir on different storage days. Superscript capital letters (^{A–D}) indicate significant differences between kefir on the same storage day ($p \leq 0.05$).

Regarding titratable acidity, kefir G24 had the highest lactic acid content (Table 2). During storage, an increase in lactic acid content was observed only in kefir L12. The stability of titratable acidity in kefir during refrigerated storage is related to the presence of yeast, which slows the proliferation of lactic acid bacteria, resulting in the production of small amounts of lactic acid [46,61]. The lactic acid content in kefir G24 was consistent with the findings of Yousefvand et al. [62] for kefir obtained with the addition of 2% KG and fermented at 28 °C for 20 h (0.88–0.91%).

The study showed that the water content for experimental kefir presented similar values to that observed in milk and oscillated around 88–90% (Table 2). The water content of kefir was stable over the duration of the experiment. Significant differences ($p < 0.05$) between the variants were recorded only on day 1. A similar range of water content in kefir was reported in a study by Setyawardani and Sumarmono [65] (86.87–89.84%) and Irigoyen et al. (2003) [46]. A higher range of water content in kefir obtained with KG supplementation, ranging from 90.30 to 90.75% was reported by Bağdat and Kahraman Ilikkan [63]. Similarly to our study, the lack of differences in water content in kefir with a freeze-dried culture and a traditional culture was found by Barukčić et al. [30], who noted the value of this indicator in a range between 88.7 and 89.2%.

The analysis of SI conducted by the centrifuge method indicated quite large discrepancies among the samples. The value of this parameter was significantly affected by both

storage time and the type of kefir (Table 2). Throughout the storage period, kefir L24 had the lowest index for syneresis, and kefir G12 had the highest. The SI of kefirs G12 and G24 increased during storage time. Only in the case of kefir L24 did the SI remain unchanged throughout storage. The lower water binding capacity of kefir produced with KGs compared with kefir made with a freeze-dried culture is associated with KG filtration. This process alters the structure of the curd, reducing its ability to hold water [52]. In a study conducted by Baranowska [66], the susceptibility to syneresis of kefirs from conventional milk stored for 28 days ranged from 19.54% to 40.00%. The author found that factors that reduced the susceptibility to syneresis were a possible increase in the fat and dry matter content of the product. In the work of Setyawardani et al. [49], syneresis results ranging from 62% to 67% were reported, higher than in our study. However, Yousefvand et al. [62] stated that syneresis in kefirs obtained with KG addition varied from 25.71 to 30.12% during a 21-day storage period, lower than our results.

The content of ethyl alcohol, the compound that shapes the aroma of kefirs, varied in all samples throughout the storage period (Table 2). The results did not indicate a higher ethanol content in kefirs made with KGs. However, different results showing a greater ability of KGs to produce ethanol compared to a freeze-dried culture were reported by Gul et al. [61]. The ethanol content of kefir is influenced by both the composition of the milk as a growth medium, the properties of the microbial culture, the size of the inoculum, and the fermentation parameters, such as temperature and time [67]. A significantly lower ethanol content in kefir (0.04–0.06%) was reported by Barukčić et al. [30]. In the study conducted by Leite et al. [68], the ethyl alcohol content reached a maximum of 1.36% over a 28-day storage period.

3.3.2. Color Parameters

The color parameters of the experimental kefirs (L , a^* , b^* , WI) are presented in Table 3. The values of lightness (L) and the WI remained stable during storage period, with significant differences over time observed only in kefir L12. It can be noted that on the last day of experiment, the samples fermented for 24 h had higher L and WI values compared with those fermented for 12 h. According to Cais-Sokolińska and Pikul [69], the lightness of fermented milk is negatively affected by acidity; however, we did not find this relationship in our study. The whiteness index (WI) is positively correlated with L and negatively correlated with b^* parameter [49]. The a^* parameter is responsible for green (negative values) or red (positive values) color. Negative values of the a^* parameter throughout the storage period indicated the greenish hue of tested kefirs. The highest value of the a^* parameter in all samples was recorded on day 7. For most of the storage period, kefir L24 had the highest a^* value. The b^* parameter represents the proportion of blue (negative values) or yellow (positive values) color. The values of this parameter fluctuated throughout storage. During the storage period, the experimental kefirs exhibited a yellow tint (positive b^* values). The b^* value is affected by the riboflavin content in organic milk, which contributes to the development of a yellowish color [49].

