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Preliminary Evaluation of the Use of Thermally-Dried Immobilized Kefir Cells in Low Alcohol Winemaking

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Abstract: Low alcohol wines (≤10.5% vol) are novel products that have gradually been gaining the consumers' and market's interest over the last decade. Taking into account the technological properties of immobilized cell systems alongside with the commercial need for dry cultures, the aim of the present study was to assess the suitability of thermally-dried immobilized kefir cells on DCM, apples pieces, and grape skins in low alcohol wine production. Storage of thermally-dried kefir culture in various temperatures (−18, 5, and 20 °C) resulted in high viability rates for immobilized cells (up to 93% for yeasts/molds immobilized on grape skins and stored at −18 °C for 6 months). Fermentation activity was maintained after storage in all cases, while high operational stability was confirmed in repeated batch fermentations for a period of 6 months. Principal Component Analysis (PCA) revealed that the fermentation temperature rather than the state of kefir culture affected significantly volatiles detected by Head Space Solid-Phase Microextraction Gas Chromatography–Mass Spectrometry analysis. Notably, all new products were of high quality and approved by the sensory panel.

Keywords: low alcohol wine; kefir culture; immobilization; thermal drying; volatiles

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1. Introduction

Low alcohol wines (≤10.5% vol) are novel products that for multiple reasons (modern lifestyle, social reasons, economic motives, etc.) have gradually been gaining the consumers' and market's interest over the last decade [1]. Based on their alcoholic strength, these products may be categorized as dealcoholized (<0.5% *v*/*v*), low alcohol (0.5–1.2% *v*/*v*), reduced alcohol (up to 6.5% *v*/*v*), or lower alcohol wines (up to 10.5% *v*/*v*), although this distinction may vary among different regions [2,3]. Low alcohol wine manufacturing can be accomplished by relying on pre-fermentation vineyard strategies (application of growth regulators, decrease of leaf-area-to-fruit-mass ratio, harvest date selection, etc.), which, however, require balanced actions, and the outcome is hard to predict [4]. On the other hand, application of post-fermentation physicochemical treatments (membrane systems, vacuum or osmotic distillation, spinning cone technology, and supercritical carbon dioxide extraction) is more efficient, but also known to affect the wine characteristics negatively [1,2]. Color loss and diminished aroma or taste characteristics have been noticed between various discrepancies in the final products, especially when ethanol removal >2% vol is sought [2,4].

In the last decades, industrial winemaking has been relying heavily on the use of starter yeast cultures, thus avoiding stuck or sluggish fermentations, and ensuring better production control and repeatability [5,6]. Regardless of the practical advantages, the continuous use of a limited number of commercial yeast strains could lead to diminished diversity, loss of typicality and flattening of wine sensory characteristics among the final products [7,8]. Modern approaches, however, follow the consumers' preferences for wine with a unique character and thus many professionals have employed the use of wild

Saccharomyces or even non-*Saccharomyces* strains [9–11], aiming to link their products with a specific "terroir". "Terroir" was initially associated only with grape variety, and regional ground and climate conditions, but recent works have highlighted the microbiological aspect of this term, emphasizing the contribution of microorganisms (present in fermentation) to the wine's final characteristics [6,12,13].

After alcoholic fermentation completion, wines traditionally produced are usually subjected to malolactic (ML) fermentation, aiming to reduce acidity, microbial stabilization, modification of sensory attributes, etc. [14]. Nevertheless, fermentation failures or delays are not uncommon, and thus the use of mixed cultures (yeasts and malolactic bacteria) for simultaneous alcoholic and ML fermentation is suggested [15]. In this vein, kefir culture (a mixture of yeasts, lactic acid bacteria, and occasionally acetic acid bacteria) originating from the traditional drink "kefir" (produced in the Caucasus regions of Russia) [16], has been previously immobilized on natural supports (apple pieces, delignified cellulosic material (DCM), and grape skins) and successfully used in low alcohol wine fermentations at a wide temperature range [14,17,18].

Worldwide, freeze-dried cultures remain steadily preferred to wet cultures, due to advantages associated with protection against microbial contamination, longer preservation times, easy to handle products during storage, etc. [19]. However, taking into account the lower equipment cost and energy demand, as well as the lack of expensive cryoprotectants and zero risk of remaining cryoprotectant residues in the final product, the application and commercialization of simpler methods, like thermal drying, is necessary [20,21].

Immobilization technology is also suggested in food applications, as it results in the maintenance of cell viability during processing and storage [19], and is linked with high operational stability, improvement of fermentation productivity, cell control, application of continuous system configurations, enhancement of cell viability, ability for cell recycling, improved final product quality, etc. [22,23].

In the present study, the suitability of a thermally-dried immobilized mixed culture (kefir culture) in low alcohol wine production was of interest. Data indicating the survival of the thermally-dried immobilized kefir cells during storage and their efficiency in simultaneous alcoholic and ML fermentations for low alcohol winemaking are presented.

2. Materials and Methods

2.1. Immobilization of Kefir Culture and Fermentation Medium

Kefir culture was isolated from a traditional kefir drink [17]. Wet kefir culture was initially grown and stored on synthetic medium (4% w/v glucose (Fluka, Buchs, Switzerland), 0.5% w/v $MgSO_4 7H_2O$ (Merck, Darmstadt, Germany), 0.1% w/v $(NH_4)_2SO_4$ (Merck), 0.1% w/v KH_2PO_4 (Fluka) and 0.4% w/v yeast extract (Fluka)) [24]. Immobilization on natural supports (DCM, apple pieces, grape skins) was performed, as recently described [14].

Concentrated grape must of Muscat Hamburg variety (Tyrnavos Cooperative Winery and Distillery, Tyrnavos, Greece) was diluted to a final $\sim 10 \pm 0.5$ °Be density ($\sim 170 \pm 8.5$ g/L sugars) and used in fermentations, as recently described [14]. Prior to use, the diluted must was sterilized at 121 °C and 1.1 atm for 15 min.

2.2. Thermal Drying and Storage of Kefir Cells

Free and immobilized kefir cells were thermally-dried overnight in an oven at 38–40 °C. Thermally-dried kefir cells (free and immobilized) were subsequently stored at ambient (20 °C), refrigerator (5 °C), and freezing environment (−18 °C) for a period up to 6 months. The cell viability was determined at time intervals of 1, 3, and 6 months.

2.3. Determination of Cell Viability of Thermally-Dried Cells

After storage, thermally-dried cells were rehydrated with sterilized water [25] and yeasts/molds, lactobacilli and lactococci counts were determined, as described previously [24]. The % survival rate was calculated as logcfu/g after thermal drying, divided by logcfu/g before thermal drying, and multiplied by 100 [17].

2.4. Fermentations

Fermentation stability of kefir cells, after thermal drying, was investigated with a series of repeated batch fermentations (250 mL each) of grape must in batch bioreactors of 0.5 or 1 L using thermally-dried free (10 g/L) or immobilized kefir culture on natural supports, as recently described [14]. After rehydration with sterilized water, three repeated batch fermentations were initially carried out at 30 °C, three repeated batch fermentations were subsequently performed at 20 °C, and finally two repeated batch fermentations were performed at 5 °C in all cases. After the end of each fermentation, both free and immobilized cells were washed with grape must (~10 ± 0.5 °Be) and reused for the next batch fermentation. Except for temperature control, no other factor (agitation, aeration, pH, etc.) was regulated on the systems.

Fermentation efficiency of (rehydrated) thermally-dried cells after storage (at 20, 5, and −18 °C) was assessed in single batch fermentations (250 mL) of concentrated grape must at 30 °C, as previously described [14].

Samples collected at the end of all fermentations were subjected to chemical analyses.

2.5. Chemical Analyses

The pH, total acidity, volatile acidity, and water activity (a_w) were determined as previously described [17].

