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Characterization of kefir-like beverages produced from vegetable juices

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A B S T R A C T

The aim of this work was to develop new non-dairy fermented beverages using vegetable juices as fermentable substrates. Carrot, fennel, melon, onion, tomato and strawberry juices underwent backslopping fermentations, carried out by water kefir microorganisms. Results indicated that lactic acid bacteria and yeasts were capable of growing in the juices tested. Melon juice registered the highest numbers of microorganisms. Almost all juices underwent a lactic fermentation. After fermentation, there was observance of a decrease of the soluble solid content and an increase of the number of volatile organic compounds. In particular, esters were present in high amounts after the fermentation, especially in strawberry, onion and melon, whereas carrot and fennel registered a significant increase of terpenes. The concentration of alcohols increased, while that of aldehydes decreased. Changes in colour attributes were registered. Strawberry, onion and tomato juices retained a high antioxidant activity after fermentation. The overall quality assessment indicated that carrot kefir-like beverage (KLB) was the product mostly appreciated by the judges. These findings support the further development of vegetable KLBs with additional benefits and functional properties.

1. Introduction

In the past few years, there has been an increased awareness of the consumers towards disease concerns related to foods. Consequently, there has been a growing interest to develop new functional foods (Prado, Parada, Pandey, & Soccol, 2008). In general, yogurt represents the main probiotic food consumed worldwide. However, due to the allergy to dairy products by several people, there has recently been an intensive research addressed to non-dairy foods. Furthermore, the ongoing trend of vegetarianism, with an increasing number of vegan vegetarian, has established a massive worldwide importance of non-dairy probiotic products (Granato, Branco, Nazzaro, Cruz, & Faria, 2010). Fruit juices, desserts and cereal-based products are suitable media for delivering probiotics (Reichert, 2008). Among vegetable probiotic beverages, there have been recent proposal for beet-based drink (Yoon, Woodams, & Hang, 2005), tomato-based drink (Yoon, Woodams, & Hang, 2004), cabbage juice (Yoon, Woodams, & Hang, 2006) and carrot juice (Nazzaro, Fratianni, Sada, & Orlando, 2008).

Since the beginning of recorded history, kefir is an ancient food attributed with exceptional health promoting and curative properties (Shavit, 2008), and in Caucasus, it is also associated with longevity (Cevikbas et al., 1994; Zourari & Anifantakis, 1988). Within non-dairy fermented beverages, water kefir is prepared with a sucrose solution with or without fruit extracts (Schneedorf, 2012) fermented by kefir grains, which consist of mainly lactic acid bacteria (LAB) and yeasts included into a polysaccharide matrix named kefiran (Rodrigues, Caputo, Carvalho, Evangelista, & Schneedorf, 2005).

Since the beginning of the third millennium, the scientific interest in kefir and the promotion of its industrial production are on the increase because of its health benefits (Anar, 2000). The concept that the foods provide not only essential nutrients needed for life but also bioactive compounds for health promotion and disease prevention is quite clear among consumers. For example, there have been demonstrations that the daily consumption of fruit and vegetables reduces the risk of stroke (He, Nowson, & MacGregor, 2006) and this medical evidence induced the change of dietary

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habits of several peoples.

Based on the several positive effects of kefir products, and vegetable and fruits, on human health, this work aimed to evaluate the characteristics of kefir-like beverages obtained after the fermentation of juices extracted from vegetables with water kefir microorganisms, in order to develop new non-dairy fermented products.

2. Materials and methods

2.1. Production of kefir-like beverages

The vegetable juices (VJ) fermented in this study were obtained from carrots (*Daucus carota* L.), fennels (*Foeniculum vulgare* Mill.), melons (*Cucumis melo* L.), onions (*Allium cepa* L.), tomatoes (*Solanum lycopersicum* L.) and strawberries (*Fragaria* x *ananassa* Duch.). Table 1 reports the characteristics of the juices, obtained by means of a centrifugal extractor (Moulinex JU650G, Milan, Italy). The commercial water kefir microorganism preparation "kefir d'acqua fai da te" (BioNova snc, Villanova sull'Arda, Italy), containing approximately 10^9 CFU/g of *Lactobacillus*, *Lactococcus*, *Leuconostoc* and *Saccharomyces*, as declared by the producer, was used to carry out the fermentation. VJs were subjected to pasteurisation at 75 °C for 5 min and cooled at room temperature before processing.