The analysis of the kefir color was also undertaken in a study conducted by Setyawardani et al. [49]. The L^* value reported in their study was lower than in ours, amounting to 73.74. Additionally, the WI noted in the cited work (67.72) was significantly lower than our findings. In the cited work, the a^* parameter was similar, while the b^* value was much higher, reaching 8.31, indicating a greater proportion of yellow color. Another study analyzing the color of conventional kefir was performed by Baranowska [66], who found significantly higher values of L (91.60–94.18) and b^* (6.49–11.70). The a^* value in her study was similar to that in our results, ranging from -0.56 to -3.11 . As in our research, Baranowska observed fluctuations in kefir color parameters during refrigerated storage. Higher values of all color parameters were recorded by Gul et al. [52] in a study on kefirs made from conventional cow milk. In the cited study, the value of L was 91.80–91.93, a^* -0.87 – -1.01 , b^* 10.15–10.61, and WI 86.76–86.9. The authors found no significant differences in the color of kefir made with conventional KGs compared to a freeze-dried culture.

Table 3. Color parameters of organic kefirs during refrigerated storage.

Day	L12	L24	G12	G24
L				
1	77.55 ^{b,B,C} ± 0.13	78.03 ^C ± 0.03	76.39 ^A ± 0.41	77.51 ^B ± 0.18
7	75.14 ^{a,A} ± 0.79	77.89 ^B ± 0.40	75.99 ^A ± 0.33	76.28 ^A ± 1.48
14	77.31 ^{b,A} ± 0.28	77.53 ^A ± 1.04	76.43 ^A ± 0.18	77.12 ^A ± 0.93
21	77.05 ^{b,B} ± 0.08	77.39 ^C ± 0.20	76.25 ^A ± 0.19	77.58 ^C ± 0.16
a*				
1	−2.18 ^{c,D} ± 0.01	−1.98 ^{c,A} ± 0.01	−2.11 ^{c,C} ± 0.01	−2.04 ^{c,B} ± 0.01
7	−1.93 ^{a,C} ± 0.05	−1.55 ^{a,A} ± 0.06	−1.77 ^{a,B} ± 0.05	−1.65 ^{a,A} ± 0.07
14	−2.13 ^{c,C} ± 0.02	−1.90 ^{b,A} ± 0.03	−2.14 ^{c,C} ± 0.02	−2.02 ^{c,B} ± 0.01
21	−2.03 ^{b,C} ± 0.02	−1.84 ^{b,A} ± 0.02	−2.01 ^{b,C} ± 0.02	−1.92 ^{b,B} ± 0.03
b*				
1	5.00 ^{b,A} ± 0.09	5.64 ^{b,C} ± 0.03	5.47 ^{c,B} ± 0.08	5.10 ^{b,c,A} ± 0.11
7	4.35 ^{a,A} ± 0.35	5.75 ^{b,C} ± 0.22	5.01 ^{a,b,B} ± 0.22	4.18 ^{a,A} ± 0.30
14	5.14 ^{b,A} ± 0.15	5.10 ^{a,A} ± 0.35	4.96 ^{a,A} ± 0.12	4.80 ^{b,A} ± 0.28
21	5.31 ^{b,A,B} ± 0.10	5.44 ^{a,b,B} ± 0.11	5.26 ^{b,c,A,B} ± 0.09	5.23 ^{c,A} ± 0.11
whiteness index (WI)				
1	76.90 ^{b,B} ± 0.10	77.23 ^B ± 0.03	75.67 ^A ± 0.39	76.85 ^B ± 0.16
7	74.69 ^{a,A} ± 0.72	77.10 ^B ± 0.34	75.41 ^A ± 0.28	75.86 ^{A,B} ± 1.42
14	76.64 ^b ± 0.24	76.87 ± 0.94	75.82 ± 0.16	76.54 ± 0.85
21	76.36 ^{b,B} ± 0.06	76.68 ^C ± 0.17	75.59 ^A ± 0.17	76.90 ^C ± 0.14