Ethanol, glycerol, residual sugars, and organic acid content were determined by HPLC analysis (Shimadzu chromatography system (Shimadzu Corp., Duisburg, Germany)). Fermentation parameters were calculated as previously described [24].

Gas Chromatography (MASTER GC Fast Gas Chromatograph (DANI Instruments S.p.A., Cologno Monzese, Italy)) [24] was used for the determination of major volatiles content and HS-SPME GC/MS analysis (6890N GC, 5973NetworkedMS MSD (Agilent Technologies, Santa Clara, CA, USA)) [14] was used for the determination of minor volatiles content.

2.6. Quality and Kinetic Parameters Determination

Residual sugars, ethanol, glycerol, and organic acids concentrations were determined using standard curves prepared by standard solutions ($R^2 \geq 0.99$).

Major volatile compounds concentrations were determined using standard curves prepared by standard solutions ($R^2 \geq 0.99$).

Minor volatile compounds concentrations were determined by dividing the peak areas of the compounds of interest with the peak area of 4-methyl-2-pentanol, which was used as an internal standard, and this ratio was multiplied by its initial concentration (expressed as mg/L). The peak areas were measured from the full scan chromatograph using the total ion current (TIC).

The fermentation parameters were calculated as follows:

Ethanol productivity: g of ethanol produced per day per liter of liquid volume of bioreactor.
Conversion: (Initial sugar conc.—Residual sugar conc.)/Initial sugar conc. * 100.
Ethanol production yield: g of ethanol produced per g of sugars utilized.
Malic acid conversion: (Initial malic acid conc.—Residual malic acid conc.)/Initial malic acid conc. * 100.

2.7. Preliminary Sensory Analysis

Wines produced were assessed for their quality characteristics (aroma, taste, and overall quality), as previously reported [18].

2.8. Statistical Analysis

All data were analyzed for statistical significance by two-way analysis of variance. The nature of the kefir culture (free or immobilized), and the fermentation temperature or the storage temperature were considered as factors. The Bonferroni correction was used to identify significant differences ($p < 0.05$) among the results. Statistical significance at

$p < 0.05$, coefficients and ANOVA tables were computed by Statistica v.12.0 (Stat Soft Inc., Tulsa, OK, USA).

Principal Component Analysis was computed by XLSTAT 2015.1 (Addinsoft, Paris, France) [24].

3. Results and Discussion

3.1. Cell Viability of Thermally-Dried Immobilized Kefir Cells

Kefir cells were initially immobilized on natural supports and thermal drying was applied, resulting in reduced populations (Table 1) compared to the wet cultures [14]. Interestingly, viable counts of thermally-dried immobilized cells were significantly higher ($p < 0.05$) compared to those of thermally-dried free cells, as the protective effect of immobilization to cell integrity is well established [20,25,26]. After inoculation (timepoint T0), yeasts/molds ranged near (or over) 7 logcfu/mL of must in fermentations with thermally-dried immobilized cells, in all cases, populations over the proposed counts recommended for starter cultures' inoculations [27], whereas lactobacilli and lactococci counts ranged 4–5 logcfu/mL of grape must, respectively. Despite the counts of thermally-dried lactobacilli and lactococci being seemingly low, concentrations of 5 logcfu/mL or even lower have previously been shown to be sufficient for malolactic fermentation to occur [28]. After all, cell rehydration and effective biomass recovery of thermally-dried cells, when found in suitable medium, has previously been reported [26,29].

Table 1. Cell counts of kefir cells immobilized on natural supports after thermal drying.

	Free Cells	Immobilized Cells on Apple Pieces	Immobilized Cells on DCM	Immobilized Cells on Grape Skins
Yeasts/Molds	5.55 ± 0.05	6.95 ± 0.09	7.07 ± 0.05	7.35 ± 0.05
Lactobacilli	3.12 ± 0.07	4.25 ± 0.06	5.36 ± 0.05	4.60 ± 0.05
Lactococci	3.02 ± 0.21	4.15 ± 0.15	5.49 ± 0.05	4.65 ± 0.08

Regardless of the storage duration (1, 3, or 6 months), all kefir populations (yeasts/molds, lactobacilli, lactococci) were affected significantly ($p < 0.05$) by both the state of the cells (free or immobilized) and the storage temperature (20, 5, or −18 °C), while strong interactions ($p < 0.05$) were noted. Notably, significantly higher counts of thermally-dried immobilized cells were obtained ($p < 0.05$) compared to those of thermally-dried free cells, at all storage intervals (Figure 1) [25]. Specifically, after 6 months of storage, thermally-dried immobilized yeasts/molds on grape skins retained the highest ($p < 0.05$) viability scores (up to 93% at −18 °C) among all supports, while the highest ($p < 0.05$) lactobacilli and lactococci survival rates were detected on DCM, also stored at −18 °C. As expected, low storage temperatures (5 and −18 °C) resulted in significantly higher ($p < 0.05$) survival rates compared to ambient temperatures in all cases [25,29]. However, a drop in microbial counts was detected as the storage duration increased, but not significant in all cases.

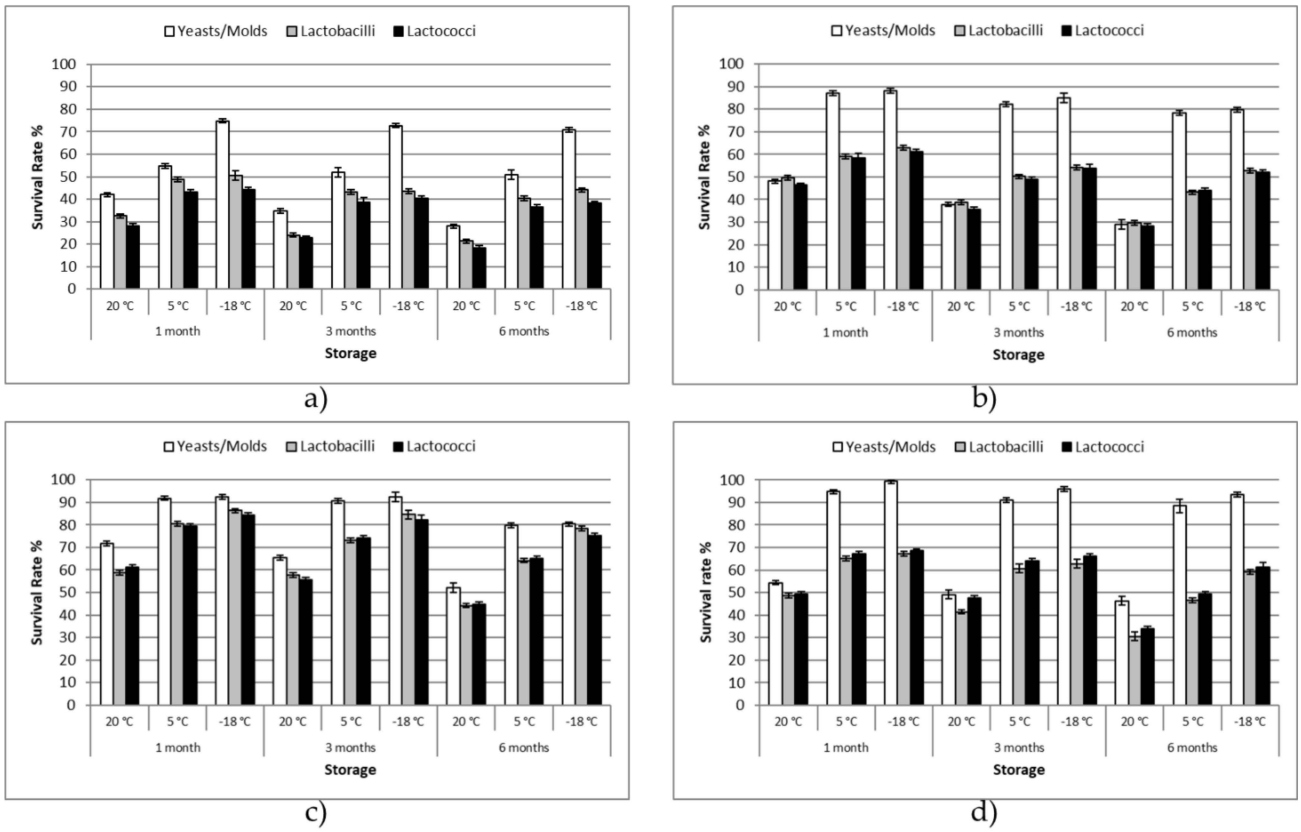

Figure 1. Survival rate (%) of thermally-dried free and immobilized kefir cells. (**a**) Free kefir culture, (**b**) immobilized kefir cells on apple pieces, (**c**) immobilized kefir cells on delignified cellulosic material (DCM), (**d**) immobilized kefir cells on grape skins.