Kefir-like beverages (KLBs) were produced by back-slopping. Aliquots of 50 mL of each VJ were inoculated with 0.125 g of the freeze-dried microbial mixture and incubated at 25 °C for 72 h to develop the active inoculants (Ins). Higher volumes of VJ (1 L) were then inoculated with the corresponding In (4% v/v) and the fermentation processes were performed at 25 °C for 48 h. Beverage productions were carried out in triplicate.

2.2. Microbiological analyses

Preparation of decimal dilutions of VJs, Ins and KLBs was in Ringer's solution (Sigma–Aldrich, Milan, Italy). The cell suspensions were used to estimate the following microbial groups: total mesophilic count (TMC) on plate count agar (PCA), incubated aerobically at 30 °C for 72 h; *Enterobacteriaceae* on double-layered violet red bile glucose agar (VRBGA), incubated aerobically at 37 °C for 24 h; pseudomonads on *Pseudomonas* agar base (PAB) supplemented with 10 mg/mL cetrimide fucidin, incubated aerobically at 20 °C for 48 h; rod LAB on de Man-Rogosa-Sharpe (MRS) agar, acidified to pH 5.4 with lactic acid (5 mol/L) and incubated anaerobically at 30 °C for 48 h; coccus LAB on M17 agar, incubated anaerobically at 30 °C for 48 h; yeasts on dichloran rose Bengal chloramphenicol (DRBC) agar, incubated aerobically at 25 °C for 48 h. All media and supplements were purchased from Oxoid (Milan, Italy). Count plates were carried out in duplicate for each independent production.

2.3. Characterization of the commercial starter preparation

Characterization of the commercial starter culture for water kefir production was at species level. Freeze-dried preparation (1 g) was diluted and analysed for LAB and yeasts, as reported above. Four colonies of yeasts and Gram-positive (determined by KOH method) and catalase negative (determined by transferring fresh colonies from a Petri dish to a glass slide and adding 5%, w/v, H_2O_2) bacteria for each morphology observed were isolated from the agar media inoculated with the highest dilutions of cell suspension. Purification of the cultures to homogeneity was by successive subculturing in the same agar media and then propagating in the corresponding broth media.

DNA from broth cultures was extracted by Instagene Matrix kit (Bio-Rad, Hercules, CA) and used as template for PCR reactions. LAB were identified by 16S rRNA gene sequencing as described by Weisburg, Barns, Pelletier, and Lane (1991). DNA fragments of about 1600 bp were purified by QIA-quick purification kit (Qiagen S.p.a., Milan, Italy) and sequenced by PRIMM (Milan, Italy). The sequences were compared to those available in the GenBank/EMBL/DDBJ database. All yeasts were grouped by restriction fragment length polymorphism (RFLP) analysis of the region spanning the internal transcribed spacers (ITS1 and ITS2) and the 5.8S rRNA gene, as reported by Esteve-Zarzoso, Belloch, Uruburu, and Querol (1999), and then identified at species level by sequencing the D1/D2 domains of

Table 1
Microbial loads (Log CFU/mL) of vegetable kefir-like beverages.

Sample		Media					
		PCA	VRBGA	PAB	MRS	M17	DRBC
Carrot	VJ	5.5 ± 0.4	< d.l.	<1	5.7 ± 0.5	5.7 ± 0.2	5.0 ± 0.5
	KLB	8.4 ± 0.5	< d.l.	<1	8.5 ± 0.2	8.5 ± 0.5	6.7 ± 0.4
		***	ns	ns	***	***	***
Fennel	VJ	5.4 ± 0.4	< d.l.	<1	6.1 ± 0.8	5.5 ± 0.4	4.2 ± 0.7
	KLB	8.5 ± 0.4	< d.l.	<1	8.6 ± 0.4	8.1 ± 0.2	5.5 ± 0.4
		***	ns	ns	***	***	**
Melon	VJ	5.4 ± 0.5	< d.l.	<1	6.1 ± 0.2	5.7 ± 0.5	5.4 ± 0.4
	KLB	9.1 ± 0.7	3.3 ± 0.5	2.3 ± 0.4	9.1 ± 0.4	9.2 ± 0.5	7.8 ± 0.8
		***	***	***	***	***	***
Onion	VJ	5.8 ± 0.3	< d.l.	<1	6.2 ± 0.7	5.2 ± 0.3	2.0 ± 0.2
	KLB	8.6 ± 0.5	< d.l.	<1	8.9 ± 0.7	8.5 ± 0.4	3.3 ± 0.4
		***	ns	ns	***	***	**
Strawberry	VJ	5.3 ± 0.7	< d.l.	<1	5.3 ± 0.4	4.9 ± 0.7	5.1 ± 0.5
	KLB	7.8 ± 0.4	< d.l.	<1	7.7 ± 0.5	6.4 ± 0.7	7.7 ± 0.6
		***	ns	ns	***	**	***
Tomato	VJ	5.7 ± 0.8	< d.l.	<1	5.4 ± 0.7	5.3 ± 0.5	5.1 ± 0.6
	KLB	9.0 ± 0.2	< d.l.	<1	8.9 ± 0.6	8.9 ± 0.2	7.1 ± 0.4
		***	ns	ns	***	***	***

