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From Milk Kefir to Water Kefir: Assessment of Fermentation Processes, Microbial Changes and Evaluation of the Produced Beverages

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Abstract: The aim of the present study was to investigate the feasibly of using traditional milk kefir grains for the production of water kefir-like beverages and assess the changes in the physicochemical characteristics and the microbial populations of the fermented beverages. To this end, experiments of milk fermentation were primarily conducted at different temperatures and upon selection of the optimal, a gradual substitution of the substrate was performed by replacing milk from a sucrose-based solution. After the successful fermentation of the sucrose substrate, fruit juices were used as fermentation substrates. Sensory evaluation of the sugar-based beverages was also performed in order to access their acceptability for consumption. According to the results, the transition from milk to water kefir is indeed feasible, leading to the production of beverages with relatively higher ethanol concentrations (up to 2.14 ± 0.12% w/v) than milk kefir and much lower lactic acid concentrations (up to 0.16 ± 0.01% w/v). During the fermentation of the sugary substrates, yeasts seemed to be dominant over lactic acid bacteria, in contrast to what was observed in the case of milk kefir, where LAB dominated. The sensory evaluation revealed that all sugar-based beverages were acceptable for consumption, with the fruit-based ones obtaining, though, a better score in all attributes.

Keywords: fermented foods; water kefir; cow milk; sugars; microbial population shift

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1. Introduction

Fermented foods are reported to provide many health benefits due their anti-oxidant, anti-microbial, anti-fungal, anti-inflammatory, anti-diabetic, and anti-atherosclerotic activities [1]. Among them, kefir is considered a beverage that contributes greatly to the well-being of consumers [2]. Kefir may be beneficial for patients with lactose intolerance, improving the immune and gastrointestinal system and lowering cholesterol [3], whereas it also has anti-cancer, antimicrobial, probiotic, and prebiotic properties [4]. Various types of milk can be used to produce kefir drink, such as goat, sheep, cow, and even soy milk, and its production can be either on a small household scale using traditional methods, or on an industrial level. For home preparation of a traditional kefir drink, live cultures of kefir in the form of granules (kefir grains) are added in pasteurized milk as initiators of the fermentation. The inoculated milk is left at room temperature, usually for 18–24 h and afterwards the granules are separated from fermented milk by filtration (sieving), to be re-used for the fermentation of a new amount of milk [5]. In the industrial production of kefir beverage, the temperature and other fermentation conditions should be strictly controlled in order to ensure the production of batches with stable organoleptic characteristics and quality. As such, the fermentation with kefir grains is limited due to the possible variations in the composition of the microflora of kefir grains and instead stable microbial populations in the form of lyophilized cultures are used [6].

Apart from milk, alternative non-dairy substrates, such as fruit and vegetable juices and molasses, have also been tested to produce fermented functional beverages with different organoleptic characteristics, the so-called water kefir or sugary kefir. This differentiation is vital for the production of new probiotic products to meet the specific needs of consumers, e.g. people following vegan diets or having intolerance to lactose. Water (sugary) kefir is based on the fermentation or sugar solutions or sugary substrates instead of milk via proper kefir grains, consisting of polysaccharides and microorganisms, and its production process is similar to that of milk kefir. According to the literature, water kefir grains (WKG) have similar microbial characteristics to traditional milk kefir grains (MKG), especially in terms of lactic acid bacteria and yeast genera such as *Lactobacillus*, *Leuconostoc*, *Kluyveromyces*, *Pichia*, and *Saccharomyces* [7]. However, the type and frequency of microbial species may vary, depending on the carbon and energy sources available for fermentation. It is indeed reported that kefir grains may adapt to new available carbon sources affecting their granulation and the microbial growth on them, as well as the microbial characteristic of the final beverage. In general, selective pressure is observed at the species level, such as metabolism stimulation of the *Saccharomyces* species, thus leading to higher alcohol content of the water kefir beverages [8]. Similarly to milk kefir, existing literature indicates important biological activities related to the consumption of sugary kefir beverages, such as anti-inflammatory, anti-ulcerogenic, antioxidant, antimicrobial, and healing ones [8].

Although water kefir drinks are household-produced around the world, an industrial process has not yet been established. In fact, further research on the microbiological, technological, and functional properties is required to produce new kefir-based probiotic products [9]. Unlike milk kefir, attempts to recreate a stable water kefir consortium by recombining individual isolates have not been so far successful. Nevertheless, proposals for the minimum number of strains that is appropriate for an efficient fermentation have been put forward, whereas the microbial safety of water kefir is assured due its low pH [10].

Into this context, the scope of the current study was to investigate the feasibility of using traditional milk MKG for the production of sugary kefir beverages, and to assess the differences in the composition and the microbial populations among the final beverages. Initially, experiments with milk were carried out at different temperatures and then the gradual substitution of the substrate was performed by replacing milk from a sucrose-based solution. Upon the successful transition from milk to the sucrose, the fermentation substrate was replaced by fruit juices. In all cases, the consumption of sugars and the concentrations of the main metabolic products of the fermentation were estimated, whereas the sensory analysis of the produced beverages was also performed to assess their acceptability for consumption. To our knowledge, it is the first time that the transition of milk kefir to water kefir has been successfully studied, and the characteristics of the produced beverages were assessed.

2. Materials and Methods

2.1. Fermentation Media and Kefir Grains

High heat-treated long-life milk with a composition per 100 mL: 3.6 ± 0.1 g fat, 3.4 ± 0.1 g protein, 5.2 ± 0.1 g sugar was used for the fermentation of milk. For the fermentation of sugar, a solution of sucrose was prepared with tap water with a concentration of 46.5 ± 0.2 g/L. Brown sugar of 97% purity was used and 10 g/L of chopped dried prunes was added with soluble sugar concentration of 3.8 ± 0.1 g/L. The final concentration of soluble sugars in the solution was 50.4 ± 0.2 g/L. For the fermentation of juices, commercial sour cherry nectar (SCN) with sugars concentration of 123.0 ± 0.5 g/L, and natural apple juice (NAJ)with sugars concentration of 92.0 ± 0.1 g/L were used. All measured sugars were expressed in glucose equivalents. The milk kefir grains used in the present study were derived from household milk kefir (Attica, Greece).

2.2. Experimental Plan

The fermentation of milk at three different temperatures was initially studied, i.e., at a constant temperature of 20 and 25 °C in an incubator (FTC 901, VELP Scientifica Srl, Italy) and at ambient conditions (temperature varying from 19 to 26 °C). Each fermentation cycle (FC) lasted ~24 h and 20 FC were performed in total, i.e., 9 cycles at 25 °C, 6 cycles at ambient temperature, and 5 cycles at 20 °C. In each FC the initial and final pH values and the increase in the kefir grains weight, on wet basis, were recorded. In each last FC of each period, frequent sampling was performed throughout the 24 h to monitor the change in pH, sugar consumption, and alterations in microbial populations in the fermentation mixture, while the fermentation products were also quantified. Based on the evaluation of the results, all subsequent fermentations were performed at a constant temperature of 25 °C. Subsequently, the milk was gradually replaced (20% daily) by a sucrose solution (SS) in order to adapt the microbial flora of the milk kefir to sucrose as substrate (instead of lactose) and evaluate whether the adapted consortium would be adequate for the production of beverages based on fruit juices. The transition from one substrate to the other took place gradually in order to allow acclimation of the microorganisms and prevent possible collapse of the culture, a case that is frequently reported in the literature. After complete substrate replacement, the fermentation process was monitored in 24 h FCs. This was followed by a study of the fermentation in 24 h FC of the two different commercial juices, SCN and NAJ. As in the milk fermentation experiments, for all sugary substrates the initial and final pH values, and the increase in weight of kefir grains were recorded, while in the last FC of each period, frequent sampling was performed to monitor the change in pH of sugars and the change in microbial populations in the fermentation mixture, while the final concentrations of the main fermentation products were also quantified. All fermentations were performed in sterilized (20 min, 121 °C) glass containers capped (not airtight) with metallic lids, whereas the initial ratio of kefir grains to fermentable liquid was 5 g/100 mL.