Explanatory notes: The table presents mean values ± standard deviation. L12—kefir fermented with a freeze-dried culture for 12 h; L24—kefir fermented with a freeze-dried culture for 24 h; G12—kefir fermented with kefir grains for 12 h; G24—kefir fermented with kefir grains for 24 h. Different superscript letters (^{a–c}) indicate significant differences in the same kefir on different storage days. Superscript capital letters (^{A–D}) indicate significant differences between kefirs at the same storage day ($p \leq 0.05$).

3.3.3. Sensory Properties

The results of sensory evaluation of organic kefirs during three weeks of refrigerated storage are presented in Table 4. The appearance ratings of the experimental kefirs were significantly influenced by the type of kefir and storage time (Table A1). Kefir L24 consistently received very high appearance scores, close to the maximum value of five, throughout the entire storage period. This kefir variant also received the highest points for taste; however, a statistical analysis showed no significant differences among the samples. In terms of odor, the sample with the highest ratings was L12, although as with taste, the differences between samples were not significant ($p > 0.05$). The final sensory quality parameters evaluated were consistency and texture, which were significantly influenced by the type of beverage but not by storage time (Table A1). The consistency and texture of kefir L24 had the highest notes, but with the exception of day 14, the differences were not statistically significant ($p > 0.05$). In summary, kefir L24 received the highest ratings for appearance, taste, and consistency; however, these results were not statistically significant. The highest acceptability of kefir L24 compared to beverages made from KGs may be due to its lower acidity, which resulted in a more delicate taste and aroma, as well as a higher viscosity index, which is associated with better consistency and texture. The higher acceptability of kefir made with a freeze-dried culture was also found by Gul et al. [52]. Similarly, studies by Kök-Taş et al. [70] and Yousefvand et al. [62] reported the higher sensory quality of kefir produced with the use of liquid natural starter culture in comparison with KG.

Table 4. The results of sensory evaluation of organic kefirs during refrigerated storage.

Day	L12	L24	G12	G24
Appearance				
1	5.00 ^{b,B} ± 0.00	4.83 ^{A,B} ± 0.29	3.50 ^A ± 0.87	4.00 ^{A,B} ± 0.50
7	4.67 ^{b,A} ± 0.58	4.83 ^A ± 0.29	3.00 ^A ± 1.00	4.17 ^A ± 1.04
14	2.83 ^{a,A} ± 0.29	4.83 ^B ± 0.29	3.67 ^{A,B} ± 0.29	4.33 ^{A,B} ± 1.15
21	2.33 ^{a,A} ± 1.15	4.67 ^C ± 0.29	2.67 ^{A,B} ± 0.76	4.33 ^{B,C} ± 0.58
Taste				
1	4.00 ± 0.00	4.33 ± 0.29	3.17 ± 1.76	3.33 ± 2.08
7	4.17 ± 1.04	4.00 ± 0.00	2.67 ± 1.53	2.83 ± 1.61
14	3.67 ± 1.53	4.33 ± 0.29	3.50 ± 1.50	3.17 ± 1.04
21	3.00 ± 1.73	4.33 ± 0.29	3.33 ± 1.53	3.67 ± 1.26
Odor				
1	5.00 ± 0.00	4.83 ± 0.29	4.50 ± 0.87	4.33 ± 1.15
7	4.83 ± 0.29	4.33 ± 0.29	3.83 ± 0.76	3.67 ± 0.29
14	4.67 ± 0.58	4.00 ± 0.00	4.33 ± 0.58	4.00 ± 1.00
21	4.17 ± 0.29	3.83 ± 0.76	3.33 ± 1.53	3.50 ± 0.87
Consistency and texture				
1	3.83 ± 1.04	4.00 ± 1.00	2.50 ± 1.32	3.33 ± 0.58
7	3.83 ± 1.04	4.17 ± 0.76	2.50 ± 0.50	3.50 ± 0.87
14	3.33 ^{A,B} ± 0.58	4.50 ^B ± 0.50	2.83 ^A ± 0.76	3.67 ^{A,B} ± 0.58
21	2.33 ± 1.15	4.00 ± 1.00	3.00 ± 1.00	3.50 ± 0.87

