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The Impact of Production Techniques on the Physicochemical Properties, Microbiological, and Consumer's Acceptance of Milk and Water Kefir Grain-Based Beverages

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Abstract: The increasing focus on a healthy lifestyle has emphasized a connection between gut microbiome and health. Consumers face the choice between consuming traditional dairy kefirs or more trendy fruit-based fermented drinks. Also, the aim of this study is to determine the similarities and differences theoretically and experimentally based on a higher ratio of grains to make a lower pH-based beverage at different inoculation times and durations. This study also aims to assess acceptability from a sensory perspective. The goal of the research was to make kefir grain-based beverages with a higher number of lactic acid bacteria (LAB) owing to their probiotic impact on the human gut. After analysis, it was found that there was the highest content of LAB in water kefir after 48 h of inoculation. However, consumer acceptance tests found traditional dairy kefir with a 24-h inoculation to be more acceptable. Although both drinks were inoculated in a 1:4 ratio, it is important to note the variations in grain origin as well as the initial composition of the milk–water suspension, which significantly affects the final product. Since the recipe of the kefir determines the benefits of the drink, the onus is on the consumers to decide which fermented drink suits their health condition best.

Keywords: kefir; lactic acid bacteria (LAB); kefir grains; milk; water; beverage

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1. Introduction

Recently, people have become increasingly aware of the importance of a healthy human gut, and as a result, the consumption of functional fermented foods is on the rise. Furthermore, research on the gut microbiome has revealed differences in microbial flora composition between healthy individuals and those diagnosed with diseases. As a result of gut dysbiosis, various diseases and health conditions occur, ranging from modern diseases like diabetes and obesity [1] to psychosomatic disorders such as multiple sclerosis [2]. Not only that, studies show there is a connection between emotional states and the gut microbiome [3]. Finally, there is also the fact that incidences of colorectal cancer are increasing [4]. In order to prevent diseases, some of which are mentioned in this study, people turn to probiotics to strengthen and maintain a healthy gut microbiome [5]. The notion of probiotic food often brings to mind milk kefir, a fermented dairy beverage. However, there is a trend toward eliminating milk due to its hard-to-digest protein compound casein and the presence of lactose, both of which are fermented by kefir grain microorganisms [6]. Some people are genuinely lactose intolerant, and consuming these products causes discomfort,

which makes the elimination of those products necessary. In addition, water-based kefir is suitable for vegans and is used as a vegan alternative to other dairy probiotic beverages.

The goal of this research is to determine the similarities and differences between water and milk kefir depending on the duration of inoculation based on two different production processes. During fermentation, two values were taken: 24 h, according to research [7], being the optimal conditions for the production of milk kefir, and 48 h, as the optimal conditions [8] for the production of water kefir. The aim of this paper was to make a beverage with a higher number of *Lactobacillus* and see their correlation with other physio–chemistry features, which withheld consumer approval. The problems and hypotheses of this research are as follows: which beverage is more acceptable, milk kefir or water-based kefir; which beverage contains a higher number of *Lactobacillus* after inoculation; and what are the similarities and differences between final beverages depending on inoculation time?

1.1. Comparison of Milk Kefir and Water-Based Beverage

In today's world, consumers are increasingly drawn to the importance of functional food with the intention of improving their health. This fact has led to a high demand for healthy probiotic foods, with traditional fermented beverages such as milk kefir and related products, as well as other water-based kefir products, standing out. Although they are produced from gelatinous particles known as kefir grains, both also have different physical, chemical, and microbiological compositions [9]. Often, even in scientific papers, there is confusion between these two types of grains and the processes of beverage production. However, there are significant differences in the structure, the microbial composition, and the final beverage, as further explained in this study. Milk kefir is a significant source of proteins, probiotics, and prebiotics, while water kefir, in addition to its probiotic and prebiotic properties, also has significant antioxidant properties and can be an important part of a vegan diet as well as suitable for lactose-intolerant individuals [9]. Also, according to Codex Alimentarius kefir, "Starter culture prepared from kefir grains, *Lactobacillus kefiri*, species of the genera *Leuconostoc*, *Lactococcus*, and *Acetobacter*, growing in a strong specific relationship. Kefir grains constitute both lactose-fermenting yeasts (*Kluyveromyces marxianus*) and non-lactose-fermenting yeasts (*Saccharomyces unisporus*, *Saccharomyces cerevisiae*, and *Saccharomyces exiguus*)".

Thanks to [10,11], research shows that *Lactobacillus hilgardii* and *L. nageli* in water grains produce exopolysaccharides such as dextran and branched glucans [12], while in milk kefir, these are formed through the action of *Lactobacillus kefiranofaciens* [13]. In their research, water-based beverages with water kefir grains are called water kefir.

Another difference lies in the disaccharide fermented by the grains. In water kefir, sucrose is broken down, while in milk kefir, it is lactose. The sucrose in water kefir is used as a carbon source, while dried fruits are added as a nitrogen source. There are numerous sources of nitrogen and carbon that can be used in water kefir production, such as dried figs, plums, raisins, and various other types of sugar used for fermentation [14]. The final recipe depends on consumer preferences and tastes.

1.2. The Role of Lactobacillus spp.

Increasingly, health conditions are being linked to the composition of the gut microbiome. According to [15], a reduced presence of *Lactobacillus* bacteria was found in individuals infected with the HIV virus [16]. In individuals with irritable bowel syndrome (IBS), besides the documented reduction, the higher content of *Lactobacillus* in the human gut reduces symptoms such as abdominal pain and bloating [17]. *Lactobacillus* levels were also reduced in individuals with type 1 diabetes [18]. In research on type 2 diabetes, consumption of kefir and other dairy products fermented with *Lactobacillus* has been shown to be a good preventive measure against the disease [5]. In mouse models with gut-brain-microbiota axis deficiencies, it has been found that probiotic administration positively affects the function of the colon, mitigating gut dysbiosis. Such results may also be important for emotional disorders, as shown by probiotic research indicating that

the *Lactobacillus* probiotic improves symptoms of human depression [3]. Cohort studies have revealed a relative reduction in intestinal *Lactobacillus* in individuals suffering from multiple sclerosis compared to healthy adults [2]. This confirms that indole aldehydes produced by *Lactobacillus* have a strong anti-inflammatory effect on brain glial cells (astrocytes), limiting inflammation in the central nervous system in a mouse model of human multiple sclerosis [19]. Lastly, as colorectal cancer has been on the rise recently, research has been conducted that shows how the presence of *Lactobacillus*, along with a reduced number of butyrogenic bacteria in the gastrointestinal system, is consistently reduced in individuals suffering from this malignant disease [20]. Certain studies have found that, while kefir does not have a direct in vitro effect on tumor cells, it does support anti-tumor activity mechanisms. Kefiran from milk kefir is supposed to suppress tumor growth through macrophages activated by lymphokines via lymphatic tissue in the gut, while water-insoluble fractions (microorganisms) increase the activity of natural killer cells (NK cells), which are known for their cytotoxic effects against tumor cells [21].