Results represent mean values ± SD of six measurements (carried out in duplicate for three independent productions).

Abbreviations: PCA, plate count agar for total mesophilic counts; VRBGA, violet red bile glucose agar for *Enterobacteriaceae*; PAB, *Pseudomonas* agar base for pseudomonads; MRS, de Man-Rogosa-Sharpe agar for rod LAB; M17, medium 17 agar for mesophilic coccus LAB; DRBC, dichloran rose Bengal chloramphenicol agar for yeasts; VJ, vegetable juice after pasteurisation; KLB, kefir-like beverage; d.l., detection level.

Significant differences among vegetable juices and fermented kefir-like beverages for each vegetable sample and each microbial load: ***, $p \leq 0.001$, **, $p \leq 0.01$; *, $p \leq 0.05$; ns, not significant.

the 26S rRNA gene using the primers NL1 and NL4 (O'Donnell 1993). Yeast DNAs were sequenced by PRIMM. BlastN search against the NCBI non-redundant sequence database located at http://www.ncbi.nlm.nih.gov determined the identity of the sequences.

2.4. Physico-chemical determinations

Physico-chemical analyses of pH, total titratable acidity (TTA) and soluble solid content (SSC) were performed according to the methodology proposed by the AOAC (2000). Total phenolic compounds (TPs) were analysed according to the Folin-Ciocalteu procedure (Slinkard & Singleton, 1977). The antioxidant activity was determined as DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity (%) (Larrauri, Sánchez-Moreno, & Saura-Calixto, 1998). The total anthocyanin content (TAC) was determined according to Fuleki and Francis (1968) with some modifications (Lee, Durst, & Wrolstad, 2005).

Ethanol, acetic and lactic acids were detected using Enzymatic BioAnalysis/Food Analysis kits (Boehringer Mannheim/R-Biopharm).

Carbon dioxide was indirectly estimated by measuring the weight loss before and after the fermentations and expressed as g/100 mL (Lombardi, Delfini, Zilio, & Tosi, 2004).

Colours of juices were measured, before and after fermentation, with a colorimeter (Chroma Metre CR-400, Minolta, Osaka, Japan) recording CIElab chromaticity coordinates (L^*, a^*, b^*).

All chemicals were purchased from WWR International (Milan, Italy), except when reported differently. Five readings were taken for each replicate of each sample.

2.5. Volatile organic compounds (VOCs)

VJs and KLBs were subjected to GC/MS analysis in order to identify the volatile organic compounds (VOCs). The extractions of VOCs were carried out using a SPME fibre of divinylbenzene/carboxen/polydimethylsiloxane (Supelco, Bellefonte, PA). Conditioning of the fibre was at 250 °C for 30 min. The fibre was then subjected to an exposure step for 30 min at 40 °C to the headspace of the sample vial. The GC–MS equipment, column and conditions described by Corona (2010) were used for analysis. 1-heptanol solution (35 mg/L 1-heptanol in 20% ethanol aqueous solution) was used as an internal standard. Identification of individual peaks was by comparing their retention indices to those of control samples and by comparing their mass spectra with those within the NIST/EPA/NIH Mass Spectral Library database (Version 2.0d, build 2005). Expression of volatile compounds was as μg/L. Determinations were carried out in triplicate for each sample.

2.6. Sensory evaluation

The final products were evaluated for their sensory profiles by fifteen untrained judges (six women and 9 men, 14 Italians and one Turkish, 25–35 years old). Serving of randomised, refrigerated (10 °C) samples of 10 mL was in clear, tulip-shaped glasses with a volume of 50 mL. The glasses were marked with three digit random numbers and covered with Petri dishes. Instruction to tasters was to cleanse their palates with a plain biscuit and cold, filtered tap water before evaluating each sample. Water kefir produced with the same microbial mixture used to produce KLBs was used as control. For each product, tasters were asked to indicate a mark on a 9-point hedonic scale related to the overall quality (9 = extremely good; 1 = extremely bad). Four samples were analysed in each session and the experiment was replicated three times, i.e., there were nine sessions in total (Magalhães et al., 2011).