3.2. Fermentations

3.2.1. Fermentation Efficiency

Thermally-dried free or immobilized kefir cells were initially evaluated regarding their fermentation efficiency in simultaneous alcoholic and ML repeated batch fermentations at 30, 20, and 5 °C for a period greater than 6 months. Although low alcohol winemaking using both wet and freeze-dried immobilized kefir culture on DCM, apple pieces, and grape skins was recently proposed [17], to the best of the authors' knowledge this is the first time the use of thermally-dried immobilized kefir cells is investigated. Fermentation kinetic data and other important parameters are presented in Table 2.

Table 2. Kinetic parameters and organic acid profile of low alcohol wines fermented by repeated batch fermentations at various temperatures (5–30 °C) using thermally-dried free or immobilized kefir culture.

Nature of Thermally-Dried Kefir Culture	Fermentation Temperature (°C)	Repeated Batches	Fermentation Time (h)	Ethanol Concentration (g/L)	Glycerol (g/L)	Residual Sugars (g/L)	Ethanol Productivity (g/L d)	Ethanol Production Yield (g/g)	Conversion (%)	Malic Acid * (g/L)	Lactic Acid (g/L)	Malic Acid Conversion (%)	Acetic Acid (g/L)	Citric Acid (g/L)	Total Acidity (g Tartaric/L)	Volatile Acidity (g Acetic/L)	pH
Free cells	30	1–3	168–216	8.6–9.2	3.9–4.5	1.0–2.2	8.0–10.1	0.40–0.43	98.7–99.2	1.3–1.5	0.1–0.2	37.2–41.3	0.5–0.9	0.6–0.9	3.4–3.6	0.43–0.51	4.1
	20	4–6	312–360	8.6–10.0	4.4–5.9	0.7–3.7	4.5–6.2	0.41–0.47	97.8–99.6	1.4–1.7	0.1	30.3–38.2	0.5–0.9	0.3–0.9	3.5–3.7	0.42–0.46	4.1
	5	7–8	1404–1476	2.9–3.1	2.9–3.1	68.6–73.2	0.4	0.23–0.25	56.9–59.6	1.4–1.5	0.1	33.8–39.1	0.4–0.5	0.9–1.0	3.6	0.39–0.40	4.0
Immobilized cells on apple pieces	30	1–3	120–144	7.3–8.4	4.4–4.8	0.9–1.1	9.7–13.3	0.34–0.39	99.3–99.5	1.7–1.9	0.1–0.2	17.7–25.0	0.5–1.1	0.5–1.0	5.1–5.2	0.44–0.47	3.7–3.8
	20	4–6	276–312	8.7–9.3	5.6–6.8	1.1–1.3	5.5–6.0	0.41–0.44	99.2–99.4	1.9–2.1	0.3–0.4	13.0–17.4	0.4–0.7	0.6–0.9	5.1–5.2	0.41–0.48	3.7–3.8
	5	7–8	1536–1584	6.7–7.1	5.0–5.3	21.3–25.1	0.8–0.9	0.37–0.38	85.2–87.4	1.6–1.8	0.2	23.7–30.4	0.5–0.6	0.8–0.9	5.1	0.38	3.7–3.8
Immobilized cells on DCM	30	1–3	144–168	7.2–7.9	4.3–5.2	0.9–1.1	8.1–10.4	0.34–0.37	99.3–99.5	1.3–1.8	0.2	21.1–41.4	0.6–1.4	0.5–0.6	5.3–5.4	0.33–0.41	3.8–3.9
	20	4–6	264–288	8.6–10.0	4.4–7.2	1.4–3.7	5.6–7.0	0.41–0.47	97.8–99.2	1.4–1.9	0.1–0.3	20.8–37.0	0.5–0.8	0.4–1.0	5.3–5.4	0.35–0.40	3.8
	5	7–8	1500–1560	4.4–5.0	3.6–4.0	55.0–62.2	0.6	0.32–0.34	63.4–67.6	1.8–1.9	0.1	16.2–21.7	0.5–0.7	1.0–1.1	5.3	0.32–0.35	3.8
Immobilized cells on grape skins	30	1–3	120–168	6.4–7.3	4.7–5.6	0.9–1.2	8.2–10.7	0.30–0.34	99.3–99.5	1.5–1.9	0.2	18.8–36.1	0.5–1.4	0.6–0.8	5.1	0.48–0.54	3.9
	20	4–6	288–312	8.7–9.1	6.2–6.5	1.3–1.5	5.3–6.0	0.41–0.43	99.1–99.2	1.7–2.0	0.2–0.3	13.0–26.1	0.6–0.8	0.5–0.7	5.1	0.50–0.57	3.9
	5	7–8	1392–1440	6.5–7.1	5.1–5.3	25.8–30.6	0.9–1.0	0.37–0.39	82.0–84.8	1.4–1.6	0.2	30.4–39.3	0.7–0.8	0.5–0.6	5.1–5.2	0.47–0.48	3.8

* Initial grape must malic acid content: 2.4 ± 0.1 g/L.

Ethanol content, ethanol productivity, ethanol production yield, and acetic acid concentration were significantly ($p < 0.05$) affected only by the fermentation temperature. On the other hand, total acidity values were significantly ($p < 0.05$) affected by the nature of the culture (free or immobilized cells). Fermentation time, residual sugars content and sugar conversion, malic acid concentration and malic acid conversion, lactic acid and glycerol concentration, volatile acidity and pH were affected significantly ($p < 0.05$) by both factors, while strong interactions ($p < 0.05$) were noted. No significant differences were observed in citric acid content.

As expected, significantly ($p < 0.05$) higher fermentation times and significantly ($p < 0.05$) lower ethanol productivity values were recorded as the fermentation temperature was lowered, in all cases [14,20,30–34]. Ethanol concentration ranged from 4.4 to 10.0% (*v/v*) in fermentations performed with immobilized kefir cells, depending on fermentation temperature, as previously reported [17]. Higher ethanol productivities (up to 15.3 g/L d) were noted in fermentations with thermally-dried immobilized cells at all fermentation temperatures in comparison to free cells [35], although not significantly in all cases, and in levels greater than usually noticed in traditional practice or similar to industrial fermentations [35,36]. Notably, high ethanol yield values (up to 0.47 g/g, accounting for over 90% of the total theoretical yield) that could be of high interest for the wine-making industry [37] were noted at 20 °C for both thermally-dried free and immobilized cells. Lower ethanol yield values were recorded at the first batches (30 °C), in all cases, but similar to yields recently associated with parallel alcohol and yeast biomass production (known as the "Crabtree effect") in grape must fermentation [38]. As stated above, thermal drying may result in diminished cell populations, mainly due to cell membrane damage and oxidative stress (caused by free radical formation) [39], thus the recovery of cell biomass at the initial stages of fermentations with thermally-dried cells is not uncommon [26]. However, the continuous improvement of fermentation kinetics as the repeated batches proceeded, indicated adaptation of the thermally-dried cells [25], an attribute that has also been noticed when other drying methods have been applied [40–42]. On the other hand, at 5 °C, ethanol yield values exhibited a substantial drop (although not significant in the case of immobilized cells), which may be attributed to a variety of causes like greater yeast susceptibility to ethanol toxicity in low temperatures [43], oxidative stress [44], changes to yeast metabolism due to cold stress [45,46], or even cell exhaustion after an increased number of repeated batches [47]. As a result, significantly lower sugar conversion values ($p < 0.05$) and significantly ($p < 0.05$) higher residual sugars were documented at 5 °C, in all cases. Nevertheless, residual sugars abundance could be exploited for novel low alcohol wine development with a distinct semi-sweet or sweet (*liquoreux*) character, as recently proposed [18].