2.3. Sampling and Processing of Samples

For the measurement of pH, sugars, and fermentation products, 2 mL of fermentation liquid was removed. Sample were then centrifuged at 4000 rpm for 15 min and filtered under vacuum via 0.7 μm pore glass fiber filters (GFF, Whatman plc, Maidstone, UK). The filtrate was collected and stored at −21 °C until analysis. For the calculation of the microbial populations, 0.1 mL of fermenting liquid was removed during stirring, and subjected to immediate decimal dilutions under aseptic conditions. At the end of each FC, the fermentation broths (beverage) were filtered via a plastic sieve under aseptic conditions. The collected kefir grains were rinsed with ~200 mL of sterile, distilled water, and were allowed to drain for 30 min at room temperature under aseptic conditions and weighed for the estimation of wet mass increase.

2.4. Microbiological Profiling Analysis

During the fermentation of the different substrates, aliquots of the fermentation broth were removed under aseptic conditions and decimal dilutions were performed for the enumeration of total mesophilic flora (TMF); yeasts, and lactic acid bacteria (LAB). Sterile tryptone water, 0.1% (Sigma-Aldrich, St. Louis, MS, USA) was used to prepare the dilutions for the microbiological analyses. TMF was quantified on plate count agar (PCA), incubated at 30 °C for 72 h; LAB counts were quantified on De Man, Rogosa, and Sharpe (MRS) agar plates, incubated at 30 ± 1 °C for 48 h. Yeast counts were quantified on Potato Dextrose Agar (PDA) plates, which were incubated at 30 ± 1 °C for 48 h. For the development of TMF and yeasts the spread plate method of 0.1 mL sample from each dilution was followed, whereas for LAB, the integration procedure was followed. Counts were expressed in total colony-forming units per milliliter (Log10 CFU/mL). For each sampling period sampling was performed in duplicate, and from each sample at least four technical replicates were made.

2.5. Sensory Evaluation

Sensory evaluation was performed to evaluate flavor, aroma, and appearance of the sugary fermented beverages [11]. Specifically, 10 panelists (aged 22–45) who were habitual consumers of commercial and/or homemade milk kefir were selected to evaluate the water kefir beverages according to 7 pre-defined attributes on a 0-10 scale. All three beverages, together with water, were offered chilled (8 ± 1 °C) in glass, colorless transparent vials, under good diffused lighting. The 7 attributes upon the evaluation were, "attractiveness, colour, aroma, sweetness, acidity, taste, and overall acceptance". Average of the intensity scores from the panelists was calculated for each attribute. The test was subjective.

2.6. Analytical Techniques

For the quantification of sugars and ethanol, a spectrophotometric method and high liquid pressure gas chromatography were used, respectively, as previously described by Ntaikou et al. [12]. Acetic acid was quantified via gas chromatography [13]. pH measurements were conducted using a portable pH meter (Metrohm 744, Metrohm AG, Switzerland), which was daily calibrated with standard solutions of pH 4 and pH 7. Lactic acid was quantified via an enzymatic method using a Megazyme L-D Lactate kit (Megazyme, Bray, Ireland).

2.7. Statistical Analysis

The data were statistically analyzed using the SPSS Inc.17 software. After checking for homogeneity of the variance (Levene's test of equality of error variances), the significant difference among values was assessed non-parametrically, using the Mann–Whitney U test ($p < 0.05$, ANOVA).

3. Results

3.1. Effect of Temperature on the Fermentation of Milk

The fermentation of milk at three different temperatures was initially studied at ~24 h FCs, at the end of which the fermentation broth was separated by the kefir grains, and the latter were used as inoculum for the next FC. In Figure 1, the changes in the weight of kefir grains and the pH during the fermentation of milk at three different temperatures for a period of about 30 days are illustrated. As shown, the weight of the kefir grains was constantly increasing, with the higher rate observed for 25 °C. In Table 1, the overall weight increase (WI), as well as the average rate of weight increase (RWI) calculated for each fermentation period of milk at different temperature profiles are shown. It seems, indeed, that the temperature affects the WI of the grains, since during the fermentation at a constant temperature of 25 °C, the RWI was almost fourfold compared to that of fermentation at 20 °C. In addition, fermentation with temperature fluctuations between these values (variable temperature 19–26 °C) also leads to an increased RWG value, which was almost threefold compared to those of fermentation at 20 °C. Figure 1b shows the change in pH by the fermentation of milk at three different temperatures (initial and final pH values of each FC), while the average final pH values for each fermentation period are shown in Table 1. The final pH values fluctuate, even for the same incubation temperatures, which can be attributed to the slight differences in the fermentation time of each FC, which was between 22 and 26 h (24 h mean). In all three cases, the average value of the final pH was in agreement with that reported by previous studies [14] and within the acceptable pH limits of the commercial milk kefir, i.e., 4.6–4.9 [15]. A slight variation in the mean values was observed as follows $pH_{25°C} < pH_{20°C} < pH_{amb}$, which can be attributed to the reducing trend of the fermentation rate and is indeed expected to lead to the production of lower amounts of fermentation products affecting pH. However, the values do not actually have statistically significant difference (see Table 1).

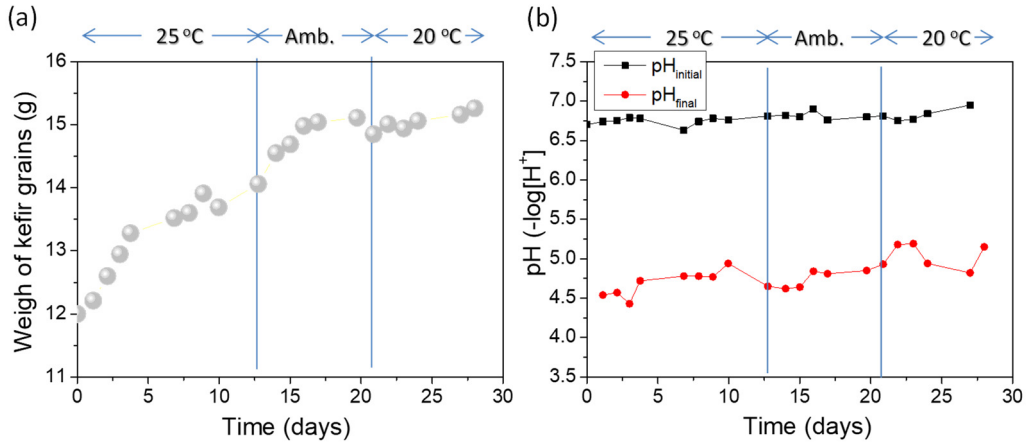

Figure 1. Weight increase of kefir grains (**a**) and pH variation (**b**), during the fermentation of milk at 24 h fermentation cycles (FC) at different temperatures.

Table 1. Total weight increase (WI) of kefir grains, mean average weight increase rate (WIR) and mean final pH values, during the fermentation of milk at 24 h fermentation cycles at different temperatures. Values in each column that share the same letter do not differ significantly from each other (Mann–Whitney U-test).

Fermentation Temperature	Total WI (g)	Average Mean WIR (g/Day)	Mean Final pH
25 °C	2.06 ± 0.02	0.28 ± 0.16	4.67 ± 0.15 ^{ab}
Ambient	0.95 ± 0.01	0.19 ± 0.12	4.78 ± 0.15 ^{ac}
20 °C	0.22 ± 0.02	0.08 ± 0.05	4.94 ± 0.16 ^{bc}

In the last FC of each period, a kinetic study of the process was carried out by frequent sampling during the day. The results in terms of sugars consumption, pH drop, and final distribution of metabolites are presented in Figure 2. The profiling of the consumption of sugars as depicted in Figure 2a seems to be faster for fermentations at 25 °C. It also appears that the consumption of sugars was not complete in either case after 24 h of fermentation, with approximately 2% w/v (20 g/L remaining in the fermentation liquid, corresponding to ~60% consumption of the available sugars). The final concentrations of sugars, as well as the exact consumption percentage are shown in Table 2. Regarding the pH drop (Figure 2b), it was higher for higher fermentation temperatures. The lowest value (4.56) is observed for 25 °C and the highest for 20 °C (5.07). It should be noted that the drop in pH for 25 °C and ambient temperature shows the exact same rate for the first 10 h of fermentation, and then appears to decrease in the case of ambient temperature. This is obviously due to the fact that during the day, the average ambient temperature was 26 ± 1 °C, while during the night and in the early morning hours, it was 19 ± 1°C. In terms of the final fermentation products, as shown in Figure 2c, lactic acid and ethanol were the main ones, whereas only traces of acetic acid were detected. The concentration of ethanol was ~0.9% w/v (~9 g/L), showing minimal difference for different fermentation temperatures and in accordance with the values reported in the literature (1–2% v/v) [16]. Lactic acid concentration was also high and similar for all fermentation temperatures, ~0.85% w/v (~8.5 g/L), which was lower than the concentrations reported in the literature reaching 2% w/v [17].