2.7. Statistical analyses

Data were analysed using a generalized linear model (GLM). The post-hoc Tukey's method ($P < 0.05$) was used to determine differences among the overall quality of KLBs. Statistical data were processed with STATISTICA software version 10 (StatSoft Inc., Tulsa, OK, USA).

3. Results and discussion

3.1. Microbial evolution of vegetable juices and fermented beverages

The levels of the six microbial populations (TMC, *Enterobacteriaceae*, pseudomonads; rod LAB; coccus LAB and yeasts) of VJs, before pasteurization, were different. Pseudomonads were undetectable in any sample, and for melon juice, the other groups were below the detection limit by the plate count method. Characterization of onion juice was by the presence of 1.6 Log CFU/mL of TMC and strawberry juice hosted 1.0 Log CFU/mL of yeasts. Carrot, fennel and tomato juices were microbiologically complex showing the presence of consistent levels of TMC (5.2–5.7 Log CFU/mL). Both LAB groups were at 10^4 CFU/mL in carrot and fennel juices. Rod and coccus LAB of tomato juice were 2.5 and 4.3 Log CFU/mL, respectively. Yeasts were 1.5, 2.5 and 3.5 Log CFU/mL, while *Enterobacteriaceae* were 1.3, 2.2 and 1.5 Log CFU/mL for tomato, carrot and fennel juice, respectively. Due to their different microbial composition, there was pasteurization of the bulks in order to provide enough volume of each VJ that is stable over time. The thermal treatment reduced all microbial groups at levels below the detection limits.

At the time of addition into KLBs, there was characterization of the active inoculants of the six VJs by 10^7 CFU/mL of TMC. Rod LAB were in the range 7.0–8.3 Log CFU/mL, while coccus LAB were in the range 6.8–7.6 Log CFU/mL. Except onion In for which a level of 3.9 Log CFU/mL was registered, yeasts ranged between 6.1 and 7.7 for the other Ins. *Enterobacteriaceae* and pseudomonads were undetectable in any Ins.

The microbiological characteristics of the KLBs are reported in Table 1. After inoculation, all microbial groups of Ins resulted as diluted by almost two orders of magnitude. At the end of fermentation, strawberry KLB contained 7.7 and 6.4 Log CFU/mL of rod and coccus LAB, respectively: melon KLB had 9.1 and 9.2 Log CFU/mL of rod and coccus LAB, respectively, while the other products hosted levels of 10^8 CFU/mL of both LAB groups. Yeasts were 3.3 Log CFU/mL for onion KLB and ranged between 5.5 and 7.8 Log CFU/mL for the other fermented juices. Although *Enterobacteriaceae* and pseudomonads were undetectable in all Ins, melon KLB was characterized by their presence (3.3 and 2.3 Log CFU/mL, respectively) at the end of fermentation. This phenomenon might be due to the presence of a very few cells in Ins which were not detected through the microbiological investigation, but transferred into KLB where they developed at detectable levels. Furthermore, there was characterization of melon juice by an almost neutral pH (Table 2) that is not inhibitory to the development of *Enterobacteriaceae* and pseudomonads.

We also verified the codominance of LAB and yeasts typical of traditional milk or water kefir (Chen, Wang, & Chen, 2008) for the vegetable kefir products tested in this study. However, yeasts in onion KLB developed at very low levels not only compared to those of LAB, but also compared to the levels of yeast counts estimated for the other KLBs of the experimentation. The high levels of sulphur compounds characterizing *Allium* species (garlic and onion) explain this finding and there are reports of them inhibiting different yeast species, including *Saccharomyces cerevisiae* (Kim, Kim, & Kyung,

Table 2

Physico-chemical analysis of vegetable juices and kefir-like beverages.