Glycerol, known for smoothing the taste and contributing to the wine "sweetness", ranged from 2.9 to 7.2 g/L, depending on the fermentation temperature, but remained well within the values usually found in table wines [48].

Mediocre malic acid conversion values (up to 41.4%) were recorded in fermentations with thermally-dried immobilized kefir culture in all cases, but were still acceptable by the industrial sector [49]. Similar malic degradation values were previously reported in winemaking and low alcohol winemaking using immobilized kefir cells [14,17,50–52].

Acetic acid concentrations up to 1.4 g/L were detected in low alcohol wines produced by thermally-dried immobilized kefir cells at 30 °C (at the first batch only), but were significantly ($p < 0.05$) reduced as batch fermentations proceeded and never exceeded 1.0 g/L. Again, this was in accordance with previously published results on wine fermentations with wet free or immobilized kefir cells [14].

Increased total acidity was observed in low alcohol wines produced with thermally-dried immobilized kefir cells compared to free cells, but in any case, remained well within normal levels for wines. Volatile acidity and pH values also ranged in typical levels for wines, in all cases [31,32].

3.2.2. Fermentation Stability

Thermally-dried free or immobilized kefir cells were further evaluated regarding their fermentation efficiency in simultaneous alcoholic and ML single batch fermentations at 30 °C, after storage at various temperatures (−18, 5, and 20 °C) for a period of 1, 3, and 6 months, respectively (Table 3). According to the results, fermentation time, ethanol content, ethanol production yield, glycerol and citric acid concentrations, and total volatile values were significantly ($p < 0.05$) affected only by the nature of thermally-dried kefir cells. Malic acid concentration and malic acid conversion, ethanol productivity values, residual sugars concentrations and sugar conversion values were also significantly ($p < 0.05$) affected by the storage temperature, while strong interactions ($p < 0.05$) were noted in some cases. On the contrary, no significant differences were observed in pH and volatile acidity values, and in acetic acid and lactic acid content.

Fermentation times were significantly ($p < 0.05$) increased after storage, for all thermally-dried kefir cells [53], most likely due to decrease in cell viability or inactivation of yeasts [25], but were still significantly ($p < 0.05$) lower when immobilized cells were used. As a result, daily ethanol productivities were reduced during storage, but not significantly in all cases, and remained several folds higher than those encountered in the traditional practice [35,54]. Interestingly, ethanol concentrations up to 10.1% (v/v) were recorded, while high sugar conversion values and low residual sugars concentrations were documented, in all cases. Adequate ethanol yield values (up to 0.47 g/g) were recorded for both thermally-dried free and immobilized kefir cells after 1 month of storage [37], but increased storage duration (regardless the conditions) resulted in yield losses, as previously reported [55]. Consequently, ethanol concentrations were reduced (although not significantly) compared to those derived after 1 month of storage, but still remained in ranges suitable for low alcohol winemaking [2]. However, sugar conversion ~99% was documented and residual sugars < 2.0 g/L were detected in all cases, indicating high rates of biomass recovery [39]. The use of aerization would potentially enable faster growth of kefir biomass (by employing sugar respiration) prior to alcoholic fermentation, but adverse effects may occur (e.g., excessive production of acetic acid and high volatile acidity), that are unacceptable in wine [56]. Moreover, lack of agitation or aerization control results in a decrease of the operational costs [18].

Malic acid conversion values (up to 34.8%) were recorded in fermentations with thermally-dried immobilized culture after storage, and an increase in malic acid degradation was observed in lower storage temperatures, as previously shown in winemaking using wet or freeze-dried kefir cells [14,17], but not significantly in all cases. Similarly, glycerol content, acetic acid and lactic acid concentrations as well as total acidity, volatile acidity and pH values remained in levels usual for wines [17,32,54], indicating the metabolic stability of the thermally-dried kefir cells during storage [53].

Table 3. Effect of storage on the kinetic parameters and profile of organic acids of low alcohol wines fermented at 30 °C using thermally-dried free or immobilized kefir culture.