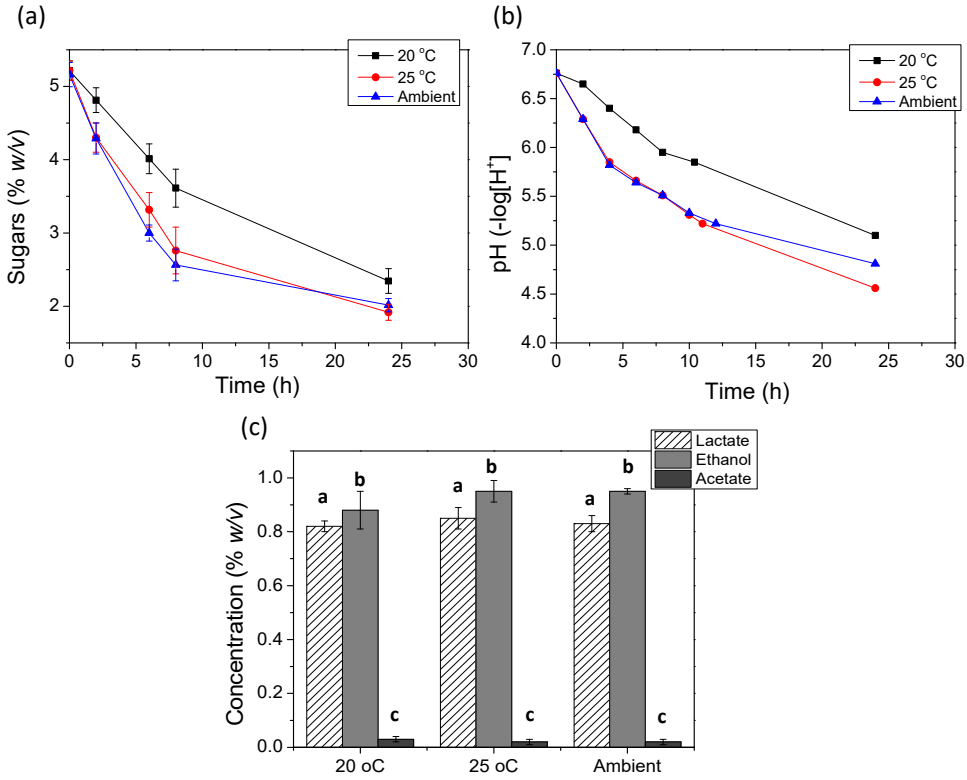

Figure 2. Consumption of sugars (**a**), pH drop (**b**), and distribution of final fermentation products (**c**) during the 24h fermentation of milk with kefir grains at different temperatures. The same letter above columns of each metabolite indicates that values do not differ significantly from each other (Mann–Whitney U-test).

Table 2. Final concentration of sugars and total estimated sugars consumption during the 24 h fermentation of milk with kefir grains at different temperatures. Values in each column that share the same letter do not differ significantly from each other (Mann–Whitney U-test).

Fermentation Temperature	Final Concentration of Sugars (% *w*/*v*)	Consumption of Sugars (% of Initial)
20 °C	2.34 ± 0.16 [a]	0.56 ± 0.01 [a]
25 °C	1.91 ± 0.11	0.64 ± 0.02
Ambient	2.15 ± 0.09 [a]	0.59 ± 0.02 [a]

In Figure 3, the microbial count in the fermentation broths is depicted versus time for the 24 h fermentation of milk at different temperatures. In the present study, no quantification of the microbial populations that were immobilized on, or trapped inside, the kefir grains was performed. However, a qualitative estimation of the microbial species of the grains has been conducted and presented in the study of Syrokou et al. [18]. As shown, in all cases, LAB seemed to dominate over yeasts, even at the beginning of fermentations, i.e., when the kefir grains were added and mixed with the milk. The populations counted may be due to the already existing microbial load of the milk but also due to the microorganisms that were on the surface of the kefir grains and were released into the milk during mixing at the beginning of the fermentation. The predominance of LAB throughout the fermentation is in line with the literature, according to which, they tend to dominate over yeasts in various types of kefir drinks [19]. It also seems that the higher temperature favors the growth of LAB in the liquid. Regarding the TMF, it appears that the populations of both the initial fermentation and the final product are within the permissible limits for consumption [20].

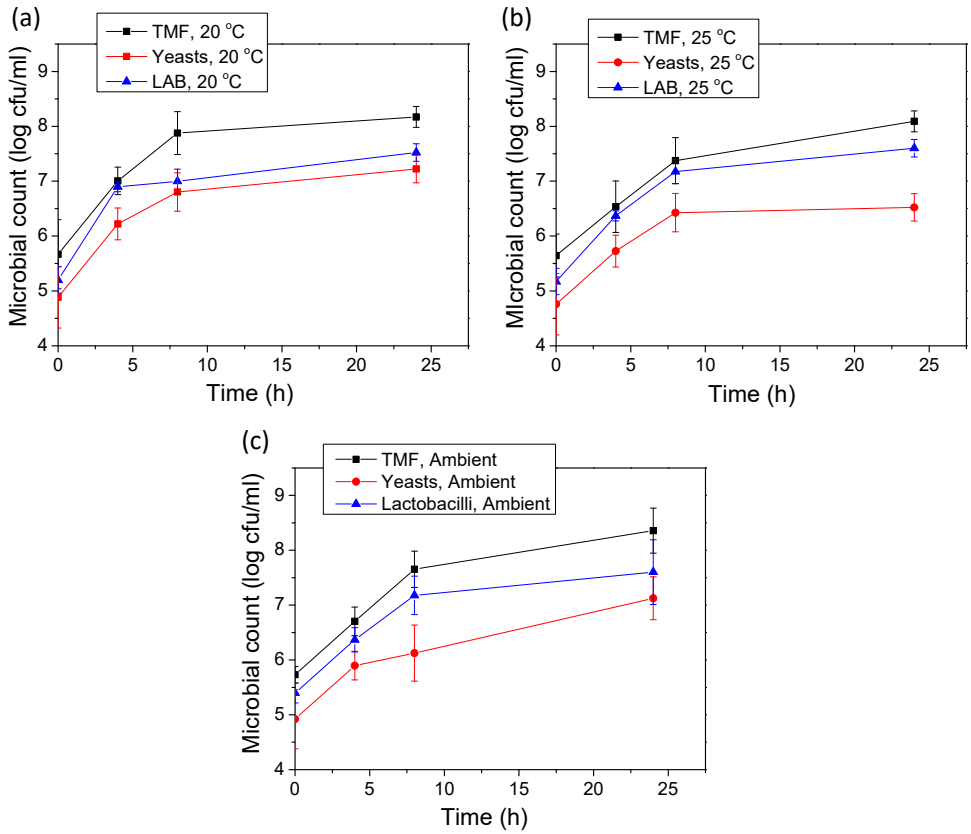

Figure 3. Microbial count for total mesophilic flora (TMF), yeasts and lactic acid bacteria (LAB) during the 24 h fermentation of milk with kefir grains at 20 °C (**a**), 25 °C (**b**), and ambient temperature (**c**).

3.2. Transition from Milk to Water Kefir—Gradul Substitution of Milk from Sucrose in the Fermentation Medium

The complete replacement of the initial fermentation substrate, milk, from SS was performed over a period of 6 days at 24 h FCs. As such, the fermentation media during the gradual substitution of the substrate had the following composition (% *v*/*v*): 80 Milk/20 SS, 60 Milk/40 SS, 40 Milk/60 SS, 20 Milk/80 SS, and, finally, 100 SS.