Explanatory notes: The table presents mean values ± standard deviation. L12—kefir fermented with a freeze-dried culture for 12 h; L24—kefir fermented with a freeze-dried culture for 24 h; G12—kefir fermented with kefir grains for 12 h; G24—kefir fermented with kefir grains for 24 h. Different superscript letters (^{a,b}) indicate significant differences in the same kefir on different storage days. Superscript capital letters (^{A–C}) indicate significant differences between kefirs at the same storage day ($p \leq 0.05$).

3.3.4. Texture Parameters

Texture is an important determinant of the quality of fermented milk and should remain stable throughout the refrigerated storage [30]. Table 5 presents the firmness, consistency, cohesiveness, and viscosity index of experimental kefirs. The firmness of the kefirs varied among the samples. Kefirs G24 and L12 had lower firmness compared with kefirs G12 and L24. The statistical analysis showed that firmness was affected only by the type of kefir, while storage time did not influence this parameter (Table A1). A lower range of kefir firmness during the 21-day refrigerated storage (17.39–18.37 g) was recorded by Bierzuńska et al. [71]. The authors noted that firmness was positively affected by total solids and casein content in milk. Regarding cohesiveness, this parameter was influenced by both the type of beverage and storage time (Table A1). Kefir L12 had significantly lower cohesiveness values during most of the storage period (days 1–14) while kefir L24 had the highest. For kefir L24, there was a gradual decrease in cohesiveness during storage. An increase in the cohesiveness of kefir during refrigerated storage was noted by Bierzuńska et al. [71], which was contrary to our findings. In the cited study, the value for this parameter ranged from −11.54 g to −12.02 g. As with cohesiveness, kefir L12 had the lowest consistency values (the least compact), while kefir L24 had the highest (the most compact). The consistency values did not change significantly during storage (Table 5). The consistency of kefir was in the range 383.01–396.47 g·s, which was similar to the L24 variant recorded by Bierzuńska et al. [71]. The final texture parameter evaluated was the viscosity index (g·s). Similar to consistency and cohesiveness, this parameter was influenced by both storage time and the type of kefir (Table A1). Kefir L12 had the lowest viscosity index values (the least negative values), while L24 had the highest (the most negative values). Bierzuńska et al. [71] recorded a viscosity index range similar to kefir G12 during the 21-day storage (−1.24 g·s to −1.76 g·s). The low values of the firmness, cohesiveness, consistency, and viscosity index of kefir L12 are presumably the result of its insufficient acidification. When comparing kefir L24 and G24, a significantly higher

viscosity index was noted for kefir made with the freeze-dried culture. Similar results were obtained by Gul et al. [52], and as in the case of the syneresis index, this can be attributed to the filtering step of the KGs, which damages the structure of the casein curd. The texture analysis showed that using a freeze-dried starter culture resulted in kefir with higher values of texture parameters compared to kefir made with traditional KGs. To achieve desirable textural properties, kefir must be fermented for a sufficiently long time.

Table 5. Texture parameters of organic kefir during refrigerated storage.