2. Materials and Methods

2.1. Sample Preparation

The preparation of the kefir milk sample used 1.5 L of cow's milk, collected at the small family farm, which is located in Nevesinje, and household kefir grains from private sources. The kefir grains were maintained and propagated by regularly preparing homemade kefir using homogenized milk with 2.8% milk fat. Before fermentation, milk is partially skimmed, removing the surface fat layer along with a dose of lactose, resulting in lower values of milk fat and lactose in milk. Since the production of kefir involves using milk directly from the family farm, this is a common practice in this method of milk production, and excess milk fat is used for making the dairy product kajmak. The pasteurization of milk was carried out at 85 °C for 2 min and then cooled down to 30 °C. After cooling down pasteurized milk, 400 mL of pasteurized milk was added to a sterilized glass jar.

For the preparation of the water-based beverage, 1.5 L of tap water, 110 ± 0.1 g of consumable brown sugar from the manufacturer "Tak", Bosnia and Herzegovina, used as the main source of sucrose, and 88 ± 0.1 g of dried raisins from the manufacturer "Bingo", its country-of-origin Bosnia and Herzegovina, were used as a nitrogen source. The sugared solution was first mixed in a shaker before being inoculated with household water grains from a local producer. Water grains contain a dextran matrix, and they are cultivated and propagated through regular production of a fermented beverage at home using a mixture of water, brown sugar, and raisins. In two sterilized jars, 400 mL samples were measured with a burette and inoculated with 100 g of kefir grains each (ratio 1:4) since the initial goal of the research was to increase the concentration of *Lactobacilli* in beverages. This concentration, for us, proved to be the most effective for forming grains after both inoculations. This helps kefir to stay acceptable to consumers and maintain a lover pH) [22], covered with sterile gauze, sealed with a rubber band, and placed in an incubator at a temperature of 22 ± 0.01 °C, one for 24 h and another for 48 h. This process was conducted on both the milk and water-based kefir samples. Three replications of all batches, samples, and analyses were performed. All analyses were provided at the Institute of Public Health of the Federation of Bosnia and Herzegovina using standard methods.

2.2. Determination of Total LAB Count

To determine the total number of LAB, a DeMan–Rogosa–Sharpe agar medium, also known as MRS agar medium, was provided by the "Biokar" company and prepared according to the given recipe. This agar is used for the growth and enumeration of *Lactobacillus* cultures, and in the literature, it is used to determine the total number of *Lactobacilli* [23]. When analyzing kefir grain samples, 5 g of rinsed kefir grains were placed in a plastic sterile bag before being crushed by rolling a round object over the outside of the bag. Afterward, 45 mL of a solution containing 8.5 g/L NaCl and 1 g/L bacteriological peptone water in a 1:1 ratio was added. This mixture was then homogenized for 15 min at

high speed using a Stomacher 400 Circulator Lab Blender (Seward Ltd., Technology Centre Easting Close, Worthing West Sussex BN14 8HQ, United Kingdom), and the resulting suspension was inoculated onto the prepared MRS nutrient agar medium and incubated at 30 °C for 48 h. Colony counting was expressed as log CFU (colony-forming units) per gram of kefir liquid. The beverage analysis was conducted equally.

2.3. Determination of Yeast Count

From an appropriate dilution, 1 mL of the sample was taken using a sterile pipette and transferred onto a Petri dish. The prepared yeast glucose chloramphenicol agar medium, cooled to approximately 45 °C, was poured over the sample while mixing. After the medium solidified, it was placed in an incubator at a temperature of 25 °C for 5 days. The colony count was then recorded after the incubation period.

2.4. Weighing of Wet Grain Weight

At the beginning of the experiment, each sample was inoculated with 100 g of kefir grains. After fermentation for a period of 24 h or 48 h, wet grains were separated from the liquid beverage by a narrow colander, and the wet mass of the grains was measured using an analytical balance (Sartorius model BP 2215, Schönwalde-Glien, Germany).

2.5. Determination of pH Value

The pH value of all samples was determined using a pH meter (SEVEN EASY S20, Mettler-Toledo, Columbus, OH 43240, USA). pH was measured in a fresh milk and water mixture first. After the 24-h fermentation period and the separation of the grains from the sample, the pH was measured from the 24-h inoculation length sample by adding 5 mL of the separated liquid into a glass container. This process was also repeated for the 48-h inoculation.

2.6. Determination of Total Sugar and Sucrose Content

The method for determining the mixture of glucose and fructose is based on the reducing properties of these monosaccharides due to their free glycosidic -OH groups. In an alkaline environment, they reduce copper sulfate ($CuSO_4$) from Luff's reagent to form red precipitates while themselves oxidizing to the corresponding acids. The left-over, unreacted amount of copper ions is then titrated with a thiosulfate solution. The amount of sugar is calculated by determining the difference between the titration of the blank and the sample, as indicated in the table. The method for determining sucrose content is based on its prior hydrolysis into reducing monosaccharides. The acid hydrolysis of sucrose results in an equivalent amount of glucose and fructose. By measuring the sugar content after hydrolysis, the total sugar content (directly reducing sugars and indirectly reducing sugars) is determined. The difference between the obtained total invert and natural invert gives the amount of reducing sugars formed by sucrose inversion.

2.7. Determination of Fat Content

For milk samples, the Gerber method was used. For water beverages, the Soxhlet method was employed.

2.8. Determination of Protein Content

After the Kjeldahl method was used to determine the total protein content, it was calculated using a conversion factor of 6.38. The obtained value (total N × 6.38) is 4–8% higher than the actual protein content because it includes non-protein nitrogen.

2.9. Determination of Dry Matter Content

The sample was neutralized with 0.1 mol/L strontium hydroxide. Phenolphthalein was used as an indicator. Drying was carried out, as in the case of the milk-based sample. An empty container with a lid and sand was dried for 2 h at 100 °C, then cooled in a

desiccator before being weighed. The dried sample was placed in the container, mixed with distilled water, and dried for 1.5 h at 100 °C. After cooling in the desiccator, it was weighed again and then left to dry for an additional hour. The amount of strontium used for neutralization was subtracted from the total dry matter of the processed sample.

2.10. Determination of Total Ash Content by Direct Ignition Method

The samples were burned directly at combustion temperatures ranging from 500 to 550 °C, typical for most foods. The crucible for combustion was preheated to the combustion temperature, cooled in a desiccator to room temperature, and weighed with an accuracy of ±0.0001 g. Two to five grams of the sample were then placed in the crucible. The sample initially began combustion at a lower temperature using a burner, and then the processing continued in a muffle furnace, where it continued to burn until a homogeneous mass was obtained, typically white ash. The crucible with ash was then cooled in a desiccator and weighed with an accuracy of ±0.0001 g. The total ash content was calculated using the following formula:

$$Ash\ content = \frac{a \times 100}{weight}(\%)$$

where a = mass of ash (g) (difference in mass between the crucible with ash and the empty crucible) (g).