The change in the weight of kefir grains during the transition process is illustrated in Figure 4. As shown, in the first four FCs, a reduction in the weight of the grains seems to take place, whereas in the last FC, conducted with 100% SS as substrate, a slight increase in the weight of the grains was observed. Overall, the weight of the kefir grains decreased by 0.4 g, while the corresponding average weight change of the grains during the fermentation of milk at the same temperature (25 °C) in the same time period was estimated to be +1 g approximately. Milk kefir granules consist mainly of a hetero-polysaccharide, which consists of equal proportions of glucose and galactose and is produced mainly by *Lactobacillus kefiranofaciens* [21]. Glucose and galactose come from the breakdown of lactose, which is the main sugar in milk. The sugar contained in the SS is sucrose, i.e., the disaccharide of glucose with fructose. As such, the observed reduction in kefir grain biomass might be attributed to the metabolic pressure and reduced ability of kefiran production by the microbial consortium due to the reduced availability of galactose during the substitution of milk as a carbon source of sucrose. WKG on the other hand, consist of glucose polysaccharides (dextrans) at about 95–97%, which can indeed be produced by the hydrolysis of sucrose [22]. It is therefore considered possible that during the transition from milk fermentation to sucrose fermentation, a gradual change in the composition of the grains' matrix takes place due to the gradual synthesis of dextrans, which might indeed be responsible for the weight gain observed in the last FC. It should be noted, however, that even at this point the morphology of the grains did not resemble the usual WKG, which are

transparent [7], and remained opaque like MKG. During the transition period, however, an alteration in the color of the grains was noted, i.e., they turned brownish (Figure 5), from opaque white to brownish, which can be attributed to the absorption of the characteristic color pigments of molasses of the brown sugar, by the kefir grains matrix. In Figure 4b the pH variation during fermentation of milk/SS mixtures in the 24 h FCs is illustrated. As shown, the initial pH values did not vary significantly for the different fermentation mixtures and were ~6.7. The final pH values of each FC were, however, relatively lower than those of milk fermentation at the same temperature, i.e., 25 °C. Specifically, the average final pH value at the end of the 24 h fermentation was 4.20 ± 0.23, while the corresponding mean value for milk fermentation was 4.65 ± 0.31, values that were not, though, statistically different (Mann–Whitney U-test).

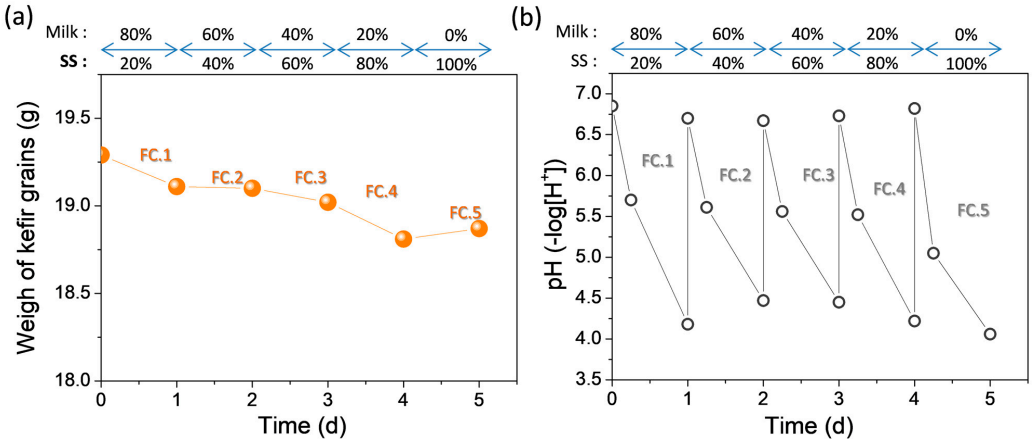

Figure 4. Weight change of kefir grains (**a**) and pH variation (**b**), during the gradual substitution of fermentation medium (from milk to sucrose solution) at 24 h fermentation cycles (FC).

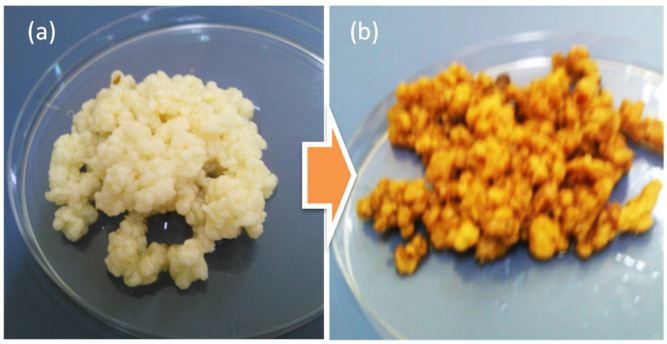

Figure 5. Morphology of kefir grains in the beginning of the initial fermentation cycle (FC) (**a**) and the end (**b**) of the final FC during the substrate substitution (milk from brown sugar solution).

3.3. Production of Water Kefir from Sugary Substrates by the Acclimated Kefir Grains

After the complete substitution of milk from the SS as fermentation substrate, successive fermentations were performed in 24 h FCs with three different sugary substrates. Specifically, the same SS that was used in the experiment of transition of substrates, sour cherry nectar (SCN) and natural apple juice (NAJ), aimed to assess the feasibility of producing beverages bearing similar characteristics to typical water kefir drinks, and also to assess their acceptability for consumption. Initially, for better acclimation of the microbial population, fermentations with SS as the sole carbon source was performed, and then fermentation with SCN (i.e., natural sour cherry juice supplemented with sugar) and NAJ (containing solely the natural sugars of apples) was carried out.

In Figure 6a, the change in kefir grains biomass during the fermentation (24 h FCs) of the sugary substrates is illustrated. As shown, the weight of kefir grains initially decreased

for the first seven FCs, while by the end of the eighth FC, this phenomenon was reversed, and weight gain was observed. During the fermentation of SCN, the weight of kefir grains increased further, i.e., no weight loss was observed as in the case of SS fermentation, but with a much lower rate compared to the subsequent fermentation of NAJ. The initial loss and subsequent gain of the weight of the kefir grains might be attributed to the possible decomposition of kefiran and gradual substitution of the polymeric matrix by dextrans after the successful acclimation of the microbial consortium to the new substrate.

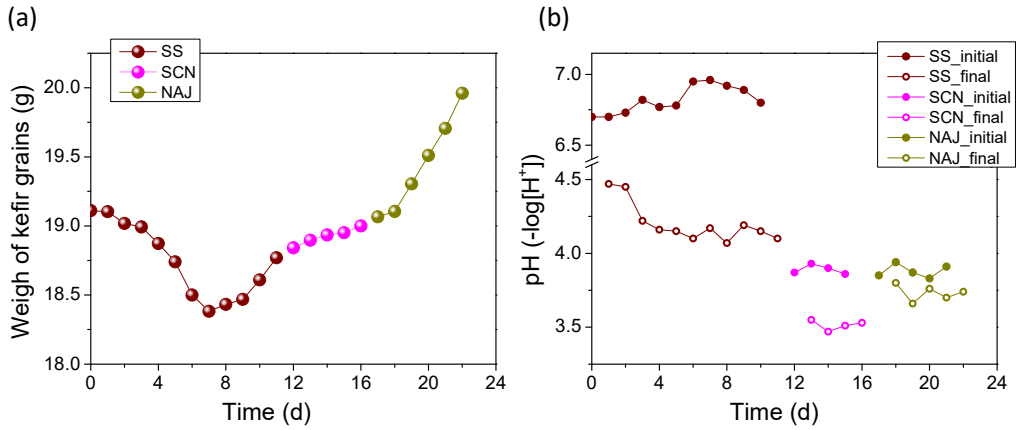

Figure 6. Weight change of kefir grains (**a**), pH variation (**b**), during the fermentation of sugary substrates at 24 h fermentation cycles (FC). SS, Sucrose solution; SCN, sour cherry nectar, NAJ, natural apple juice.

The biomass increase for the free different substrates was quantified via the estimation of WI and RWI, the values of which are presented in Table 3. As shown, both absolute weight gain, and rate were maximum for the NAJ and minimum for SC. Nevertheless, even the higher WIR observed for NAJ seems to be lower than that of milk fermentation at the same temperature. The pH values at the beginning and at the end of each FC are illustrated in Figure 6b, whereas the average final pH values for each substrate are given in Table 3. The mean initial pH of the FCs with SS was 6.82 ± 0.10, whereas the final pH was 4.20 ± 0.13, i.e., a pH drop of more than 2.5 units was noted, which was more severe than that observed during the fermentation of milk. It can also be observed that during the first days of SS fermentation, the pH values at the end of the FCs exhibit a constantly decreasing tendency, following the pattern of kefir grains biomass decrease. It could be assumed, thus, that the two phenomena were associated. The final pH values for SCN and NAJ were both even lower than those of SS fermentation, but the pH drop was less severe, since the starting values of pH were also much lower than those for SS fermentation, 3.89 ± 0.03, and 3.88 ± 0.04, respectively. Similar values have also been reported for mixed sugars (sucrose, fructose, and sucrose) fermentation with water kefir grain [23], during which the pH dropped to 3.7 at 24 h and 3.3 at 72 h, from 4.3.