Nature of Thermally-Dried Kefir Culture	Months of Storage	Storage Temperature (°C)	Fermentation Time (h)	Ethanol Concentration (g/L)	Glycerol (g/L)	Residual Sugars (g/L)	Ethanol Productivity (g/L d)	Ethanol Production Yield (g/g)	Conversion (%)	Malic Acid * (g/L)	Lactic Acid (g/L)	Malic Acid Conversion (%)	Acetic Acid (g/L)	Citric Acid (g/L)	Total Acidity (g Tartaric/L)	Volatile Acidity (g Acetic/L)	pH
Free cells	1	20	360 ± 51	8.7 ± 1.4	4.2 ± 0.8	4.3 ± 1.7	4.6 ± 0.1	0.44 ± 0.06	95.8 ± 2.1	2.0 ± 0.1	0.2 ± 0.1	13.0 ± 6.1	0.4 ± 0.1	1.3 ± 0.4	3.5 ± 0.2	0.44 ± 0.07	4.0 ± 0.1
		5	312 ± 44	9.7 ± 1.0	4.6 ± 0.7	5.0 ± 1.5	5.9 ± 0.3	0.46 ± 0.04	96.5 ± 0.1	1.8 ± 0.1	0.2 ± 0.1	21.7 ± 5.5	0.5 ± 0.1	1.5 ± 0.4	3.5 ± 0.2	0.45 ± 0.07	4.1 ± 0.1
		−18	312 ± 44	9.3 ± 1.4	5.6 ± 0.9	1.9 ± 0.6	5.6 ± 0.1	0.44 ± 0.07	98.7 ± 0.1	1.9 ± 0.1	0.3 ± 0.1	17.4 ± 5.8	0.5 ± 0.1	1.6 ± 0.5	3.4 ± 0.3	0.44 ± 0.06	4.0 ± 0.1
	3	20	408 ± 58	8.9 ± 1.8	4.6 ± 1.4	1.4 ± 0.4	4.1 ± 0.2	0.42 ± 0.08	99.2 ± 0.2	1.7 ± 0.2	0.2 ± 0.1	26.1 ± 8.4	0.4 ± 0.1	0.6 ± 0.2	3.3 ± 0.4	0.44 ± 0.06	4.0 ± 0.1
		5	360 ± 41	9.9 ± 0.7	4.9 ± 1.0	1.5 ± 0.4	5.2 ± 0.1	0.46 ± 0.04	99.1 ± 0.3	1.6 ± 0.1	0.2 ± 0.1	28.6 ± 3.1	0.5 ± 0.1	0.6 ± 0.2	3.3 ± 0.4	0.50 ± 0.08	4.1 ± 0.1
		−18	360 ± 41	10.1 ± 0.5	5.2 ± 0.9	1.5 ± 0.4	5.3 ± 0.3	0.47 ± 0.03	99.1 ± 0.3	1.6 ± 0.1	0.2 ± 0.1	31.6 ± 3.0	0.5 ± 0.1	0.9 ± 0.3	3.4 ± 0.4	0.50 ± 0.07	4.1 ± 0.1
	6	20	408 ± 75	9.4 ± 1.1	5.0 ± 1.3	2.0 ± 0.6	4.6 ± 0.3	0.46 ± 0.05	98.8 ± 0.3	2.1 ± 0.1	0.2 ± 0.1	8.7 ± 3.9	0.4 ± 0.1	0.7 ± 0.2	3.4 ± 0.6	0.48 ± 0.07	4.1 ± 0.1
		5	408 ± 58	9.5 ± 1.2	5.2 ± 0.9	1.9 ± 0.6	4.4 ± 0.1	0.45 ± 0.06	98.9 ± 0.3	2.0 ± 0.1	0.2 ± 0.1	13.0 ± 4.9	0.4 ± 0.1	0.9 ± 0.3	3.4 ± 0.6	0.51 ± 0.08	4.0 ± 0.1
		−18	360 ± 51	10.1 ± 0.4	5.9 ± 0.8	1.7 ± 0.5	5.5 ± 0.5	0.46 ± 0.02	99.0 ± 0.3	1.8 ± 0.1	0.4 ± 0.1	25.0 ± 4.2	0.5 ± 0.1	1.0 ± 0.3	3.5 ± 0.6	0.55 ± 0.09	4.0 ± 0.1
Immobilized cells on apple pieces	1	20	240 ± 34	8.6 ± 1.8	6.4 ± 1.3	1.3 ± 0.5	6.8 ± 0.5	0.40 ± 0.08	99.2 ± 0.3	2.1 ± 0.1	0.4 ± 0.2	8.7 ± 2.6	0.4 ± 0.1	1.7 ± 0.5	5.1 ± 0.4	0.48 ± 0.08	3.8 ± 0.1
		5	240 ± 34	8.8 ± 1.2	6.2 ± 1.2	1.3 ± 0.5	7.0 ± 0.1	0.41 ± 0.06	99.2 ± 0.3	2.0 ± 0.1	0.5 ± 0.2	13.0 ± 2.5	0.4 ± 0.1	1.7 ± 0.5	5.0 ± 0.4	0.45 ± 0.08	3.8 ± 0.1
		−18	240 ± 31	9.3 ± 1.3	6.1 ± 1.2	1.6 ± 0.6	7.3 ± 0.1	0.44 ± 0.06	99.1 ± 0.3	1.8 ± 0.1	0.4 ± 0.2	21.7 ± 2.2	0.4 ± 0.1	1.6 ± 0.5	5.1 ± 0.4	0.43 ± 0.07	3.8 ± 0.1
	3	20	264 ± 52	7.2 ± 2.1	4.7 ± 1.5	1.1 ± 0.4	5.1 ± 0.5	0.34 ± 0.10	99.4 ± 0.2	2.0 ± 0.1	0.3 ± 0.1	13.0 ± 2.5	0.2 ± 0.1	0.9 ± 0.3	4.8 ± 0.5	0.47 ± 0.10	3.8 ± 0.1
		5	240 ± 31	8.3 ± 1.4	5.8 ± 1.0	1.3 ± 0.5	6.5 ± 0.3	0.39 ± 0.07	99.2 ± 0.3	1.9 ± 0.1	0.3 ± 0.1	17.4 ± 2.3	0.4 ± 0.1	0.9 ± 0.3	5.0 ± 0.6	0.38 ± 0.08	3.8 ± 0.1
		−18	240 ± 27	7.8 ± 1.2	5.6 ± 1.0	1.2 ± 0.4	6.1 ± 0.3	0.36 ± 0.06	99.3 ± 0.2	1.5 ± 0.1	0.2 ± 0.1	34.8 ± 1.8	0.4 ± 0.1	1.0 ± 0.3	5.0 ± 0.6	0.41 ± 0.09	3.8 ± 0.1
	6	20	264 ± 56	7.6 ± 1.8	5.1 ± 1.4	1.2 ± 0.4	5.4 ± 0.2	0.36 ± 0.08	99.3 ± 0.2	2.2 ± 0.1	0.3 ± 0.1	4.3 ± 2.7	0.3 ± 0.1	0.7 ± 0.2	5.0 ± 0.4	0.45 ± 0.10	3.8 ± 0.1
		5	240 ± 34	7.8 ± 1.4	5.3 ± 0.7	1.2 ± 0.4	6.1 ± 0.2	0.36 ± 0.07	99.3 ± 0.2	2.0 ± 0.1	0.3 ± 0.1	13.0 ± 3.7	0.4 ± 0.1	1.0 ± 0.3	5.1 ± 0.4	0.50 ± 0.07	3.8 ± 0.1
		−18	240 ± 34	7.4 ± 1.0	5.9 ± 0.8	1.1 ± 0.4	5.8 ± 0.1	0.35 ± 0.05	99.4 ± 0.2	1.8 ± 0.1	0.5 ± 0.2	21.7 ± 2.2	0.4 ± 0.1	0.9 ± 0.3	5.1 ± 0.4	0.48 ± 0.07	3.8 ± 0.1
Immobilized cells on DCM	1	20	240 ± 32	8.0 ± 0.8	6.6 ± 0.7	1.4 ± 0.4	6.3 ± 0.2	0.37 ± 0.04	99.2 ± 0.3	2.0 ± 0.1	0.3 ± 0.1	13.0 ± 5.5	0.5 ± 0.1	0.3 ± 0.1	5.4 ± 0.6	0.40 ± 0.08	3.9 ± 0.1
		5	240 ± 32	7.7 ± 0.8	6.6 ± 0.5	1.3 ± 0.4	6.1 ± 0.2	0.36 ± 0.03	99.2 ± 0.2	2.0 ± 0.1	0.4 ± 0.2	13.0 ± 4.3	0.5 ± 0.1	0.3 ± 0.1	5.4 ± 0.6	0.38 ± 0.05	3.9 ± 0.1
		−18	240 ± 27	9.4 ± 0.9	6.6 ± 0.5	1.4 ± 0.4	7.4 ± 0.1	0.44 ± 0.04	99.2 ± 0.3	1.9 ± 0.1	0.4 ± 0.2	17.4 ± 3.5	0.7 ± 0.1	0.4 ± 0.1	5.4 ± 0.6	0.40 ± 0.06	3.8 ± 0.1
	3	20	240 ± 48	7.1 ± 1.1	5.3 ± 1.1	1.2 ± 0.4	5.6 ± 0.2	0.33 ± 0.05	99.3 ± 0.2	2.0 ± 0.1	0.3 ± 0.1	13.0 ± 4.9	0.3 ± 0.1	0.1 ± 0.1	5.4 ± 0.5	0.40 ± 0.07	3.9 ± 0.1
		5	240 ± 42	5.6 ± 0.8	5.0 ± 0.7	0.8 ± 0.2	4.4 ± 0.2	0.26 ± 0.04	99.5 ± 0.1	2.0 ± 0.1	0.2 ± 0.1	13.0 ± 3.7	0.3 ± 0.1	0.2 ± 0.1	5.4 ± 0.5	0.38 ± 0.05	3.8 ± 0.1
		−18	240 ± 34	6.3 ± 0.9	5.4 ± 0.8	1.0 ± 0.3	5.0 ± 0.1	0.29 ± 0.04	99.4 ± 0.2	1.9 ± 0.1	0.3 ± 0.1	17.4 ± 3.5	0.4 ± 0.1	0.1 ± 0.1	5.3 ± 0.4	0.40 ± 0.05	3.8 ± 0.1
	6	20	264 ± 56	7.2 ± 1.8	5.4 ± 1.6	1.1 ± 0.3	5.1 ± 0.2	0.34 ± 0.09	99.4 ± 0.2	2.2 ± 0.1	0.2 ± 0.1	4.3 ± 2.7	0.3 ± 0.1	0.4 ± 0.1	5.3 ± 0.4	0.45 ± 0.08	3.8 ± 0.1
		5	240 ± 37	6.5 ± 1.1	5.4 ± 0.9	1.0 ± 0.3	5.1 ± 0.1	0.30 ± 0.05	99.4 ± 0.2	2.0 ± 0.1	0.2 ± 0.1	13.0 ± 2.5	0.3 ± 0.1	0.2 ± 0.1	5.4 ± 0.5	0.40 ± 0.07	3.8 ± 0.1
		−18	240 ± 37	6.7 ± 0.9	5.3 ± 0.8	1.0 ± 0.3	5.3 ± 0.1	0.31 ± 0.04	99.4 ± 0.2	1.9 ± 0.1	0.4 ± 0.2	17.4 ± 2.3	0.3 ± 0.1	0.2 ± 0.1	5.4 ± 0.5	0.38 ± 0.05	3.8 ± 0.1
Immobilized cells on grape skins	1	20	264 ± 35	9.2 ± 0.9	7.0 ± 0.8	1.8 ± 0.6	6.6 ± 0.2	0.43 ± 0.04	98.9 ± 0.3	1.7 ± 0.1	0.3 ± 0.1	26.1 ± 5.2	0.5 ± 0.1	0.3 ± 0.1	5.1 ± 0.6	0.58 ± 0.08	3.9 ± 0.1
		5	240 ± 32	9.2 ± 0.9	7.1 ± 0.8	1.8 ± 0.6	7.3 ± 0.3	0.43 ± 0.04	98.9 ± 0.3	1.5 ± 0.1	0.4 ± 0.2	34.8 ± 4.6	0.5 ± 0.1	0.3 ± 0.1	5.0 ± 0.6	0.51 ± 0.07	3.9 ± 0.1
		−18	240 ± 27	10.1 ± 0.7	7.4 ± 0.5	2.0 ± 0.6	7.9 ± 0.3	0.47 ± 0.03	98.8 ± 0.4	1.6 ± 0.1	0.4 ± 0.2	33.3 ± 3.8	0.5 ± 0.1	0.4 ± 0.1	5.1 ± 0.6	0.54 ± 0.08	3.9 ± 0.1
	3	20	312 ± 62	8.2 ± 1.3	4.7 ± 1.0	1.3 ± 0.4	5.0 ± 0.2	0.38 ± 0.06	99.2 ± 0.2	1.8 ± 0.1	0.3 ± 0.1	21.7 ± 5.5	0.4 ± 0.1	0.1 ± 0.1	4.5 ± 0.4	0.45 ± 0.06	3.9 ± 0.1
		5	264 ± 47	7.8 ± 1.1	4.9 ± 1.0	1.2 ± 0.4	5.6 ± 0.2	0.36 ± 0.05	99.3 ± 0.2	1.6 ± 0.1	0.3 ± 0.1	30.4 ± 4.4	0.4 ± 0.1	0.2 ± 0.1	5.0 ± 0.4	0.47 ± 0.07	3.9 ± 0.1
		−18	264 ± 47	8.1 ± 1.1	5.2 ± 1.1	1.2 ± 0.4	5.8 ± 0.2	0.38 ± 0.05	99.3 ± 0.2	1.5 ± 0.1	0.3 ± 0.1	34.8 ± 3.7	0.3 ± 0.1	0.1 ± 0.1	5.1 ± 0.4	0.51 ± 0.07	3.9 ± 0.1
	6	20	312 ± 71	8.0 ± 1.7	5.0 ± 1.5	1.5 ± 0.5	4.9 ± 0.1	0.37 ± 0.08	99.1 ± 0.3	2.1 ± 0.1	0.3 ± 0.1	8.7 ± 3.9	0.4 ± 0.1	0.4 ± 0.1	5.1 ± 0.6	0.47 ± 0.08	3.9 ± 0.1
		5	264 ± 56	8.5 ± 1.2	5.4 ± 1.1	1.5 ± 0.5	6.2 ± 0.4	0.40 ± 0.06	99.1 ± 0.3	2.0 ± 0.1	0.3 ± 0.1	13.0 ± 3.1	0.4 ± 0.1	0.2 ± 0.1	5.1 ± 0.6	0.56 ± 0.10	3.9 ± 0.1
		−18	264 ± 49	7.8 ± 0.6	5.0 ± 0.9	1.3 ± 0.4	5.7 ± 0.6	0.37 ± 0.02	99.2 ± 0.2	2.0 ± 0.1	0.3 ± 0.1	13.0 ± 3.7	0.4 ± 0.1	0.2 ± 0.1	5.0 ± 0.6	0.50 ± 0.08	3.9 ± 0.1