2.11. Determining the Acidity Level

A specific amount of the sample was weighed and then diluted in the sample of milk kefir with distilled water (due to its thicker consistency). A phenolphthalein indicator was added and titrated with a 0.1 mol/L solution of NaOH until a pink color appeared. The acidity was calculated using the formula according to the Soxhlet–Henkel method:

$$Acidity\ level\ (^{\circ}sH) = a' \times F \times 2\ (\%)$$

where a' = the number of milliliters of 0.1 mol/L NaOH solution consumed to neutralize 20 g of the sample, and F = the molarity factor of the 0.1 mol/L NaOH solution.

2.12. Determining the Content of Lactic Acid

A total of 25 mL of the fermented beverage was placed in a 200 mL Erlenmeyer flask, where a few drops of phenolphthalein were added before being titrated with a 0.1 M NaOH solution and stopping at the first appearance of a purple color. Each mL of 0.1 M NaOH is equivalent to 90.08 mg of lactic acid. The mass concentration of lactic acid (mg/mL) is calculated according to the equation:

$$Lactic\ acid: \gamma(CH_3CH(OH)COOH) = \frac{V(NaOH) \cdot M(NaOH) \cdot 90.08}{V\ (sample)}$$

where $V(NaOH)$ = the volume of 0.1 M NaOH solution used (mL); $M(NaOH)$ = molarity of NaOH (0.1 M); and $V(sample)$ = volume of the sample (mL).

2.13. Determination of Acetic Acid Content

In a different 200 mL Erlenmeyer flask, 1 mL of the fermented beverage sample was added to 20 mL of water, and a few drops of phenolphthalein were added. The prepared sample was titrated with a 0.1 M NaOH solution until the first appearance of a purple color. The mass concentration of acetic acid (g/L) is calculated using the formula:

$$Acetic\ acid: \gamma(CH_3COOH) = V(NaOH) \times f(NaOH) \times V(sample) \times 6$$

where $V(NaOH)$ = the volume of 0.1 M NaOH solution used (mL); $f(NaOH)$ = the factor for 0.1 M NaOH (1.000); and $V(sample)$ = the volume of the sample (1 mL).

2.14. Determination of Gluconic Acid Content

In another 200 mL Erlenmeyer flask, 25 mL of the fermented beverage sample was added, and a few drops of phenolphthalein were added. The prepared sample was titrated with a 0.1 M NaOH solution until the first appearance of a purple color. The mass concentration of gluconic acid (g/L) is calculated using the formula:

$$Gluconic\ acid\text{: } \gamma(C_6H_{12}O_7) = (V(NaOH) \times M(NaOH) \cdot 1.97) / V(sample)$$

where $V(NaOH)$ = the volume of 0.1 M NaOH solution used (mL); $M(NaOH)$ = molarity of NaOH (0.1 M); and $V(sample)$ = the volume of the sample (mL).

2.15. Microbiological Analyses of Beverages

2.15.1. Presence of *Salmonella* spp.

The presence of *Salmonella* spp. was tested using the standard method [24].

2.15.2. Presence of *Staphylococcus aureus*

The presence of *Staphylococcus aureus* was tested using the standard method [25].

2.15.3. Presence of *Listeria monocytogenes*

The presence of *Listeria monocytogenes* was tested using the standard method [26].

2.16. Consumer Acceptance Test

The sensory properties of prepared samples of milk kefir and the water beverage were evaluated using a 9-point hedonic scale. This scale consisted of nine points, or degrees of preference, toward a specific attribute of the tested sample. The ratings given by the participants ranged from 'extremely liked' with a neutral midpoint of 'neither liked nor disliked' to 'extremely disliked'. The sensory properties assessed included aroma, appearance, texture, taste, and overall impression. The panel consisted of five participants. The results were processed using the standard deviation, and in the end, an average rating was obtained for each beverage.

2.17. Statistical Data Analysis

The experimental data were analyzed using analysis of variance (ANOVA) and Fisher's least significant difference (LSD), with statistical significance defined at $p < 0.05$. Statistical analysis was conducted using the Statistica 12.7 software (2015, StatSoft Inc., Tulsa, OK, USA) and Microsoft Office Excel 2013 (Microsoft, Redmond, WA, USA).

3. Results and Discussion

Analyses were conducted on eight groups of samples: milk kefir grains (MG), water kefir grains (WG), pasteurized milk (M), water suspension (WB), milk kefir after 24 h of inoculation (MK24h), water beverage after 24 h of inoculation (WB24h), milk kefir after 48 h of inoculation (MK48h), and water beverage after 48 h of inoculation (WB48h). Three samples from each group were prepared for analysis. All measurements were conducted in three repetitions. The analyses included nine measurements, where physical-chemical, microbiological, and consumer acceptance tests were performed on the samples, the results of which are presented in the attached Tables 1–12 and Figure 1. In the milk samples analyzed, a statistically significant difference ($p < 0.05$) was observed among all three sample groups in terms of the colony count of the LAB. The lowest colony count (log CFU/g) was found in milk kefir after 24 h of inoculation (4.455 $\pm$ 0.5 log CFU/g), while the highest count was found in milk grains (7.27 $\pm$ 0.5 log CFU/g) (Table 1).

Table 1. The values of LAB and yeast in dairy samples depend on the length of inoculation.

	LAB (log CFU/g)	Yeasts (log CFU/g)
MG	7.27 [c]	6.66 [c]
MK24h	4.45 [a]	5.29 [a]
MK48h	6.77 [b]	6.51 [b]

The shown results are a mean value of nine measurements; differences in values within the column marked with the same letter (a–c) are not statistically significant ($p < 0.05$); MG—milk grains; MK24h—milk kefir after 24 h inoculation; MK48h—milk kefir after 48 h inoculation.

The colony count of LAB increased when comparing samples MK24h and MK48h, indicating an increase over the time of inoculation (Table 1). Although studies [27] and [28] reported a higher number of lactobacilli (8.94 log CFU/g in grains and 7.45 log CFU/g after 24 h of fermentation) in comparison to our sample MK24h, these differences could be due to the differences in fermentation conditions and milk composition in our experiment. Looking at the analyzed water beverage samples, the highest number of LAB was found in the group of WB48h samples, with a statistically significant difference ($p < 0.05$), while the other two sample groups (WG and WB24h) did not show a statistically significant difference (Table 2).

Table 2. The values of LAB and yeast in water samples depend on the length of inoculation.

	LAB (log CFU/g)	Yeasts (log CFU/g)
WG	7.43 [a]	6.80 [c]
WB24h	7.32 [a]	4.32 [a]
WB48h	9.09 [b]	5.80 [b]

The shown results are a mean value of nine measurements; differences in values within the column marked with the same letter (a–c) are not statistically significant ($p < 0.05$); WG—water grains; WB24h—water beverage after 24 h inoculation; WB48h—water beverage after 48 h inoculation.