Table 3. Total weight increase (WI) of kefir grains, mean average weight increase rate (WIR), and mean final pH values, during the fermentation of sugary substrates at 24 h fermentation cycles (FC). SS, Sucrose solution; SCN, sour cherry nectar, NAJ, natural apple juice. Values in each column that share the same letter do not differ significantly from each other (Mann–Whitney U-test).

Fermentation Temperature	Total WI (g)	Average Mean WIR (g/d)	Mean Final pH
SS	0.34 ± 0.01 *	0.10 ± 0.08 [a,*]	4.20 ± 0.13
SCN	0.16 ± 0.01	0.04 ± 0.02 [a]	3.52 ± 0.03
NAJ	0.89 ± 0.01	0.18 ± 0.08 [a]	3.73 ± 0.05

* From day 7 until day 11, during which WI was noticed.

In the last FC of each substrate, several samples were collected so as to follow the consumption of sugars and the pH drop. The concentrations of metabolic products were also quantified by the end of the fermentation. The results are illustrated in Figure 7.

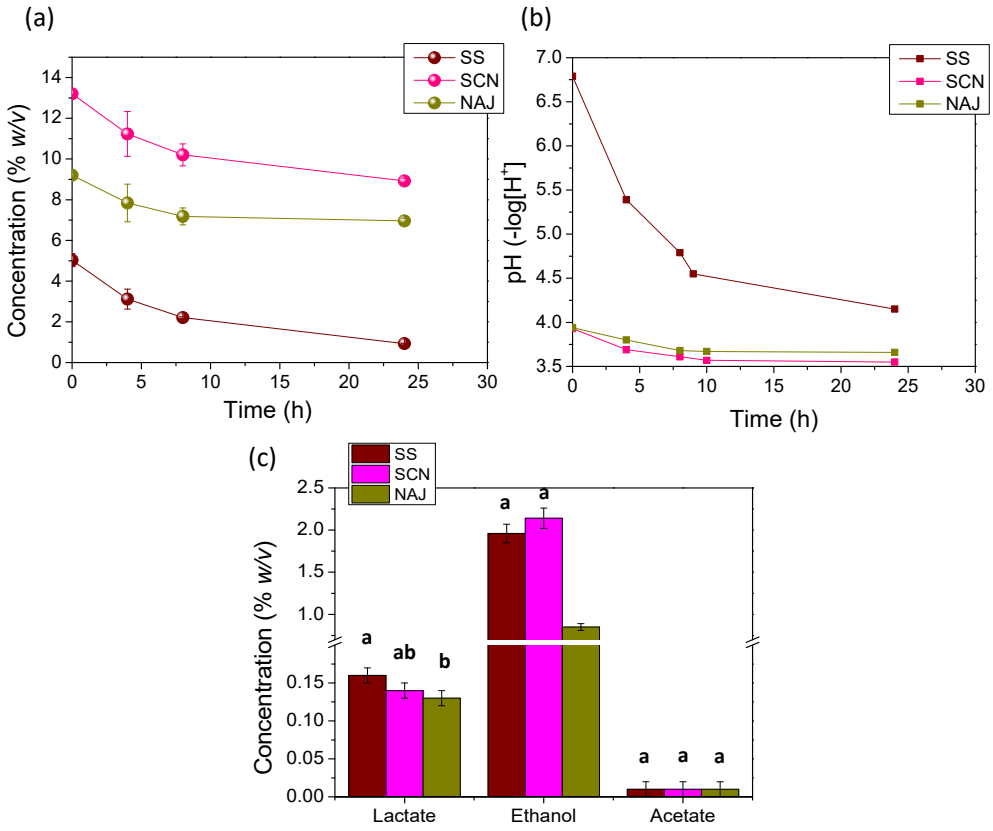

Figure 7. Consumption of sugars (**a**), pH drop (**b**), and distribution of final fermentation products (**c**), during the 24 h fermentation of fermentation of sugary substrates. SS, Sucrose solution; SCN, sour cherry nectar, NAJ, natural apple juice. The same letter above columns of each metabolite, indicate that values do not differ significantly from each other (Mann–Whitney U-test).

As depicted in Figure 7a, during the first hours of fermentation the consumption rate of sugars does not seem to differentiate among SS and SCN, with the overall consumption being approximately 4% w/v (~40g/L) after 24 h (Table 4), which is also in agreement with the results of the kinetic study performed by Laureys et al. [23]. In the case of NAJ, though the overall consumed sugars after 24 h is significantly lower. In the case milk fermentation, the consumption of sugars was not complete in either case after 24 h of fermentation, resulting in a residual concentration of sugars of 0.93 ± 0.11, 8.92 ± 0.10, and 6.96 ± 0.07 for SS, SCN, and NAJ, respectively.

Table 4. Final concentration of sugars and total estimated sugars consumption during the 24 h fermentation of different sugary solutions with acclimated kefir grains. SS, Sucrose solution; SCN, sour cherry nectar, NAJ, natural apple juice. Values in each column that share the same letter do not differ significantly from each other (Mann–Whitney U-test).

Substrate	Final Concentration of Sugars (% *w*/*v*)	Consumed Sugars (% *w*/*v*)	Consumption of Sugars (% of Initial)
SS	0.93 ± 0.11	4.10 ± 0.37 ^{a}	0.82 ± 0.09
SCN	8.92 ± 0.10	4.28 ± 0.28 ^{ab}	0.32 ± 0.02
NAJ	6.96 ± 0.07	2.24 ± 0.19 ^{b}	0.24 ± 0.02

As it regards the pH (Figure 7b), its profile is similar for SCN and NAJ dropping from 3.94 to approximately 3.60, whereas it is differentiated in the case of SS for which it drops to 4.15 from 6.79. The distribution of the fermentation products is shown in Figure 7c. The main final product detected in the fermentation broths was ethanol, reaching 2.14 ± 0.12% *w*/*v*, 1.96 ± 0.11% *w*/*v*, and 0.85 ± 0.04% *w*/*v* for SS, SCN, and NAJ, respectively, i.e., for SS and SCN, ethanol production was almost doubled compared to the fermentation of milk. On the contrary, the concentration of lactic acid was much lower than in the case of milk fermentation for all three substrates, reaching up to 0.16 ± 0.01% *w*/*v*. Traces of acetic acid were also detected (0.01 ± 0.00% *w*/*v*). Such a distribution of metabolic products is in line with the literature, with ethanol dominating over lactate as the main end product [8,23].

In Figure 8, the microbial counts for TMF, yeasts, and LAB during the 24h fermentation of the three sugary substrates are illustrated. It is apparent from the distribution of the different types of microorganisms in the fermentation broths of the sugary substrates that yeast populations are dominant to, or are equal with, the LAB populations, in contrast to what was observed in the case of milk kefir, where LAB dominated for all three fermentation temperatures. Specifically, for the SS and SCN fermentations yeasts seem to dominate slightly over LAB throughout the 24 h fermentation, reaching final values of 7.66 ± 0.19 log CFU yeasts/mL and 7.39 ± 0.16 log CFU LAB/mL and 8.04 ± 0.21 log CFU yeasts/mL and 7.66 ± 0.19 log CFU LAB/mL, respectively. In NAJ the populations of yeasts and LAB seemed to be equal, reaching final values of 8.01 ± 0.22 log CFU yeasts/mL, and 8.04 ± 0.32 log CFU LAB/mL. It should be noted that growth rate for both yeasts and LAB seems to be maximum during the first 8 h of fermentation for all three substrates, after which populations increase only slightly. Regarding the TMF counts, they also seem to increase mainly during the first hours of fermentation, whereas the values are within the permissible limits for consumption for all three beverages.