* Initial grape must malic acid content: 2.4 ± 0.1 g/L.

3.3. Volatiles

3.3.1. Major Volatiles

The major volatile by-products detected are presented in Table 4. Isoamyl alcohol, 1-propanol, and isobutanol concentrations were significantly ($p < 0.05$) affected by both the fermentation temperature and the nature of kefir cells (free or immobilized). Ethyl acetate, acetaldehyde, and amyl alcohol concentrations were significantly ($p < 0.05$) affected only by the fermentation temperature, while 1-hexanol content was significantly ($p < 0.05$) affected by the nature of the cells, and strong interactions ($p < 0.05$) were detected between the factors. On the contrary, the methanol content was not affected by any factor.

Table 4. Major volatiles of low alcohol wines fermented by repeated batch fermentations at various temperatures (5–30 °C) using thermally-dried free or immobilized kefir culture.

Nature of Thermally-Dried Kefir Culture	Fermentation Temperature (°C)	Repeated Batches	Acetaldehyde (mg/L)	Ethyl Acetate (mg/L)	1-Propanol (mg/L)	Isobutanol (mg/L)	1-Hexanol (mg/L)	Amyl Alcohol (mg/L)	Isoamyl Alcohol (mg/L)	Methanol (mg/L)
Free cells	30	1–3	44–88	20–32	26–37	33–42	1–3	18–22	60–87	9–21
	20	4–6	20–50	10–36	23–32	28–38	2–4	14–22	51–77	5–18
	5	7–8	13–18	12–15	11–14	10–12	5–7	6–8	18–23	12–13
Immobilized cells on apple pieces	30	1–3	63–96	28–35	36–71	38–85	2–3	18–56	150–180	7–15
	20	4–6	15–21	33–67	57–100	51–94	3	24–70	89–220	8–21
	5	7–8	18–25	6–7	23–30	21–25	2	11–13	48–55	5–7
Immobilized cells on DCM	30	1–3	50–97	18–37	35–61	38–83	2–3	17–51	137–166	4–17
	20	4–6	7–15	33–65	56–57	51–67	1–3	24–40	89–146	5–8
	5	7–8	14–15	11–12	14–19	14–20	2	8–10	38–41	12–15
Immobilized cells on grape skins	30	1–3	29–41	7–17	31–44	30–47	2–3	14–38	70–133	10–21
	20	4–6	9–15	37–56	32–69	29–58	1–2	15–42	60–139	6–14
	5	7–8	14–23	10–12	27–39	22–30	1–2	13–17	60–66	3–5

Acetaldehyde, the major wine aldehyde, was detected in significantly higher ($p < 0.05$) levels (up to 97 mg/L) in fermentations performed at 30 °C with thermally-dried free and immobilized kefir cells on apple pieces and DCM compared to lower temperatures. However, it never raised above the limits considered to be acceptable in wines (100–125 mg/L) [57]. On the contrary, low acetaldehyde concentrations (with no significant changes between different fermentation temperatures) were detected in fermentations performed with thermally-dried immobilized cells on grape skins, thus contributing pleasantly to the wine's aromatic complexity [54].

Increased ethyl acetate levels were detected in fermentations performed at 20 °C compared to other temperatures, when thermally-dried immobilized cells were used. However, they never exceeded ~50 mg/L, adding pleasant notes [54], considering that concentrations up to 150 mg/L have a positive influence by contributing predominant fruity notes [58].