Over the time of inoculation, the number of LAB also increased statistically significantly ($p < 0.05$) in all sample groups. Research on water grains [29] determined a count of 7.7 ± 0.5 log CFU/g of lactobacilli within the grains, which is similar to the results of this study (7.43 ± 0.5 log CFU/g). Another study [30] reported a count of 8.2 ± 0.5 log CFU/mL within the grains while in the beverage itself, and after 24 h of fermentation, they obtained 6.9 ± 0.5 log CFU/mL. A reason for the variation in results could be the different origins of the grains used. When comparing milk and water kefir grains, no statistically significant difference ($p < 0.05$) in the colony count of LAB was found in the grain analysis (7.27 MG and 7.43 WG) (Table 9). Depending on the production technology, i.e., the time of inoculation, a statistically significant difference ($p < 0.05$) was observed in samples MK24h and WB24h, where a higher number of LAB was found in the water beverage (Table 10) after 24 h of fermentation. Additionally, when comparing samples MK48h and WB48h, a higher number of LABs were found in the water beverage. In the study [31], a count of 7.25 ± 0.3 log CFU/g of *Lactobacillus* was found in water grains and 7.70 ± 0.21 log CFU/g of *Lactobacillus* in milk grains, which is a lower count for water and a higher count for milk compared to this research. The reason is the variation in the origin of the grains. In numerous studies, the *Lactobacillus* content has been identified in the range between 7.38 and 9.05 log CFU/g [7,22]. The total yeast count among samples MG, MK24h, and MK48h showed a statistically significant difference ($p < 0.05$) among all three samples, with the highest count being found in milk grains and the lowest in MK24h, indicating an increase in the yeast count with a longer inoculation time, with a statistically significant difference ($p < 0.05$). The values were as follows: 6.66 log CFU/g at grains, 5.29 log CFU/g MK24h, and 6.51 log CFU/g MK48h (Table 1). Authors [32] reported that yeast levels within the grains vary widely, ranging from 5.18 to 8.57 log CFU/mL. In the water beverage, the highest yeast count was found in the grains themselves (WG), while the lowest was in the WB24h sample, i.e., the first fermented sample, with a statistically significant difference ($p < 0.05$). However, with a longer inoculation time (48 h) in the WB48h sample, the yeast

count also increased with a statistically significant difference ($p < 0.05$): 6.80 WG, 4.32 WB24h, and 5.80 WB48h (log CFU/g), as shown in Table 2. According to [33], after 24 h of fermentation in a water beverage, a yeast count of 7.31 ± 0.07 log CFU/mL was determined, which is higher compared to this research. When comparing samples MG and WG, a higher yeast count was found in the WG sample with a statistically significant difference ($p < 0.05$), amounting to 6.66 MG and 6.80 WG (Table 9). Between the MK24h and WB24h beverages, as well as between MK48h and WB48h, a significantly higher yeast count was found in the water beverages ($p < 0.05$) (Table 10). The authors [9] also reported a higher yeast count in water grains compared to milk grains. By measuring the wet grain mass, the initial mass of MG increased after 24 h of inoculation (MK24h) and after 48 h (MK48h) with a significant statistical difference ($p < 0.05$) among all three samples: from the initial mass of 99.95 g, it markedly increased to 109.54 MK24h and 112.43 MK48h (Table 3). Wet grain weight was measured after every inoculation described in Section 2.

Table 3. The wet grain weight values of milk particles depend on the length of inoculation.

	Wet Grain Weight (g)
MG	99.95 [a]
MK24h	109.54 [b]
MK48h	112.43 [c]

The shown results are a mean value of nine measurements; differences in values within the column marked with the same letter (a–c) are not statistically significant ($p < 0.05$); MG—milk grains; MK24h—milk kefir after 24 h inoculation; MK48h—milk kefir after 48 h inoculation.

The biomass of MK increases by 3–3.5% after each fermentation period, according to the research by [34]. Authors [30] found that the initial mass of grains increased from 16.4 ± 0.5 g to 28.6 ± 0.6 g after a 24-h inoculation of milk grains in milk. In the case of water grains, there was no statistically significant difference in mass between WG and WB24h (100.00 initial to 100.02 g WB24h) ($p < 0.05$). In fact, the mass of WB48h grains decreased significantly compared to WG and WB48h, as shown in Table 4.

Table 4. The wet grain weight values of water particles depend on the length of inoculation.

	Wet Grain Weight (g)
WG	100.00 [b]
WB24h	100.02 [b]
WB48h	96.12 [a]

The shown results are a mean value of nine measurements; differences in values within the column marked with the same letter (a, b) are not statistically significant ($p < 0.05$); WG—water grains; WB24h—water beverage after 24 h inoculation; WB48h—water beverage after 48 h inoculation.

According to [29], a minimal increase in the weight of the water grains was also observed at the beginning of inoculation, but the lower pH value led to a decrease in the mass of WB48h grains. Comparing the masses after 24 h and 48 h (MK24h and WB24h, as well as MK48h and WB48h), a higher mass was recorded in MK24h and MK48h with a statistically significant difference ($p < 0.05$). This supports the fact that the mass of milk grains increased regardless of the decreasing pH, while the mass of water grains only started to decrease in the acidic medium. In chemical analyses, it was determined that the fat content decreased in MK24h compared to M, but after extending the inoculation time to MK48h, it increased compared to MK24h in all three cases with a statistically significant difference ($p < 0.05$) in all three samples. The highest value was recorded in the M sample group and the lowest in the MK24h sample group: 2.32% at milk, 2.03% MK24h, and 2.23% MK45h (Table 5). The milk used for the experiment had a lower fat content because of the earlier milk kefir inoculation type.

Table 5. Representation of changes in the basic chemical composition values depending on the length of inoculation in milk.

	Fats (%)	Proteins (%)	Sugars (%)	Sucrose (%)	Dry Matter (%)	Ash (%)
M	2.32 ^{c}	3.63 ^{c}	2.63 ^{a}	0.045 ^{a}	11.15 ^{b}	0.66 ^{b}
MK24h	2.03 ^{a}	3.44 ^{b}	0.24 ^{a}	0.055 ^{a}	9.51 ^{a}	0.61 ^{ab}
MK48h	2.23 ^{b}	3.32 ^{a}	0.26 ^{a}	0.055 ^{a}	10.25 ^{a}	0.56 ^{b}

The shown results are a mean value of nine measurements; differences in values within the column marked with the same letter (a–c) are not statistically significant ($p < 0.05$); M—milk; MK24h—milk kefir after 24 h inoculation; MK48h—milk kefir after 48 h inoculation.