Day	L12	L24	G12	G24
Firmness, g				
1	28.28 ± 6.25	35.06 ^b ± 1.73	32.09 ± 1.56	27.51 ± 0.79
7	27.73 ^A ± 2.58	32.49 ^{a,b,B} ± 0.31	31.94 ^B ± 0.93	27.66 ^A ± 0.19
14	29.55 ± 2.30	31.32 ^a ± 0.83	29.84 ± 1.86	27.33 ± 0.11
21	28.35 ^{A,B} ± 0.74	30.16 ^{a,B} ± 1.34	32.38 ^C ± 0.33	28.09 ^A ± 0.19
Cohesiveness, g				
1	−5.41 ^A ± 0.71	−20.87 ^{b,c,C} ± 0.72	−6.06 ^{A,B} ± 1.13	−8.28 ^B ± 0.76
7	−4.94 ^A ± 0.49	−22.18 ^{c,C} ± 1.64	−7.62 ^{A,B} ± 1.15	−8.75 ^B ± 0.98
14	−4.83 ^A ± 0.60	−17.86 ^{a,b,C} ± 1.36	−6.75 ^{A,B} ± 0.68	−8.68 ^B ± 0.89
21	−5.12 ^A ± 1.43	−16.88 ^{a,B} ± 0.82	−6.61 ^A ± 0.89	−7.55 ^A ± 0.88
Consistency, g·s				
1	285.62 ^A ± 15.37	380.89 ^C ± 18.24	349.61 ^{B,C} ± 7.55	323.01 ^B ± 10.39
7	295.86 ^A ± 6.60	371.34 ^B ± 7.40	361.00 ^B ± 16.21	318.04 ^A ± 9.37
14	296.35 ± 7.69	334.96 ± 31.73	336.66 ± 5.98	316.66 ± 8.64
21	308.07 ^A ± 3.40	343.89 ^C ± 6.46	356.66 ^C ± 9.06	324.84 ^B ± 3.83
Viscosity index, g·s				
1	−0.63 ^A ± 0.44	−25.98 ^{b,B} ± 2.41	−1.14 ^A ± 1.08	−2.76 ^{a,A} ± 0.28
7	−0.51 ^A ± 0.15	−27.67 ^{b,B} ± 1.99	−2.62 ^A ± 0.52	−2.53 ^{a,A} ± 0.09
14	−0.70 ^A ± 0.30	−19.29 ^{a,B} ± 1.75	−2.02 ^B ± 0.71	−3.72 ^{b,B} ± 0.16
21	−0.53 ^A ± 0.19	−17.03 ^{a,B} ± 2.80	−1.84 ^A ± 0.79	−2.80 ^{a,A} ± 0.59

Explanatory notes: The table presents mean values ± standard deviation. L12—kefir fermented with a freeze-dried culture for 12 h; L24—kefir fermented with a freeze-dried culture for 24 h; G12—kefir fermented with kefir grains for 12 h; G24—kefir fermented with kefir grains for 24 h. Different superscript letters (^{a–c}) indicate significant differences in the same kefir on different storage days. Superscript capital letters (^{A–C}) indicate significant differences between kefir at the same storage day ($p \leq 0.05$).

3.3.5. Microbiological Analysis

Common LAB species found in traditional kefir and kefir grains include species from the lactobacilli group: *Lactobacillus kefirianofaciens*, *Lentilactobacillus kefir* (previously known as *Lactobacillus kefir*), *Lactobacillus acidophilus*, *Levilactobacillus brevis* (previously *Lactobacillus brevis*), and *Lentilactobacillus parakefir* (previously *Lactobacillus parakefir*), as well as lactococci: *Lactococcus lactis* and *Leuconostoc mesenteroides* [32,37]. Samples of organic kefir were analyzed for their microbiological features immediately after inoculation (time 0) and during the fermentation process (Table 6). The counts of microorganisms were also determined during the 3-week refrigerated storage (Table 7).