Higher alcohols like amyl alcohol, isoamyl alcohol, isobutanol, and 1-propanol are known to contribute to the product's odor complexity by adding fruity characters, when found in low levels [59,60]. Higher concentrations were detected when thermally-dried immobilized cells were used compared to free cells, although not significantly in all cases. Higher alcohols' formation is known to be greatly affected by high fermentation temperatures [54]. Significantly lower values were detected in fermentations performed at 5 °C in most cases, except for fermentations with thermally-dried immobilized cells on grape skins, where no concentration fluctuations were detected in general. On the other hand, 1-hexanol and methanol, were detected in very low levels (<50 mg/L) in all samples.

3.3.2. Minor Volatiles

In total, 30 compounds (including esters, organic acids, and alcohols) were identified (Table 5). The nature of kefir culture and the fermentation temperature significantly affected ($p < 0.05$) ester, alcohol, miscellaneous, and total volatiles' content, while strong interactions ($p < 0.05$) between the two factors were observed in most cases. On the contrary, organic acids concentration was significantly ($p < 0.05$) affected only by the nature of the kefir

culture. In general, a decrease was observed in total volatiles' concentration at lower fermentation temperatures, although not significantly in all cases [20].

Fatty acid esters like ethyl hexanoate, ethyl octanoate, ethyl decanoate, and 2-phenylethyl acetate (responsible for the fruity notes) are considered important for wine bouquet [61], and were detected in all samples. Their synthesis is favored on higher temperatures, while an increase of their levels has previously been associated with cell immobilization [18,54,62]. Likewise, 3-methylbutyl acetate (known to contribute banana-like scents) was detected in all samples, while ethyl butyrate (responsible for apple-peel attributes), 2-methylbutyl acetate (responsible for peer flavors), ethyl dodecanoate (responsible for dried fruit, smokey, earthy, and toasty aromas) [54,58,63,64], ethyl propanoate and isobutyl acetate (known for their fresh and fruity character), were detected in most of the samples. Interestingly, the highest ester concentrations ($p < 0.05$) were observed in fermentations performed at 30 °C with immobilized cells on DCM, compared to wines produced by immobilized cells on other supports and free cells.

Organic acids are known for their low odor threshold limit and their potential impact on wine flavor [64]. Octanoic acid was identified in most of the samples, while *n*-decanoic acid was only found in fermentations with free cells at 30 °C.

Regarding alcohols, characteristic compounds like 2-phenyl-ethanol (with a characteristic rose aroma), and α-terpineol (with a distinct lilac scent) were found in all wines. Linalool, providing lime tree notes, and citronellol, providing sweet, citrus, and floral scents [65,66], were also identified in most cases. In addition, 2,3-butanediol (mostly known for its bittersweet taste) was found in some of the samples, but is most likely of low sensory importance for wine [54,67].

As for miscellaneous compounds, 1,1-diethoxy-ethane, identified in most of the samples, is known for its fruity-green and refreshing scent, and is most likely the only acetal that may contribute to the wine bouquet [54,58].

HS-SPME GC/MS results were also subjected to PCA, which showed that the fermentation temperature rather than the state of the kefir culture significantly affected the volatile composition, as distinct groups were obvious in the plot (Figure 2).

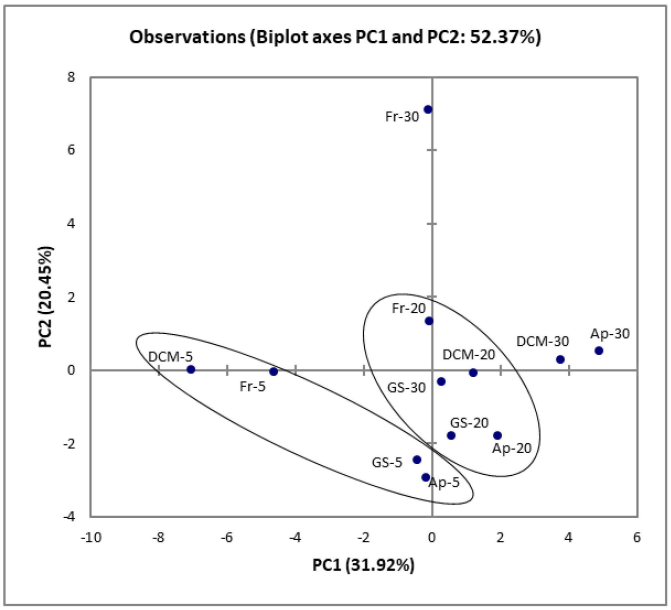

Figure 2. Principal component analysis (PCA) plot of minor volatiles isolated by low alcohol wines fermented by thermally-dried kefir cells. Fr: low alcohol wine fermented by free kefir culture, Ap: low alcohol wine fermented by immobilized kefir culture on apple pieces, DCM: low alcohol wine fermented by immobilized kefir culture on delignified cellulosic material (DCM), GS: low alcohol wine fermented by immobilized kefir culture on grape skins. The fermentation temperature is indicated at the end of the sample code.

Table 5. Minor volatile compounds (mg/L) identified by HS-SPME GC/MS in low alcohol wines fermented at various temperatures (5–30 °C) by thermally-dried free or immobilized kefir culture. Volatiles were semi-quantified using 4-methyl-2-pentanol as the internal standard.