Authors [35], in the aforementioned study, reported results of 3.62% fat in MK24h and 3.63% in MK48h. In the case of the WB, WB24h, and WB48h samples, no statistically significant differences ($p < 0.05$) in fat content were observed. That said, according to [33], the fat content of Brazilian kefir had been determined to be 2.34% in their study. The variations in results compared to this research align with the fact that the composition of the beverage can vary widely based on the country of origin and the specific recipe used in kefir production. When comparing samples MK24h and WB24h, as well as MK48h and WB48h, the highest fat content was found in the milk kefir samples (MK24h, MK48h) compared to the water beverage (WB24h, WB48h) with a statistically significant difference ($p < 0.05$): 2.02% MK24h—0.05% WB24h, and 2.23% MK48h—0.05% WB48h (Tables 9 and 10). The highest protein content was found in the milk-based samples and decreased with fermentation and longer inoculation, with statistically significant differences ($p < 0.05$). Therefore, the lowest protein content was in MK48h: 3.63% in milk, 3.44% in MK24h, and 3.32% in MK48h (Table 5). According to [36], the protein content in kefir should be around 3%, while the study conducted by [35] recorded protein contents of 3.06% after 24 h and 3.08% after 48 h of inoculation, which is similar to the findings obtained in this research.

When it comes to the water beverage, there were no statistically significant differences ($p < 0.05$) among the parameters (Table 6). According to the results of [33], the protein content was determined to be 0.4 ± 0.1%, and while lower than the findings of this research, this discrepancy can be explained by variations in the composition depending on the recipe used. In both milk and water beverages, both inoculations resulted in higher protein content in milk samples with statistically significant differences ($p < 0.05$) (Tables 10 and 11). However, the difference lies in the fact that in milk kefir, this content decreased with longer inoculation, while in the water beverage, it increased. The highest levels of sugar, dry matter, and ash were found in M, and these values decreased with longer inoculation compared to the fermented beverage. The results were higher values, which allowed for a high concentration of the grains. Therefore, there was not a statistically significant difference between MK24h and MK48h ($p < 0.05$) (Table 5). That said, an exception will have to be made for the ash content of the milk kefirs. While MK48h's ash content was not statistically significant ($p < 0.05$) and dissimilar from that of M, the ash contents of M and MK24h are statistically significant ($p < 0.05$) and disparate from each other (Table 5). In the water beverage, on the other hand, the sugar and dry matter content displayed a pronounced decrease in relation to longer inoculation, while the ash content decreased after 24 h and increased after 48 h, albeit not statistically significant ($p < 0.05$) (Table 5). To compare these findings with other studies, [36] reported a sugar content of 6% and an ash content of 0.7% in milk kefir, while [33] determined a dry matter content of 9.62% in Brazilian kefir through their analysis. In comparison to this research, the sugar and dry matter content of our samples were lower, while the ash content was higher. When comparing milk and water beverages, the sugar content was markedly higher in WB24h compared to MK24h. After 48 h, the difference in sugar was no longer statistically significant ($p < 0.05$) (Tables 10 and 11). The dry matter content was demonstrably higher in MK24h and MK48h, while the ash content was significantly ($p < 0.05$) higher in MK24h. However, after 48 h, this variance between the samples had become very minute (Tables 9 and 10). The amount

of sucrose in milk samples (M, MK24h, and MK48h) increased, but not to a large extent (Table 5). In water beverages (WB, WB24h, and WB48h), after 24 h of inoculation, there was a significant increase in sucrose ($p < 0.05$), but it decreased in WB48h compared to WB and WB24h, and this difference was statistically notable; the lowest decrease in WB48h was from 0.24 to 0.065% (Table 6). Authors [37] noted that sucrose almost completely ferments (<1 g/L) after 24, 48, and 144 h of fermentation. Acetic, gluconic, and lactic acids, as well as acidity, increased with longer inoculation with statistically significant differences ($p < 0.05$), except for acetic acid, which showed little difference between MK24h and MK48h (Table 7).

Table 6. Representation of changes in the basic chemical composition values depending on the length of inoculation in a water-based beverage.

	Fats (%)	Proteins (%)	Sugars (%)	Sucrose (%)	Dry matter (%)	Ash (%)
WB	0.04 ^{a}	0.73 ^{a}	7.04 ^{c}	0.24 ^{b}	25.31 ^{c}	0.21 ^{a}
WB24h	0.03 ^{a}	0.77 ^{ab}	2.55 ^{b}	0.45 ^{c}	4.75 ^{b}	0.15 ^{a}
WB48h	0.05 ^{a}	0.84 ^{b}	0.27 ^{a}	0.06 ^{a}	2.72 ^{a}	0.29 ^{a}

The shown results are a mean value of nine measurements; differences in values within the column marked with the same letter (a–c) are not statistically significant ($p < 0.05$); WB—water beverage; WB24h—water beverage after 24 h inoculation; WB48h—water beverage after 48 h inoculation.

Table 7. Representation of changes in the acidity level, acetic, gluconic, and lactic acid values, as well as pH, depending on the length of inoculation in milk kefir.

	Acidity (°sH)	Acetic Acid (g/L)	Gluconic Acid (g/L)	Lactic Acid (mg/mL)	pH
M	6.10 ^{a}	0.60 ^{a}	0.03 ^{a}	1.35 ^{a}	6.60 ^{b}
MK24h	77.75 ^{b}	13.05 ^{b}	0.37 ^{b}	17.50 ^{b}	3.58 ^{a}
MK48h	92.95 ^{c}	13.95 ^{b}	0.45 ^{c}	20.95 ^{c}	3.39 ^{a}

The shown results are a mean value of nine measurements; differences in values within the column marked with the same letter (a–c) are not statistically significant ($p < 0.05$); M—milk; MK24h—milk kefir after 24 h inoculation; MK48h—milk kefir after 48 h inoculation.

In the case of water beverages, the quantity of acetic and lactic acids statistically significantly increased with longer inoculation, while the quantity of gluconic acid increased rather insignificantly after 24 h (WB24h) but then more considerably after 48 h (WB48h), rising from 0.02 to 0.08% (Table 8). Authors [33] reported results of 2 mg/mL lactic acid and 2.72 mg/mL acetic acid in WB24h, which is of a lower acidity compared to this research. Furthermore, when looking at the milk and water beverages, a significantly higher amount of acetic, gluconic, and lactic acids, as well as acidity, was identified in MK24h and MK48h samples compared to WB24h and WB48h (Tables 9 and 10).

Table 8. Representation of changes in acidity level, acetic, gluconic, and lactic acid values, as well as pH, depending on the length of inoculation in a water-based beverage.

	Acidity (°sH)	Acetic Acid (g/L)	Gluconic Acid (g/L)	Lactic Acid (mg/mL)	pH
WB	4.30 ^{a}	0.75 ^{a}	0.02 ^{a}	0.97 ^{a}	5.96 ^{c}
WB24h	9.70 ^{b}	1.80 ^{b}	0.04 ^{a}	2.20 ^{b}	3.87 ^{b}
WB48h	16.85 ^{c}	2.40 ^{c}	0.08 ^{b}	3.80 ^{c}	3.45 ^{a}

The shown results are a mean value of nine measurements; differences in values within the column marked with the same letter (a–c) are not statistically significant ($p < 0.05$); WB—water beverage; WB24h—water beverage after 24 h inoculation; WB48h—water beverage after 48 h inoculation.

Table 9. Comparison of lactobacilli, yeast values, and wet mass of milk and water particles.