The counts of lactobacilli, lactococci, and yeasts were higher in organic milk inoculated with KGs than in milk inoculated with the freeze-dried culture (Table 6). The counts of the mentioned microorganisms increased throughout the fermentation process in milk with the freeze-dried starter culture, while in milk with KGs, the counts were more stable. The initial counts of lactobacilli, lactococci, and yeasts were distinctly higher in samples inoculated with KGs. However, the results showed that these groups of microorganisms did not proliferate in milk with KG. Only microorganisms originating from the freeze-dried culture were able to multiply in milk. This observation may result from the fact that, in the case of milk with kefir grains, proliferation occurs inside the structure of the grains, causing the KGs' weight gain. In the microbiological assessment, milk was tested after the separation of the grains; thus, an increase in microorganisms was not observed. However,

in the case of the milk with the freeze-dried starter culture, microorganisms proliferated throughout the entire volume of the milk, and an increase was noted. In the microbiological analysis, the count of non-LAB bacteria was also evaluated. This group includes acetic acid bacteria (AAB), which are one of the groups of kefir microflora. The count of this group of microorganisms was distinctly higher in milk inoculated with the commercial freeze-dried culture. In the study of Gul et al. [61], the level of microorganism after the fermentation of kefir with a commercial culture was similar to our findings; however, regarding kefir with KGs, the cited work presented lower counts of lactobacilli, lactococci, and yeasts. La Torre et al. [64], after 24 h of fermentation of kefir with KGs, noted levels of lactobacilli, lactococci, and yeasts lower than in our results, along with a higher level of non-LAB bacteria. In turn, in the work of Kök-Taş et al. [70], immediately after fermentation of kefir with KGs or a natural kefir starter, the counts of lactobacilli and lactococci were higher than in our results ($\log 9$ cfu/mL), while the count of yeasts was $4.7\text{--}5.5 \log$ cfu/mL, which is lower than in our kefirs with KGs. Sulmiyati et al. [47] tested the count of LAB in goat milk kefir made with the use of KGs. The level of bacteria after the production of kefir was $\log 7$ cfu/mL, which is in agreement with our results. Moreover, the cited work proved that the abundance of LAB in kefir was significantly affected by the level of KG supplementation.

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Demir [35] compared the microorganism composition and inhibitory effects of kefir made with traditional kefir grains and a commercial kefir culture. The scientist found that traditional kefir has a higher diversity of microorganism species, contained a higher quantity of probiotics, and had a more effective inhibitory effect on pathogens. The high count of lactobacilli and lactococci in kefir G24, exceeding $\log 6$ cfu/g throughout the entire storage period, enables us to recommend this product as a natural source of beneficial probiotic microorganisms [70].

Table 6. Contents of lactobacilli, lactococci, yeasts, and molds and non-LAB bacteria during fermentation of organic kefirs.

Hours	L	G
Lactobacilli, log cfu/g		
0	4.83 ^{a,A} ± 0.07	7.69 ^B ± 0.35
12	7.68 ^c ± 0.14	7.75 ± 0.24
24	6.59 ^{b,A} ± 0.21	7.57 ^B ± 0.27
Lactococci, log cfu/g		
0	3.95 ^{a,A} ± 0.59	7.04 ^{b,B} ± 0.05
12	5.39 ^{b,A} ± 0.02	6.49 ^{a,B} ± 0.36
24	5.54 ^{b,A} ± 0.23	7.67 ^{c,B} ± 0.15
Yeasts and molds, log cfu/g		
0	3.53 ^{a,A} ± 0.15	9.45 ^{c,B} ± 0.07
12	4.42 ^{b,A} ± 0.40	6.90 ^{a,B} ± 0.11
24	3.70 ^{a,A} ± 0.14	7.58 ^{b,B} ± 0.24
Non-LAB, log cfu/g		
0	2.56 ^{a,B} ± 0.13	<2 ^{a,A}
12	3.54 ^{b,B} ± 0.57	<2 ^{a,A}
24	3.67 ^{b,B} ± 0.29	2.28 ^{b,A} ± 0.04

Explanatory notes: The table presents mean values ± standard deviation. L—kefir fermented with a freeze-dried culture; G—kefir fermented with kefir grains. Different superscript letters (^{a-c}) indicate significant differences in the same kefir at different fermentation time. Superscript capital letters (^{A,B}) indicate significant differences between kefirs at the same fermentation time ($p \leq 0.05$).