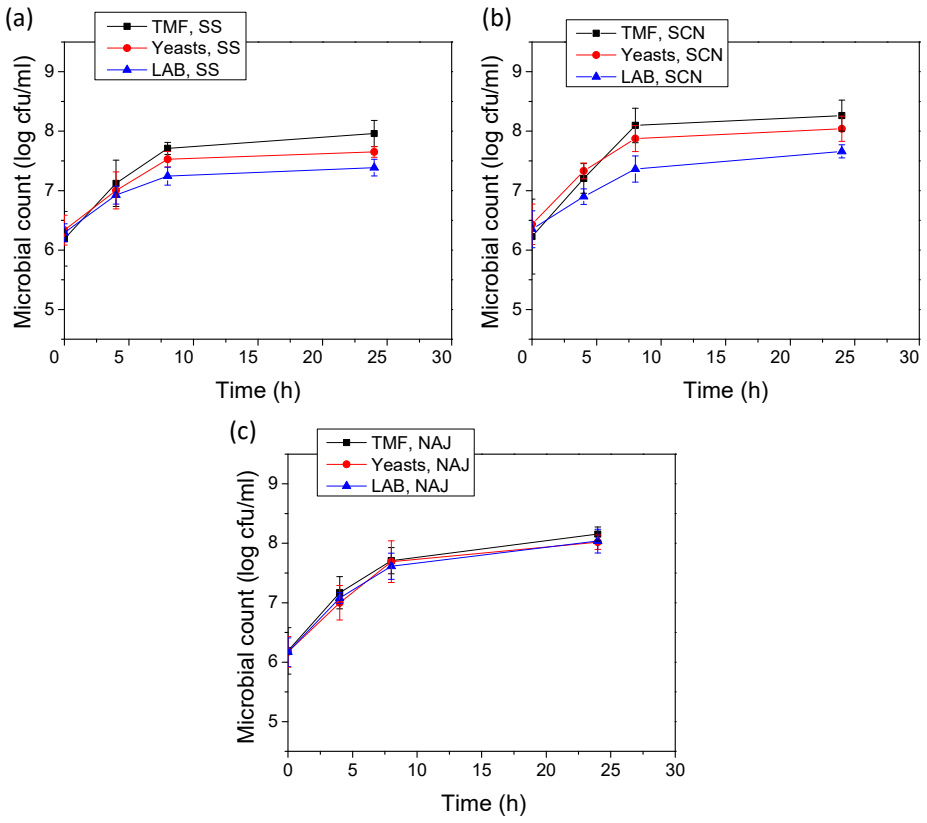

Figure 8. Microbial counts for total mesophilic flora (TMF) and yeasts and lactic acid bacteria (LAB), during the 24 h fermentation of fermentation of Sucrose solution, SS (**a**); sour cherry nectar, SCN (**b**) and natural apple juice, NAJ (**c**).

3.4. Sensory Evaluation of the Water Kefir Beverages

The sensory properties of the sugar-based beverages (water kefir) were evaluated on a scale from 0 to 10 in terms of attractiveness, color, aroma, sweetness, acidity, taste, and overall acceptance. The average scores for each attribute as estimated based by the evaluation of 10 panelists are presented in Figure 9. It is apparent that the fruit-based beverages received higher scores than the SS beverage, for all attributes but acidity. In terms of the latter, the scores were similar for all three beverages. The highest scores were obtained for the color, aroma, and taste of SCN beverage, with values, 8.8 ± 0.5, 8.2 ± 1.1, and 8.1 ± 1.6, respectively. The attractiveness, which indicates the willingness of the consumer to taste the beverage based on its appearance and smell, also received a high score, 7.9 ± 1.1. It should be noted that the SCN beverage maintained the initial color (dark pink) and aroma (fruity) of the SCN after the fermentation process. It is expected, thus, that the maintenance of those appealing characteristics also positively affected the taste of the final beverage that was positively evaluated. The lowest attractiveness, 2.8 ± 0.8, was noted for the SS beverage, which is associated mainly with the low score received for its color, i.e., 2.3 ± 0.5. Its aroma, though, was better accepted, resulting, most probably, in the comparatively higher score that it received for its taste (4.9 ± 0.8). In terms of the sweetness, the SS beverage received the lowest score, which can be linked to the low concentration of sugars at the end of the fermentation (see Table 4). On the other hand, the evaluation of the sweetness of the SCN and the NAJ beverages did not correspond to their sugar content, i.e., NAJ beverage was evaluated as sweeter than SCN beverage even though its concentration in sugars was significantly lower. That might be partially attributed to the higher acidity of SCN beverage, and the fact that the commercial SCN that was used as a fermentation substrate is supplemented with citric acid, which creates a sourer taste. In terms of the overall acceptability, the SCN and NAJ beverages were rated more preferable than the SS beverage, which might be due to their higher sweetness and also to the presence of the aromatic compounds of the juices, which are of extreme importance for the determination of the sensory character of beverages [24].

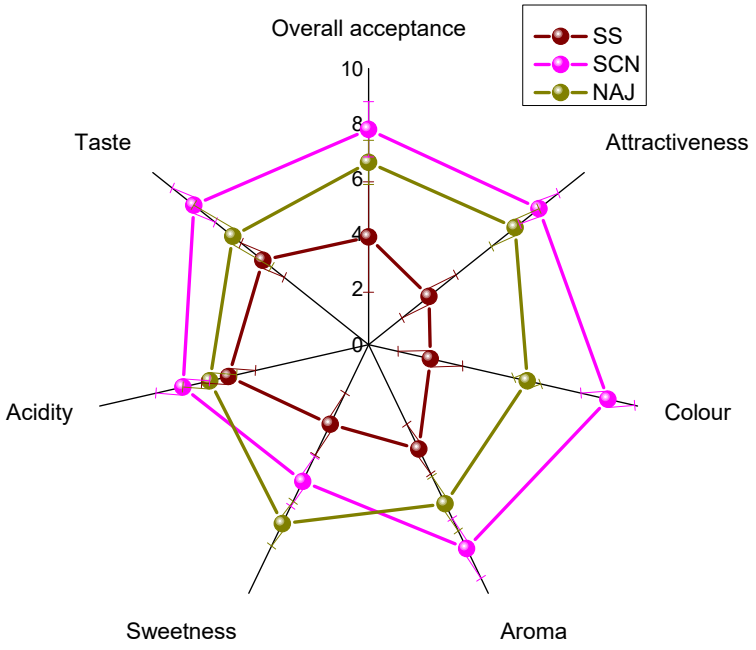

Figure 9. Sensory analysis of the water kefir beverages (fermented sugary substrates). SS, Sucrose solution; SCN, sour cherry nectar, NAJ, natural apple juice.

4. Discussion

The idea of using MKG for the production of water kefir beverages is not completely new. A survey of the internet revealed that such as transition has been proposed by some non-scientific sources [25,26], indicating the possible feasibility of the attempt. The study of Salazar Alzate et al. [27] was the first one to support such an assumption, using a reconstructed consortium derived from MKG for the successful production of a non-dairy beverage from sugarcane concentrate, bearing properties for the prevention of gastrointestinal diseases and strengthening the immune system. A few years later, Zongo et al. [28] reported the successful production of a novel kefir drink from pasteurized palm sap, a sucrose-rich sugar solution produced by palm trees, which is prone to alcoholic fermentation [29]. It that study [29] the fermentation of palm sap with 4% *w*/*v* of either MKG and WKG for the production of beverages was assessed, which were shown to bear similar physicochemical characteristics. Although none of the above studies proceeded with the evaluation of the organoleptic or sensory characteristics of the produced beverages, they provide some evidence that the efficient transition of MKG to ferment non-diary substrates, may indeed be feasible. It should be noted that such a task may not be always successful due to different characteristics, microbial species, and distribution of microbial populations of MKG and WKG [8] and may require careful planning and handling to lead to acceptable beverages. In the current study, the transition of MKG for the production of water kefir-like beverages via the fermentation of sugary, was achieved by the gradual substitution from one substrate to the other and also the multiple repetition of 24 h FCs for each substrate tested, as a fermentation strategy, thus providing time to the microbial consortium to the MKG to adapt to the new conditions.