	LAB	Yeast	Wgw
MG	7.27 ^{a}	6.66 ^{a}	99.95 ^{a}
WG	7.43 ^{a}	6.80 ^{b}	100.00 ^{a}

The shown results are a mean value of nine measurements; differences in values within the column marked with the same letter (a, b) are not statistically significant ($p < 0.05$); MG—milk grains; WG—water grains; LAB—lactic acid bacteria; Wgw—wet grain weight.

Table 10. Comparison of milk kefir and a water-based beverage after a 24-h inoculation.

	LAB	Yst	Wgw	pH	Acidity	Fats	Prot	Sug	Sucr	Aa	Ga	La	Dm	Ash
MK24h	4.45 ^{a}	5.29 ^{b}	109.50 ^{b}	3.58 ^{a}	77.75 ^{b}	2.02 ^{b}	3.47 ^{a}	0.28 ^{a}	0.07 ^{a}	13.05 ^{b}	0.36 ^{b}	17.30 ^{b}	9.60 ^{b}	0.60 ^{b}
WB24h	7.32 ^{b}	4.32 ^{a}	100.02 ^{a}	3.87 ^{b}	9.70 ^{a}	0.05 ^{a}	0.79 ^{b}	2.35 ^{b}	0.55 ^{b}	1.60 ^{a}	0.07 ^{a}	2.35 ^{a}	5.05 ^{a}	0.15 ^{a}

The shown results are a mean value of nine measurements; differences in values within the column marked with the same letter (a, b) are not statistically significant ($p < 0.05$); MK24h—milk kefir after 24 h inoculation; WB24h—water beverage after 24 h inoculation; LAB—lactic acid bacteria; Yst—yeast; Wgw—wet grain weight; Prot—proteins; Sug—sugars; Sucr—sucrose; Aa—acetic acid; Ga—gluconic acid; La—lactic acid; Dm—dry matter.

The pH values of the samples in both milk and water beverages decreased significantly ($p < 0.05$) over the longer inoculation periods, while the difference in the occurrence of fermentation between MK48h and MK24h was statistically minimal (Table 7). According to [38], the pH of milk kefir varies tendentially between 4.2 and 4.6, while analysis [35] shows a decrease in milk pH from 6.6 to kefir pH 3.40 after 24 h of fermentation. When comparing our findings for MK24h and WB24h, a markedly higher pH value was recorded for MK24h, while after 48 h, this value was only minimally larger for MK48h when compared with that of WB48h (Tables 10 and 11).

Table 11. Comparison of milk kefir and a water-based beverage after a 48-h inoculation.

	LAB	Yst	Wgw	pH	Acidity	Fats	Prot	Sug	Sucr	Aa	Ga	La	Dm	Ash
MK48h	6.77 ^{a}	6.51 ^{b}	112.40 ^{b}	3.39 ^{a}	92.95 ^{b}	2.28 ^{b}	3.33 ^{b}	0.26 ^{a}	0.06 ^{a}	13.95 ^{b}	0.47 ^{b}	20.95 ^{b}	10.25 ^{b}	0.56 ^{a}
WB48h	9.09 ^{b}	5.80 ^{a}	96.12 ^{a}	3.45 ^{a}	16.85 ^{a}	0.05 ^{a}	0.87 ^{a}	0.28 ^{a}	0.04 ^{a}	2.60 ^{a}	0.07 ^{a}	3.70 ^{a}	2.70 ^{a}	0.47 ^{a}

The shown results are a mean value of nine measurements; differences in values within the column marked with the same letter (a, b) are not statistically significant ($p < 0.05$); MK48h—milk kefir after 48 h inoculation; WB48h—water beverage after 48 h inoculation; LAB—lactic acid bacteria; Yst—yeast; Wgw—wet grain weight; Prot—proteins; Sug—sugars; Sucr—sucrose; Aa—acetic acid; Ga—gluconic acid; La—lactic acid; Dm—dry matter.

According to [37], a lower pH value consequently leads to slower grain growth, which would explain why there was a reduction in the quantity of water grains after 48 h. According to the research by these authors, the pH value decreased in the water beverage from 5.88 ± 0.05 to 3.76 ± 0.03 after 48 h. They also concluded in their research on water beverages that acetic and lactic acids increase as the pH value decreases, which is consistent with the findings of this study.

Analyses regarding the microbiological safety of the samples did not detect any tested pathogenic microorganisms, indicating they would not be harmful if consumed (Table 12). Research by [35] also shows that no pathogenic microorganisms were found in their dairy samples and highlights the inability of tested pathogenic microorganisms to develop when the pH value is below 4.2.

Table 12. Health safety assessment of grain samples, milk kefir, and water-based beverages.

	Salmonella spp.	*Staphylococcus aureus*	*Listeria monocytogenes*
M	Ø	Ø	Ø
MK24h	Ø	Ø	Ø
MK48h	Ø	Ø	Ø
WB	Ø	Ø	Ø
WB24h	Ø	Ø	Ø
WB48h	Ø	Ø	Ø

M—milk; MK24h—milk kefir after 24 h inoculation; MK48h—milk kefir after 48 h inoculation; WB—water beverage; WB24h—water beverage after 24 h inoculation; WB48h—water beverage after 48 h inoculation. Ø—not isolated.

Through the consumer acceptance analysis conducted by five panelists using a 9-point hedonistic scale, the highest average ratings for aroma, appearance, taste, texture, and overall impression were obtained for the milk kefir fermented for 24 h. The fruit beverage fermented for 48 h scored second highest overall, followed by the fruit beverage fermented for 24 h. In contrast, the milk kefir fermented for 48 h received the lowest ratings from the selected panelists. These results are presented graphically (Figure 1). The acetic acid found in the kefirs contributes to providing a pleasant taste and plays a role in inhibiting unwanted microorganisms [39], which explains why beverages with a higher content of acetic acid were preferred by the panelists.

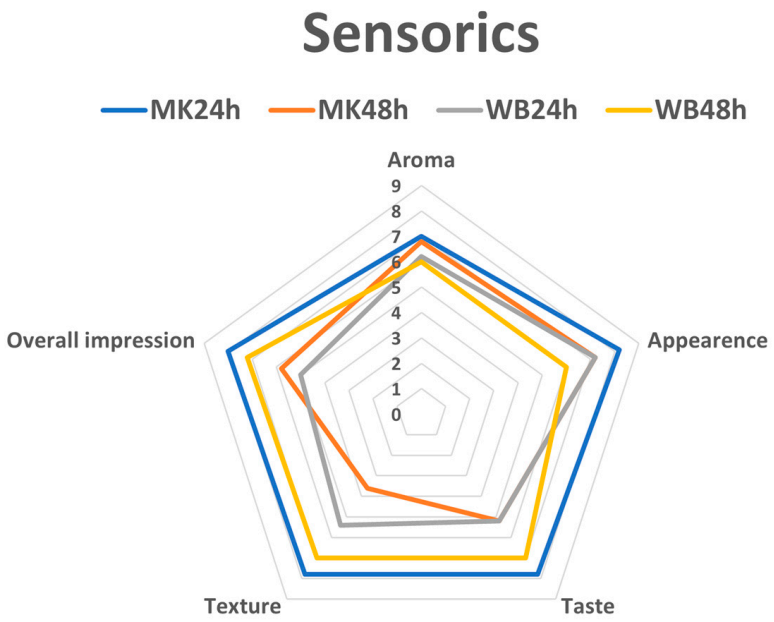

Figure 1. Consumer acceptance test.