Table 7. Contents of lactobacilli, lactococci, yeasts, and molds and non-LAB bacteria in organic kefirs during refrigerated storage.

Day	L12	L24	G12	G24
Lactobacilli, log cfu/g				
7	8.06 ^{c,C} ± 0.08	6.93 ^{b,B} ± 0.02	7.11 ^{c,B} ± 0.22	6.53 ^{b,A} ± 0.09
14	4.51 ^{b,A,B} ± 0.48	4.28 ^{a,A} ± 0.17	4.97 ^{a,B} ± 0.02	6.32 ^{a,b,C} ± 0.37
21	3.87 ^{a,A} ± 0.03	4.29 ^{a,B} ± 0.10	5.74 ^{b,C} ± 0.15	6.11 ^{a,D} ± 0.03
Lactococci, log cfu/g				
7	7.07 ^{c,B} ± 0.10	5.40 ^{c,A} ± 0.24	7.79 ^{c,C} ± 0.45	7.67 ^{b,C} ± 0.02
14	4.33 ^{b,A} ± 0.22	5.12 ^{b,B} ± 0.14	6.34 ^{a,C} ± 0.35	6.83 ^{a,D} ± 0.20
21	3.76 ^{a,A} ± 0.06	4.81 ^{a,B} ± 0.03	6.94 ^{b,C} ± 0.01	7.77 ^{b,D} ± 0.08
Yeasts and molds, log cfu/g				
7	4.57 ^{b,A} ± 0.33	4.71 ^{c,A} ± 0.09	5.80 ^B ± 0.41	6.88 ^{b,C} ± 0.61
14	4.72 ^{b,B} ± 0.16	4.22 ^{a,A} ± 0.09	5.92 ^C ± 0.10	6.37 ^{a,b,D} ± 0.06
21	3.81 ^{a,A} ± 0.07	4.36 ^{b,B} ± 0.06	5.69 ^C ± 0.37	6.10 ^{a,D} ± 0.23
Non-LAB, log cfu/g				
7	3.63 ^{b,C} ± 0.24	4.51 ^{c,D} ± 0.09	<2 ^A	3.03 ^{c,B} ± 0.09
14	2.95 ^{a,C} ± 0.03	3.43 ^{a,D} ± 0.17	<2 ^A	2.35 ^{b,B} ± 0.12
21	2.73 ^{a,B} ± 0.11	3.78 ^{b,C} ± 0.09	<2 ^A	1.42 ^{a,A} ± 0.26

Explanatory notes: The table presents mean values ± standard deviation. L12—kefir fermented with a freeze-dried culture for 12 h; L24—kefir fermented with a freeze-dried culture for 24 h; G12—kefir fermented with kefir grains for 12 h; G24—kefir fermented with kefir grains for 24 h. Different superscript letters (^{a-c}) indicate significant differences in the same kefir on different storage days. Superscript capital letters (^{A-D}) indicate significant differences between kefirs at the same storage day ($p \leq 0.05$).

4. Conclusions

The results of the study allow us to conclude that organic cow milk is a suitable raw material for kefir production. Both the type of starter culture and the fermentation time affected the quality characteristics of organic kefir. By using the freeze-dried culture Kefir-31 for the production of organic kefir and fermentation time of 24 h at 30 °C, it was possible to obtain a product with the lowest tendency for syneresis, the best textural

properties, and the desired sensory characteristics. A 12 h fermentation of kefir based on a freeze-dried culture proved to be insufficient to obtain an acceptable quality product. By using a traditional culture in the form of kefir grains, products with a lower pH value were obtained, indicating the better acidification ability of that culture. In terms of quality characteristics, kefir G24 was found to have better properties than kefir G12. Kefir G24 had a lower tendency for syneresis, a higher ethanol content, and higher cohesiveness than kefir G12. These results indicate that a longer fermentation time of 24 h is more appropriate for both the use of traditional kefir grains and a freeze-dried culture. Moreover, kefir fermented with kefir grains had a higher count of yeasts and lactic acid bacteria, especially at the end of the storage period, and may be considered as natural probiotic products.