Although the fermentations were successful for all three sugary substrates (SS, SCN, and NAJ) tested, the increase in the weight of the kefir grains during the successive 24 h FCs, and which is usually noted for either fermentation of milk via MKG [14] or non-diary substrates via WKG [30], was not initially noted. Actually, a decrease in the biomass of the kefir grains was observed throughout the transition process and also during the first cycles of fermentation of the SS. That effect was attributed to the inability of kefiran, the hetero-polysaccharide forming the cauliflower-like structure of the MKG, to be synthesized to the same extent as during milk fermentation. Indeed, kefiran is formed during the polymerization of equal amounts of glucose and galactose derived from the disaccharide lactose, the main sugar of milk by *Lactobacillus kefiranofaciens* [21]). During the experiment of gradual substitution of milk from sucrose as a fermentative substrate, lactose eventually became scarce and totally absent during the subsequent fermentations of sugary substrates and, as such, new kefiran cannot be produced. On the other hand, the water kefir grains, consisting of glucose polysaccharides (dextrans) at about 95%–97%, can indeed be produced by the hydrolysis of sucrose [21]. It is therefore considered possible that during the transition from milk fermentation to sucrose fermentation, a gradual change in the composition of the grains' matrix takes place due to the gradual synthesis of dextran-polysaccharides, which might be responsible for the weight gain observed in the last FC. The following observed increase in the kefir grain mass during the last FCs of SS fermentation and subsequent fermentation of juices may be attributed to the ability of the newly adapted consortium to convert sucrose into the dextran EPS by glucansucrases of proper lactobacilli strains, forming the typical WKG grains [31,32]. The observed decreasing rate of kefir biomass growth during the replacement of SS as a fermentation substrate by SCN, containing relative higher amount of glucose and fructose than sucrose, is also in line with previous studies reporting that the complete substitution of sucrose with glucose and/or fructose resulted in the absence of growth of WKG [30].

The analysis of the beverages resulting from the fermentation of the sugary substrates indicated that there were differentiated from the milk kefir produced from the original culture both in terms of the distribution of the major metabolic products and the dominating microbial populations. In particular, ethanol prevailed as a fermentation product over lactic acid and reached significantly higher concentrations than those detected in milk kefir,

while the concentration of lactic acid was extremely reduced. The high percentages of ethanol in water kefir are in line with the existing literature, reporting values up to 10% on the final volume [8,23] investigated the water kefir production process from unrefined cane sugar and fig extract solution (~8% w/v initial sugars concentration) using 15% (w/v) WKG as inoculum, similar to the findings of the current study, which observed that ethanol dominated as a metabolic product of the fermentation reaching 0.2% w/v, whereas lactic acid concentration was 0.49% w/v and acetate only 0.1% w/v. Moreover, Zongo et al. [28] reported higher ethanol concentration than lactate during the fermentation of palm sap from either MKG or WKG. Specifically in that study [28], final concentrations of 1.66 ± 0.25% w/v ethanol and 0.93 ± 0.02% w/v lactate from MKG and 1.73 ± 0.21% w/v ethanol and 0.52 ± 0.02% w/v lactate from WKG were obtained, with the initial concentration of lactate at the beginning of fermentations being 0.28 ± 0.01 w/v, whereas ethanol was not detected.

Regarding the distribution of microbial populations in the fermentation broths, the predominance of LABs over yeasts was observed for milk fermentation at all three fermentation temperatures, even at the beginning of fermentations, i.e., when the kefir grains were added and mixed with the milk, which is in accordance with other studies [2,33,34]. The subsequent observed domination of yeasts over LAB in the sugary based beverages, is not though in full agreement with the literature on water kefir, since LAB are reported as the dominant type of microorganisms in both WKG and water kefir with yeast reaching 6.3 ± 0.2 log CFU/mL and 7.4 ± 0.1 log CFU/mL in water kefir liquor and WKG, respectively, and LAB reaching 6.9 ± 0.1 log CFU/mL and 8.2 ± 0.1 log CFU/mL in water kefir liquor and WKG, respectively [23]. The opposing results of the current study may be attributed to the unconventional approach for producing water kefir drinks from adapted MKG and also to the fact that the consortium was subjected to a more acidic pH compared to that of milk fermentation, which is expected to favor the growth of yeasts over LAB [35,36]. Indeed, during SS fermentation, a faster and more severe pH drop is observed, whereas during both SCN and NAJ fermentations, the pH is very acidic throughout the 24 h fermentation

During the sensory evaluation of the three sugary based beverages, it became evident that the ones produced from the fermentation juices, SCN and NAJ, received a significantly higher score in terms of their overall acceptance compared to the SS-based beverage. This was attributed partly to the more attractive appearance and smell, as confirmed by the higher scores in terms of color and attractiveness, but also to their more intense and appealing aromas and taste due to the presence of aromatic substances that are already contained in these juices before the fermentation [37–39]. The highly rated sensory acceptance of fruit-based water kefir was also reported by other researchers. In the study of Randazzo et al., [38] an apple juice-based water kefir received the highest score, 5.2/9 (corresponding to 5.8/10) compared to other fruit kefir beverages (grape, kiwi, pomegranate, and quince). Highly rated overall acceptance was also reported for fruit-flavored milk kefir, with one of the highest scoped 53.1/60 (corresponding to 8.85/10) being noted for fresh berry-flavored kefir [40].

5. Conclusions

The present study has demonstrated the feasibility of producing acceptable-for-consumption beverages from sugar solution and fruit juices, which bear similar characteristics to water kefir, using milk kefir grains for the fermentation. The successful result was attributed to the fermentation strategy followed, i.e., the initial gradual substitution of milk from sucrose as a fermentation substrate, and also the performance of multiple 24 h FCs with each different substrate before the evaluation of each final product. This strategy was considered to provide the opportunity and time for the microbial population of the kefir grains to acclimate to the new conditions imposed, and lead to the successful production of a new non-kefiran-based polymeric matrix of kefir grains from the sugary substrates. Although the final drinks do not have exactly the same abiotic and biotic composition with water kefir, i.e., exhibiting relatively lower concentration of lactic acid

and lower LAB counts, the overall acceptance of the sugary beverages during the sensory evaluation confirms the success of the fermentation process proposed, even though the extremely high score of the fruit-based beverages was attributed to a large extent to the original appealing aromas of the juices before fermentation.