4. Conclusions

In the findings of this experiment, the initial water kefir grains had a higher number of lactobacilli compared to milk kefir grains. After a 24-h inoculation, the water beverage had a higher number of lactobacilli compared to the milk kefir, and after 48 h, the water beverage had the highest number of lactobacilli. The amount of yeast was also higher in water grains than compared to that in milk grains, but after a 24-h inoculation, the number of yeasts was higher in milk kefir and remained so after 48 h. The weight of wet milk grains increased during the entire 48-h inoculation period, while in wet water grains, it only decreased after 48 h. This occurrence can be explained as lowering the pH value and increasing the acidity level, which can lead to effects on the mass of the grains. Regardless of the decrease, the water beverage after 48 h contained the highest number of desirable bacteria (*Lactobacilli*). The pH value also decreased during the inoculation period, which

correlated with the observed increase in acidity in both beverages. The sugar content significantly decreased in the water beverage after 48 h of inoculation, which is consistent with the standard preparation process for the 48-h water beverage. Although there was a higher amount of acetic, lactic, and gluconic acids in milk samples, the water beverages experienced a greater total increase in the values of these acids. Based on their feedback, panelists demonstrated a preference for the traditional 24-h milk kefir preparation over the vegan alternative, even though the standard 48-h water beverage preparation had the advantage of higher levels of lactobacilli. Acetic acid contributes to a pungent taste and plays a role in inhibiting unwanted microorganisms, explaining why beverages with a higher content of acetic acid in milk were preferred by the panelists. For milk kefir, the 24-h inoculation proved more desirable, while for the water beverage, 48 h was favored, perhaps as that was the necessary time for sugar fermentation to begin. A statistically higher value of gluconic acid in milk kefir led to a sour taste of kefir and better consumer acceptance. In the case of WB24h (a water beverage prepared with a 24-h inoculation), there was a slight increase in wet grain mass, and the sugar content did not decrease but rather increased. In WB48h (a water beverage prepared with a 48-h inoculation), after 48 h of inoculation, the sugar content significantly decreased, the wet grain mass also decreased compared to the initial mass, and the acidity level and acetic acid were at their highest.

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References

1. Tiss, M.; Souiy, Z.; Bem Abdeljelil, N.; Njima, M.; Achour, L.; Hamden, K. Fermented soy milk prepared using kefir grains prevents and ameliorates obesity, type 2 diabetes, hyperlipidemia and Liver-Kidney toxicities in HFFD-rats. *J. Funct. Foods* **2020**, *67*, 103869. [CrossRef]
2. Chen, J.; Chia, N.; Kalari, K.R.; Yao, J.Z.; Novotna, M.; Paz Soldan, M.M.; Luckey, D.H.; Marietta, E.V.; Jeraldo, P.R.; Chen, X.; et al. Multiple sclerosis patients have a distinct gut microbiota compared to healthy controls. *Sci. Rep.* **2016**, *6*, 28484. [CrossRef]
3. Huang, R.; Wang, K.; Hu, J. Effect of Probiotics on Depression: A Systematic Review and Meta-Analysis of Randomized Controlled Trials. *Nutrients* **2016**, *8*, 483. [CrossRef]
4. Milani, C.; Duranti, S.; Bottacini, F.; Casey, E.; Turroni, F.; Mahony, J.; Belzer, C.; Delgado Palacio, S.; Arboleya Montes, S.; Mancabelli, L.; et al. The First Microbial Colonizers of the Human Gut: Composition, Activities, and Health Implications of the Infant Gut Microbiota. *Microbiol. Mol. Biol. Rev.* **2017**, *81*, 10–1128. [CrossRef] [PubMed]
5. Marco, M.L.; Heeney, D.; Binda, S.; Cifelli, C.J.; Cotter, P.D.; Foligné, B.; Gänzle, M.; Kort, R.; Pasin, G.; Pihlanto, A.; et al. Health benefits of fermented foods: Microbiota and beyond. *Curr. Opin. Biotechnol.* **2017**, *44*, 94–102. [CrossRef]
6. Dallas, D.C.; Citerne, F.; Tian, T.; Silva, V.L.; Kalanetra, K.M.; Frese, S.A.; Robinson, R.C.; Mills, D.A.; Barile, D. Peptidomic analysis reveals proteolytic activity of kefir microorganisms on bovine milk proteins. *Food Chem.* **2016**, *197*, 273–284. [CrossRef] [PubMed]
7. Guzel-Seydim, Z.; Wyffels, J.T.; Seydim, A.C.; Greene, A.K. Turkish kefir and kefir grains: Microbial enumeration and electron microscobic observation. *Int. J. Dairy Technol.* **2005**, *58*, 25–29. [CrossRef]
8. Randazzo, W.; Corona, O.; Guarcello, R.; Francesca, N.; Germanà, M.A.; Erten, H.; Moschetti, G.; Settanni, L. Development of new non-dairy beverages from Mediterranean fruit juices fermented with water kefir microorganisms. *Food Microbiol.* **2016**, *54*, 40–51. [CrossRef]
9. Guzel-Seydim, Z.B.; Gökırmaklı, Ç.; Greene, A.K. A comparison of milk kefir and water kefir: Physical, chemical, microbiological and functional properties. *Trends Food Sci. Technol.* **2021**, *113*, 42–53. [CrossRef]
10. Waldherr, F.W.; Doll, V.M.; Meißner, D.; Vogel, R.F. Identification and characterization of a glucan-producing enzyme from Lactobacillus hilgardii TMW 1.828 involved in granule formation of water kefir. *Food Microbiol.* **2010**, *27*, 672–678. [CrossRef]