Compounds Detected	KI	30 °C				20 °C				5 °C			
		Fr	Ap	DCM	GS	Fr	Ap	DCM	GS	Fr	Ap	DCM	GS
Esters													
Ethyl acetate	<700	0.5–1.6	3.0–5.0	2.5–3.1	0.8–1.6	1.6–6.6	5.0–7.3	6.4–8.1	4.8–8.2	1.0–1.2	5.0–6.3	3.5–4.0	7.9–9.0
Ethyl propanoate	707	0.1	0.2–0.4	0.2	0.1	0.0–0.1	0.1–0.5	0.1–0.4	0.2–0.3	0.2–1.0	0.5–0.6	0.2–0.5	0.5–0.6
Isobutyl acetate	745	Nd	Nd	Nd	Nd	Nd	0.0–0.1	Nd	0.0–0.1	Nd	0.1	0.0–0.1	Nd
Ethyl butyrate	803	<0.1–0.1	Nd	0.0–0.1	0.0–0.1	0.1–0.3	Nd	0.2	0.0–0.1	0.1–0.2	Nd	0.2–0.3	Nd
3-methylbutyl acetate	867	0.1–1.4	2.5–4.0	1.9–3.7	0.5–1.0	0.5–0.9	2.7–4.0	1.9–3.2	1.8–2.3	0.3–0.4	1.7–2.0	2.0–2.3	1.0
2-methylbutyl acetate	869	<0.1–0.1	Nd	Nd	Nd	0.0–0.1	0.0–0.2	Nd	0.0–0.1	<0.1–0.1	Nd	0.1–1.2	0.0
Ethyl hexanoate	1002	1.6–4.6	0.9–4.7	1.0–1.6	0.7–1.1	1.8–2.5	1.0–1.7	1.2–1.9	0.7–1.1	2.4–3.0	0.9–1.0	5.0–6.0	0.8–1.0
Diethyl butanedioate	1191	<0.1–0.3	1.0–2.1	1.0–3.5	0.3–1.8	Nd	0.1–1.3	1.0–1.6	Nd	Nd	Nd	1.2–1.5	0.0
Ethyl octanoate	1202	10.6–28.6	4.0–8.3	3.1–9.0	2.3–6.2	7.1–10.4	4.0–6.5	6.2–8.9	1.7–6.5	10.0–12.0	2.0–2.5	18.7–20.1	3.0
2-phenylethyl acetate	1263	1.1–2.8	0.9–1.4	39.7–55.0	1.1–2.3	0.6–1.7	0.4–0.5	3.7–5.4	0.6–2.9	0.3	0.1–0.2	0.9–1.0	0.7–1.0
Phenylethyl isobutyrate	1360	Nd	Nd	0.1–0.6	Nd	Nd	Nd	Nd	Nd	Nd	Nd	0.1–0.2	Nd
Ethyl 9-decanoate	1390	0.5–1.2	0.1–0.9	0.0–0.3	Nd	0.3–0.4	Nd	0.0–0.5	Nd	0.1	Nd	Nd	Nd
Ethyl decanoate	1398	11.0–20.7	2.6–5.9	1.2–3.0	0.6–1.0	1.8–5.8	1.5–2.4	2.0–7.2	0.4–1.2	0.1–0.2	0.5–0.6	0.4–0.5	1.0–1.1
3-methylbutyl octanoate	1453	0.0–0.1	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd
Ethyl dodecanoate	1595	0.0–0.1	0.1–0.4	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd
Total esters		28.0–61.5	19.7–29.7	52.3–79.3	8.5–13.4	18.3–23.9	15.6–24.1	24.5–37.0	10.9–20.2	16.0–17.1	12.2–12.9	34.6–35.4	15.8–16.5
Organic acids													
Octanoic acid	1198	0.1–0.6	Nd	0.2–0.3	0.1–0.3	0.5–0.9	Nd	0.5–0.9	Nd	0.6–0.7	Nd	0.5–0.6	Nd
Decanoic acid	1381	0.0–0.3	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd
Total organic acids		0.1–0.9	Nd	0.2–0.3	0.1–0.3	0.5–0.9	Nd	0.5–0.9	Nd	0.6–0.7	Nd	0.5–0.6	Nd
Alcohols													
2-methyl-1-propanol (isobutanol)	<700	0.5–1.2	0.8–1.5	0.9–1.6	0.4–0.9	0.3–1.3	0.6–1.7	0.7–1.2	0.4–0.6	Nd	0.8–1.0	0.3–0.5	0.4–0.5
3-methyl-1-butanol (isoamyl alcohol)	721	11.7–26.6	32.6–36.0	25.4–40.5	13.9–31.1	15.8–24.5	32.8–45.4	20.9–28.8	19.7–29.9	7.6–8.0	24.4–25.0	10.0–11.4	9.9–11.7
2-methyl-1-butanol (amyl alcohol)	722	4.5–10.3	12.8–15.3	10.0–12.9	4.9–8.5	2.6–9.7	6.5–13.6	6.2–10.5	5.6–8.7	0.9–1.0	5.0–6.0	3.0–3.2	3.0–3.1
2,3-butanediol	756	<0.1–0.5	Nd	0.1–0.3	<0.1–0.2	0.0–0.1	0.0–0.2	Nd	Nd	0.1	0.1–0.2	Nd	Nd
3,7-dimethyl-1,6-octadien-3-ol (linalool)	1123	0.1–0.3	Nd	Nd	0.0–0.2	Nd	0.0–0.2	Nd	0.1–0.2	0.4	Nd	0.5	Nd
2-phenylethanol	1133	6.5–13.6	16.0–20.8	10.0–25.8	8.4–9.9	1.4–2.3	3.3–7.4	3.4–5.2	3.2–8.3	0.9–1.0	1.0–1.3	3.7–4.0	1.7–2.0
α-terpineol	1192	0.4–1.1	0.4–0.7	0.3	0.2–0.3	0.4–0.8	0.1–0.3	0.4–0.8	0.2–0.5	0.5	0.2–0.4	Nd	0.3–0.5
3,7-dimethyl-6-octen-1-ol (citronellol)	1235	0.0–0.1	Nd	Nd	0.0–0.1	0.1	Nd	Nd	Nd	Nd	Nd	0.1	Nd
Total alcohols		24.9–52.9	63.6–72.9	54.6–70.7	28.8–46.4	24.0–37.8	46.8–68.5	31.8–45.0	32.7–39.7	10.4–11.0	32.8–33.3	18.3–19.3	16.0–17.2
Miscellaneous compounds													
2-fluoro-1-propene	<700	0.3–1.0	0.7–1.2	0.4–1.0	0.2–0.6	1.1–1.6	0.3–1.1	0.7–1.4	0.3–0.9	Nd	0.5–0.6	0.1	0.2
2,5-dimethyl-furan	704	Nd	Nd	Nd	Nd	0.0–<0.1	Nd	Nd	0.0–<0.1	0.1	0.1	0.1	Nd
1,1-diethoxy-ethane (acetal)	716	0.3–1.4	0.9–4.4	1.3–3.5	0.1–0.6	0.9–2.1	Nd	Nd	0.0–0.1	Nd	1.2–1.5	0.1	0.2
1,3,5-trimethyl-benzene (mesitylene)	956	0.0–0.1	Nd	Nd	0.0–0.1	0.0–0.1	0.0–0.1	Nd	0.0–0.1	0.1	Nd	0.1–0.2	Nd
1,3-bis(1,1-dimethylethyl)-benzene (m-Di-tert-butylbenzene)	1258	0.1–0.7	0.2–1.4	0.2–0.8	0.1–0.4	0.3–0.6	0.3–0.4	0.4–0.6	0.3–0.8	0.2	Nd	Nd	0.2–0.3
Total miscellaneous compounds		1.5–2.5	3.1–5.3	1.9–4.9	0.8–1.4	2.3–4.2	0.8–1.5	1.1–1.8	0.9–1.6	0.4	1.9–2.1	0.5	0.6–0.7
Total volatiles		58.5–93.4	88.9–105.7	121.7–155.1	39.1–60.7	46.1–66.4	63.1–93.9	58.1–75.2	44.5–60.9	28.0–28.6	47.6	54.7–54.9	33.1–33.7

KI: Kovats retention index; Nd: Not detected; Fr: low alcohol wine fermented by thermally-dried free kefir culture, Ap: low alcohol wine fermented by thermally-dried immobilized kefir culture on apple pieces, DCM: low alcohol wine fermented by thermally-dried immobilized kefir culture on delignified cellulosic material (DCM), GS: low alcohol wine fermented by thermally-dried immobilized kefir culture on grape skins.

3.4. Preliminary Sensory Evaluation

Low alcohol wines produced with thermally-dried kefir cells were assessed for their sensory characteristics (see Table S1 on Supplementary Materials). The aroma and the overall quality of all products were significantly ($p < 0.05$) affected by both the fermentation temperature and the nature of the kefir culture, while taste was affected ($p < 0.05$) only by the nature of kefir culture. Regarding aroma, the majority of low alcohol wines were characterized by fruity and wine-like scents, while piquant notes were identified in samples fermented with thermally-dried immobilized cells on DCM. As for taste, low alcohol wines produced at ambient and higher temperatures were mostly characterized as sour, and were light-bodied. At 5 °C, however, due to the increased residual sugar concentrations, all wine products had a predominant sweet taste and a pleasant smooth aftertaste with a medium body. Remarkably, wines fermented by thermally-dried immobilized cells on DCM scored the highest overall quality ranking, although not significantly in all cases. All new products were characterized by high clarity and were approved by the sensory panel.

4. Conclusions

Thermally-dried immobilized kefir culture on natural supports was found to be suitable for low alcohol wine production, performing simultaneously alcoholic and ML fermentation. Thermal drying of immobilized kefir cells resulted in high survival rates and maintenance of fermentation activity, even after storage for 6 months in various temperatures (−18, 5, and 20 °C). Repeated batch fermentations using thermally-dried kefir cells continued for a period >6 months, suggesting high operational stability of all systems, while the fermentation temperature rather than the state of the kefir culture significantly affected the volatile composition. The proposed technology could potentially provide the option for low-cost production of dried mixed cultures, maintenance of cell viability, and fermentation efficiency of thermally-dried cultures, as well as novel product development with unique characteristics. However, more research is still required in order to verify the suitability of the thermally-dried kefir culture in industrial practice, and allow commercialization. Important factors like the maintenance of cell viability between different enological periods and monitoring the volatile composition during long-term storage, are yet to be investigated.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/app12126176/s1, Table S1: Sensory evaluation of low alcohol wines produced by thermally-dried kefir culture at various temperatures (5–30 °C).

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