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Appendix A

The results of the two-factor analysis of variance (ANOVA) of quality characteristics of organic kefir.

Table A1. The results of the two-factor ANOVA analysis of organic kefir quality characteristics.

Properties	Factor	F	p-Value	Test F
Physicochemical	pH	Time	722.025	0.0000 *
		Sample	7382.173	0.0000 *
		Interaction	282.486	0.0000 *
	Titratable acidity	Time	9.663	0.0001 *
		Sample	48.087	0.0000 *
		Interaction	30.212	0.0000 *
	Water content	Time	1.000	0.4054
		Sample	1.000	0.4055
		Interaction	1.000	0.4600
	Ethanol content	Time	132.098	0.0000 *
		Sample	261.409	0.0000 *
		Interaction	69.309	0.0000 *
	Syneresis index	Time	5.829	0.0027 *
		Sample	833.664	0.0000 *
		Interaction	26.410	0.0000 *

Table A1. Cont.

Properties	Factor	F	p-Value	Test F
Color	L	Time	11.644	0.0000 *
		Sample	21.562	0.0000 *
		Interaction	4.339	0.0002 *
	a*	Time	468.619	0.0000 *
		Sample	218.590	0.0000 *
		Interaction	8.559	0.0000 *
	b*	Time	30.028	0.0000 *
		Sample	43.478	0.0000 *
		Interaction	13.261	0.0000 *
	WI	Time	10.268	0.0000 *
		Sample	22.395	0.0000 *
		Interaction	4.034	0.0004 *
Sensory	Appearance	Time	3.363	0.0306 *
		Sample	11.778	0.0000 *
		Interaction	3.126	0.0082 *
	Taste	Time	0.122	0.9466
		Sample	1.815	0.1643
		Interaction	0.291	0.9723
	Odor	Time	3.521	0.0260 *
		Sample	2.782	0.0568
		Interaction	0.230	0.9875
	Consistency and texture	Time	0.400	0.7536
		Sample	5.555	0.0035 *
		Interaction	0.687	0.7152
Texture	Firmness	Time	0.837	0.4836
		Sample	15.221	0.0000 *
		Interaction	1.313	0.2688
	Cohesiveness	Time	7.627	0.0006 *
		Sample	512.218	0.0000 *
		Interaction	4.511	0.0007 *
	Consistency	Time	3.732	0.0209 *
		Sample	61.454	0.0000 *
		Interaction	3.143	0.0079 *
	Viscosity index	Time	12.251	0.0000 *
		Sample	861.824	0.0000 *
		Interaction	13.919	0.0000 *
Microbiology– fermentation process	Lactobacilli	Time	6.377	0.0060 *
		Sample	19.723	0.0002 *
		Interaction	2.278	0.1243
	Lactococci	Time	34.177	0.0000 *
		Sample	76.980	0.0000 *
		Interaction	33.478	0.0000 *
	Yeasts and molds	Time	214.922	0.0000 *
		Sample	226.716	0.0000 *
		Interaction	214.928	0.0000 *
	Non-LAB	Time	1.753	0.1947
		Sample	7.349	0.0122 *
		Interaction	1.723	0.1999

Table A1. Cont.

Properties	Factor	F	p-Value	Test F
Microbiology– storage	Lactobacilli	Time	176.136	0.0000 *
		Sample	100.868	0.0000 *
		Interaction	106.144	0.0000 *
	Lactococci	Time	33.079	0.0000 *
		Sample	33.524	0.0000 *
		Interaction	16.860	0.0000 *
	Yeasts and molds	Time	2.894	0.0651
		Sample	5.561	0.0023 *
		Interaction	2.813	0.0199 *
	Non-LAB	Time	109.972	0.0000 *
		Sample	153.653	0.0000 *
		Interaction	71.580	0.0000 *

* indicates a statistically significant effect of the factor.

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