11. Laureys, D.; Van Jean, A.; Dumont, J.; De Vuyst, L. Investigation of the instability and low water kefir grain growth during an industrial water kefir fermentation process. *Appl. Microbiol. Biotechnol.* **2017**, *101*, 2811–2819. [CrossRef] [PubMed]
12. Fels, L.; Jakob, F.; Vogel, R.F.; Wefers, D. Structural characterization of the exopolysaccharides from water kefir. *Carbohydr. Polym.* **2018**, *189*, 296–303. [CrossRef] [PubMed]
13. Zajšek, K.; Kolar, M.; Goršek, A. Characterisation of the exopolysaccharide kefiran produced by lactic acid bacteria entrapped within natural kefir grains. *Int. J. Dairy Technol.* **2011**, *64*, 544–548. [CrossRef]
14. Tireki, S. Physicochemical and Sensory Parameters of Vegan Water Kefir Beverages Fermented with Different Fruits. *Eur. J. Res. Dev.* **2022**, *2*, 1–12. [CrossRef]
15. Heeney, D.D.; Gareau, M.G.; Marco, M.L. Intestinal Lactobacillus in health and disease, a driver or just along for the ride? *Curr. Opin. Biotechnol.* **2018**, *49*, 140–147. [CrossRef]
16. Yang, X.; Su, B.; Zhang, X.; Liu, Y.; Wu, H.; Zhang, T. Incomplete immune reconstitution in HIV/AIDS patients on antiretroviral therapy: Challenges of immunological non-responders. *J. Leukoc. Biol.* **2020**, *107*, 597–612. [CrossRef]
17. Liu, H.-N.C.; Wu, H.A.; DE Srjhfb Chen, Y.-Z.; Chen, Y.-J.; Shen, X.-Z.; Liu, T.-T. Altered molecular signature of intestinal microbiota in irritable bowel syndrome patients compared with healthy controls: A systematic review and meta-analysis. *Dig. Liver Dis.* **2017**, *49*, 331–337. [CrossRef]
18. Uusitalo, U.; Liu, X.; Yang, J.; Hummel, S.; Butterworth, M.; Rewers, M.; Hagopian, W.; She, J.; Simell, O.; Toppari, J.; et al. Association of early exposure of probiotics and islet autimmunity in the TEDDY study. *JAMA Pediatr.* **2016**, *170*, 20–28. [CrossRef]
19. Rothhammer, V.; Mascanfroni, I.D.; Bunse, L.; Takenaka, M.C.; Kenison, J.E.; Mayo, L.; Chao, C.-C.; Patel, B.; Yan, R.; Blain, M.; et al. Type I interferons and microbial metabolites of tryptophan modulate astrocyte activity and central nervous system inflammation via the aryl hydrocarbon receptor. *Nat. Med.* **2016**, *22*, 586–597. [CrossRef]
20. Borges-Canha, M.; Portela-Cidade, J.P.; Dinis-Ribeiro, M.; Leite-Moreira, A.F.; Pimentel-Nunes, P. Role of colonic microbiota in colorectal carcinogenesis: A systematic review. *Rev. Española Enfermedades Dig.* **2015**, *107*, 659–671. [CrossRef]
21. Farnworth, E.R.; Mainville, I. Kefir: A fermented milk product. In *Handbook of Fermented Functional Foods*; CRC Press: Boca Raton, FL, USA, 2003; pp. 89–127.
22. Garrote, G.L.; Abraham, A.G.; DE Antoni, G.L. Characteristics of kefir prepared with different grain[ratio]milk ratios. *J. Dairy Res.* **1998**, *65*, 149–154. [CrossRef]
23. Irigoyen, A.; Arana, I.; Castiella, M.; Torre, P.; Ibáñez, F. Microbiological, physicochemical, and sensory characteristics of kefir during storage. *Food Chem.* **2004**, *90*, 613–620. [CrossRef]
24. *ISO 6579:2002/Amd 1:2007*; Microbiology of Food and Animal Feeding Stuffs—Horizontal Method for the Detection of *Salmonella* spp. AMENDMENT 1: Annex D: Detection of *Salmonella* spp. in Animal Faeces and in Environmental Samples from the Primary Production Stage. International Organization for Standardization: Geneva, Switzerland, 2007.
25. *ISO 6888-1:2021*; Microbiology of the Food Chain—Horizontal Method for the Enumeration of Coagulase-Positive Staphylococci (*Staphylococcus aureus* and Other Species)—Part 1: Method Using Baird-Parker Agar Medium. International Organization for Standardization: Geneva, Switzerland, 2021.
26. *ISO 11290-1:2017*; Horizontal Method for the Detection and Enumeration of *Listeria monocytogenes* and of *Listeria* spp. International Organization for Standardization: Geneva, Switzerland, 2017.
27. Kooiman, P.; Schmidt, K.; la Riviére, J.W.M. Kefiran, a novel polysaccharide produced in the kefir grain by Lactobacillus brevis. *Arch. Microbiol.* **1967**, *59*, 269–278. [CrossRef]
28. Koroleva, N.S.; Rozhkova, I.V.; Bavina, N.A. Basic factors affecting the microflora and quality of kefir. In Proceedings of the XX International Dairy Congress, Paris, France, 26–30 June 1978; p. 843.
29. Laureys, D.; De Vuyst, L. The water kefir grain inoculum determines the characteristics of the resulting water kefir fermentation process. *J. Appl. Microbiol.* **2017**, *122*, 719–732. [CrossRef]
30. Laureys, D.; De Vuyst, L. Microbial species diversity, community dynamics, and metabolite kinetics of water kefir fer-mentation. *Appl. Environ. Microbiol.* **2014**, *80*, 2564–2572. [CrossRef] [PubMed]
31. Gökırmaklı, Ç.; Güzel-Seydim, Z.B. Water Kefir Grains vs. Milk Kefir Grains: Physical, Microbial and Chemical Comparison. *J. Appl. Microbiol.* **2022**, *132*, 4349–4358. [CrossRef] [PubMed]
32. Witthuhn, R.C.; Schoeman, T.; Britz, T.J. Isolation and characterization of the microbial population of different South African kefir grains. *Int. J. Dairy Technol.* **2004**, *57*, 33–37. [CrossRef]
33. Magalhães, K.T.; Pereira, G.V.D.M.; Campos, C.R.; Dragone, G.; Schwan, R.F. Brazilian kefir: Structure, microbial communities and chemical composition. *Braz. J. Microbiol.* **2011**, *42*, 693–702. [CrossRef]
34. Guzel-Seydim, Z.B.; Kok-Tas, T.; Greene, A.K.; Seydim, A.C. Review: Functional properties of kefir. *Crit. Rev. Food Sci. Nutr.* **2011**, *51*, 261–268. [CrossRef]
35. Sekkal-Taleb, N. Chemical and microbiological composition of Kefir and its natural benefits. *Mediterr. J. Biosci.* **2016**, *1*, 174–183. Available online: https://api.semanticscholar.org/CorpusID:55598752 (accessed on 4 April 2023).
36. Sarkar, S. Potential of kefir as a dietetic beverage—A review. *Br. Food J.* **2007**, *109*, 280–290. [CrossRef]
37. Laureys, D.; Cnockaert, M.; De Vuyst, L.; Vandamme, P. Bifidobacterium aquikefiri sp. nov., isolated from water kefir. *Int. J. Syst. Evol. Microbiol.* **2016**, *66*, 1281–1286. [CrossRef] [PubMed]

38. Saloff Coste, C.J. Kefir. Nutritional and health benefits of yogurt and fermented milks. *Danone World Newsl.* **1996**, *11*, 1–7.
39. Puerari, C.; Magalhães, K.T.; Schwan, R.F. New cocoa pulp-based kefir beverages: Microbiological, chemical composition and sensory analysis. *Food Res. Int.* **2012**, *48*, 634–640. [CrossRef]