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References

1. Şanlier, N.; Gökcen, B.B.; Sezgin, A.C. Health benefits of fermented foods. *Crit. Rev. Food Sci. Nutr.* **2019**, *59*, 506–527. [CrossRef] [PubMed]
2. Plessas, S.; Nouska, C.; Mantzourani, I.; Kourkoutas, Y.; Alexopoulos, A.; Bezirtzoglou, E. Microbiological Exploration of Different Types of Kefir Grains. *Fermentation* **2016**, *3*, 1. [CrossRef]
3. Hertzler, S.R.; Clancy, S.M. Kefir improves lactose digestion and tolerance in adults with lactose maldigestion. *J. Am. Diet. Assoc.* **2003**, *103*, 582–587. [CrossRef] [PubMed]
4. Rosa, D.D.; Dias, M.M.S.; Grześkowiak, Ł.M.; Reis, S.A.; Conceição, L.L.; Maria do Carmo, G.P. Milk kefir: Nutritional, microbiological and health benefits. *Nutr. Res. Rev.* **2017**, *30*, 82–96. [CrossRef] [PubMed]
5. Lopitz-Otsoa, F.; Rementeria, A.; Elguezabal, N.; Garaizar, J. Kefir: A symbiotic yeasts-bacteria community with alleged healthy capabilities. *Rev. Iberoam. Micol.* **2006**, *23*, 67–74. [CrossRef]
6. Assadi, M.; Pourahmad, R.; Moazami, N. Use of isolated kefir starter cultures in kefir production. *World J. Microbiol. Biotechnol.* **2000**, *16*, 541–543. [CrossRef]
7. Açik, M.; Çakiroğlu, F.P.; Altan, M.; BAYBO, T. Alternative source of probiotics for lactose intolerance and vegan individuals: Sugary kefir. *Food Sci. Technol.* **2020**, *40*, 523–531. [CrossRef]
8. Fiorda, F.A.; Pereira, G.; Soccol, V.T.; Rakshit, S.K.; Pagnoncelli, M.G.B.; Vandenberghe, L.; Soccol, C.R. Microbiological, biochemical, and functional aspects of sugary kefir fermentation—A review. *Food Microbiol.* **2017**, *66*, 86–95. [CrossRef] [PubMed]
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. Lynch, K.M.; Wilkinson, S.; Daenen, L.; Arendt, E.K. An update on water kefir: Microbiology, composition and production. *Int. J. Food Microbiol.* **2021**, *345*, 109128. [CrossRef]
11. Luning, P.A.; Vries, R.V.D.V.D.; Yuksel, D.; Ebbenhorst-Seller, T.; Wichers, H.J.; Roozen, J.P. Combined Instrumental and Sensory Evaluation of Flavor of Fresh Bell Peppers (*Capsicum annuum*) Harvested at Three Maturation Stages. *J. Agric. Food Chem.* **1994**, *42*, 2855–2861. [CrossRef]
12. Ntaikou, I.; Siankevich, S.; Lyberatos, G. Effect of thermo-chemical pretreatment on the saccharification and enzymatic digestibility of olive mill stones and their bioconversion towards alcohols. *Environ. Sci. Pollut. Res.* **2020**, *28*, 24570–24579. [CrossRef]
13. Dounavis, A.S.; Ntaikou, I.; Kamilari, M.; Lyberatos, G. Production of Bio-Based Hydrogen Enriched Methane from Waste Glycerol in a Two Stage Continuous System. *Waste Biomass Valorization* **2016**, *7*, 677–689. [CrossRef]
14. Apar, D.K.; Demirhan, E.; Özel, B.; Özbek, B. Kefir Grain Biomass Production: Influence of Different Culturing Conditions and Examination of Growth Kinetic Models. *J. Food Process Eng.* **2016**, *40*, e12332. [CrossRef]
15. Ribeiro, A.C.; Lemos, T.; Lopes, R.P.; Mota, M.J.; Inácio, R.S.; Gomes, A.M.P.; Sousa, S.; Delgadillo, I.; Saraiva, J.A. The Combined Effect of Pressure and Temperature on Kefir Production—A Case Study of Food Fermentation in Unconventional Conditions. *Foods* **2020**, *9*, 1133. [CrossRef] [PubMed]
16. Gul, O.; Mortas, M.; Atalar, I.; Dervisoglu, M.; Kahyaoglu, T. Manufacture and characterization of kefir made from cow and buffalo milk, using kefir grain and starter culture. *J. Dairy Sci.* **2015**, *98*, 1517–1525. [CrossRef] [PubMed]
17. Londero, A.; Hamet, M.F.; De Antoni, G.L.; Garrote, G.L.; Abraham, A.G. Kefir grains as a starter for whey fermentation at different temperatures: Chemical and microbiological characterisation. *J. Dairy Res.* **2012**, *79*, 262–271. [CrossRef]

18. Syrokou, M.K.; Papadelli, M.; Ntaikou, I.; Paramithiotis, S.; Drosinos, E.H. Sugary Kefir: Microbial Identification and Biotechnological Properties. *Beverages* **2019**, *5*, 61. [CrossRef]
19. Bourrie, B.C.T.; Willing, B.P.; Cotter, P.D. The Microbiota and Health Promoting Characteristics of the Fermented Beverage Kefir. *Front. Microbiol.* **2016**, *7*, 647. [CrossRef]
20. Rezac, S.; Kok, C.R.; Heermann, M.; Hutkins, R. Fermented Foods as a Dietary Source of Live Organisms. *Front. Microbiol.* **2018**, *9*, 1785. [CrossRef]
21. 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]
22. Gulitz, A.; Stadie, J.; Wenning, M.; Ehrmann, M.A.; Vogel, R.F. The microbial diversity of water kefir. *Int. J. Food Microbiol.* **2011**, *151*, 284–288. [CrossRef] [PubMed]
23. Laureys, D.; De Vuyst, L. Microbial Species Diversity, Community Dynamics, and Metabolite Kinetics of Water Kefir Fermentation. *Appl. Environ. Microbiol.* **2014**, *80*, 2564–2572. [CrossRef] [PubMed]
24. Agarbati, A.; Ciani, M.; Canonico, L.; Galli, E.; Comitini, F. Exploitation of Yeasts with Probiotic Traits for Kefir Production: Effectiveness of the Microbial Consortium. *Fermentation* **2021**, *8*, 9. [CrossRef]
25. How to Make Water Kefir with Milk Kefir Grains. Available online: https://northernhomestead.com/how-to-make-water-kefir-with-milk-kefir-grains (accessed on 10 December 2021).
26. Converting Milk Kefir Grains into Water Kefir Grains. Available online: https://www.thecheesemaker.com/converting-milk-kefir-grains-into-water-kefir-grains (accessed on 10 December 2021).
27. Salazar Alzate, B.C.; Rodríguez, M.C.; Campuzano, O.M. Identification of some kefir microorganisms and optimization of their production in sugarcane juice. *Rev. Fac. Nac. Agron. Medellín* **2016**, *69*, 7935–7943. [CrossRef]
28. Zongo, O.; Cruvellier, N.; Leray, F.; Bideaux, C.; Lesage, J.; Zongo, C.; Traoré, Y.; Savadogo, A.; Guillouet, S. Physicochemical composition and fermentation kinetics of a novel Palm Sap-based Kefir Beverage from the fermentation of *Borassus aethiopum* Mart. fresh sap with kefir grains and ferments. *Sci. Afr.* **2020**, *10*, e00631. [CrossRef]
29. Ben Atitallah, I.; Ntaikou, I.; Antonopoulou, G.; Alexandropoulou, M.; Brysch-Herzberg, M.; Nasri, M.; Lyberatos, G.; Mechichi, T. Evaluation of the non-conventional yeast strain *Wickerhamomyces anomalus* (*Pichia anomala*) X19 for enhanced bioethanol production using date palm sap as renewable feedstock. *Renew. Energy* **2020**, *154*, 71–81. [CrossRef]
30. Laureys, D.; Leroy, F.; Hauffman, T.; Raes, M.; Aerts, M.; Vandamme, P.; De Vuyst, L. The Type and Concentration of Inoculum and Substrate as Well as the Presence of Oxygen Impact the Water Kefir Fermentation Process. *Front. Microbiol.* **2021**, *12*. [CrossRef]
31. Pidoux, M. The microbial flora of sugary kefir grain (the gingerbeer plant): Biosynthesis of the grain fromLactobacillus hilgardii producing a polysaccharide gel. *World J. Microbiol. Biotechnol.* **1989**, *5*, 223–238. [CrossRef]
32. 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]
33. Chen, Z.; Liu, T.; Ye, T.; Yang, X.; Xue, Y.; Shen, Y.; Zhang, Q.; Zheng, X. Effect of lactic acid bacteria and yeasts on the structure and fermentation properties of Tibetan kefir grains. *Int. Dairy J.* **2020**, *114*, 104943. [CrossRef]
34. Georgalaki, M.; Zoumpopoulou, G.; Anastasiou, R.; Kazou, M.; Tsakalidou, E. *Lactobacillus kefiranofaciens*: From Isolation and Taxonomy to Probiotic Properties and Applications. *Microorganisms* **2021**, *9*, 2158. [CrossRef]
35. Adamberg, K. The effect of temperature and pH on the growth of lactic acid bacteria: A pH-auxostat study. *Int. J. Food Microbiol.* **2003**, *85*, 171–183. [CrossRef]
36. Miranda-Castilleja, D.E.; Aldrete-Tapia, J.A.; Arvizu-Medrano, S.M.; Hernades-Iturriaga, M.; Soto-Muñoz, L.; Martinez-Peniche, R.A. Growth Kinetics for the Selection of Yeast Strains for Fermented Beverages. In *Yeast—Industrial Applications Conversion*; InTech: London, UK, 2017. [CrossRef]
37. Mehinagic, E.; Royer, G.; Symoneaux, R.; Jourjon, A.F.; Prost, C. Characterization of Odor-Active Volatiles in Apples: Influence of Cultivars and Maturity Stage. *J. Agric. Food Chem.* **2006**, *54*, 2678–2687. [CrossRef] [PubMed]
38. Randazzo, W.; Corona, O.; Guarcello, R.; Francesca, N.; Germana, 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]
39. Nowicka, P.; Wojdyło, A. Bioactive compounds and sensory attributes of sour cherry puree sweetened with natural sweeteners. *Int. J. Food Sci. Technol.* **2014**, *50*, 585–591. [CrossRef]
40. Yilmaz, L.; Yilsay, T.; Bayizit, A.A. The sensory characteristics of berry-flavoured kefir. *Czech J. Food Sci.* **2011**, *24*, 26–32. [CrossRef]