Sample		pH	Ethanol (% v/v)	Lactic acid (g/L)	Acetic acid (g/L)	CO_2 (g/100 mL)	TTA[a] (g/L citric acid)	SSC (°Brix)	TP (mg/L)	DPPH (%)	TAC (mg/L Cy-3-glc)	Colour					
												L*	a*	b*	Croma	Hue	ΔE
Carrot	VJ	5.3 ± 0.0	n.d.	n.d.	n.d.	n.d.	9.85 ± 0.49	8.15 ± 0.21	194.25 ± 6.36	15.49 ± 0.03	n.d.	49.28 ± 0.59	23.21 ± 0.64	39.01 ± 1.00	45.39 ± 1.18	59.25 ± 0.07	
	KLB	4.1 ± 0.0	3.00 ± 0.14	4.81 ± 0.65	1.90 ± 0.71	1.51 ± 0.18	10.23 ± 0.25	3.38 ± 0.10	206.40 ± 18.60	14.53 ± 1.67	n.d.	50.22 ± 0.38	25.33 ± 1.11	40.78 ± 1.22	48.00 ± 1.62	58.17 ± 0.37	2.94 ± 0.32
		***	***	***	***	***	ns	***	ns	ns		***	***	**	**	***	
Fennel	VJ	5.5 ± 0.0	n.d.	n.d.	n.d.	n.d.	6.75 ± 0.21	4.45 ± 0.07	208.07 ± 1.64	22.56 ± 0.08	n.d.	37.09 ± 0.68	−0.55 ± 0.20	2.46 ± 0.79	2.54 ± 0.74	104.08 ± 6.66	
	KLB	4.4 ± 0.0	0.63 ± 0.03	3.55 ± 0.66	0.18 ± 0.10	0.87 ± 0.13	4.47 ± 0.06	1.87 ± 0.06	101.83 ± 10.11	20.12 ± 0.11	n.d.	48.20 ± 2.15	−1.37 ± 0.14	5.02 ± 1.23	5.24 ± 1.13	107.99 ± 7.80	11.55 ± 0.46
		***	***	***	**	***	***	***	***	***		***	***	*	*	ns	
Melon	VJ	6.4 ± 0.1	n.d.	n.d.	n.d.	n.d.	3.60 ± 0.01	10.05 ± 0.07	185.90 ± 20.15	18.42 ± 2.80	n.d.	35.70 ± 0.69	−0.11 ± 0.04	4.02 ± 0.52	4.02 ± 0.52	91.60 ± 0.43	
	KLB	4.4 ± 0.0	2.56 ± 0.62	4.80 ± 0.52	0.59 ± 0.23	3.39 ± 0.47	5.33 ± 0.31	3.83 ± 0.06	160.03 ± 5.05	20.24 ± 0.98	n.d.	43.69 ± 2.15	−1.42 ± 0.25	7.32 ± 1.18	7.46 ± 1.20	100.95 ± 0.86	8.76 ± 0.68
		***	***	***	***	***	**	***	ns	*		***	***	***	***	***	
Onion	VJ	5.0 ± 0.0	n.d.	n.d.	n.d.	n.d.	2.11 ± 0.42	9.95 ± 0.07	714.55 ± 85.32	81.78 ± 9.22	37.14 ± 2.92	33.17 ± 0.18	8.35 ± 0.30	−7.86 ± 0.16	11.47 ± 0.12	316.72 ± 1.62	
	KLB	5.0 ± 0.6	0.09 ± 0.02	1.24 ± 0.56	0.03 ± 0.02	0.14 ± 0.06	1.50 ± 0.06	9.17 ± 0.15	515.94 ± 45.91	78.67 ± 0.02	9.36 ± 1.32	32.74 ± 2.01	11.39 ± 1.13	−7.78 ± 1.38	13.97 ± 1.10	324.99 ± 9.17	3.91 ± 0.24
		ns	***	***	*	*	***	ns	*	ns	**	ns	**	ns	***	ns	
Strawberry	VJ	3.2 ± 0.0	n.d.	n.d.	n.d.	n.d.	7.20 ± 0.73	5.95 ± 0.07	813.79 ± 42.69	95.27 ± 1.23	90.20 ± 2.87	42.35 ± 0.35	19.61 ± 1.61	9.99 ± 1.23	22.01 ± 1.99	26.94 ± 1.06	
	KLB	3.6 ± 0.0	2.35 ± 0.26	0.58 ± 0.02	0.10 ± 0.03	1.71 ± 0.27	8.82 ± 1.40	2.47 ± 0.06	619.86 ± 41.23	95.38 ± 0.43	24.79 ± 2.85	43.81 ± 3.12	17.60 ± 1.52	8.30 ± 1.44	19.64 ± 2.56	24.52 ± 2.75	5.47 ± 0.31
		***	***	***	**	***	*	***	*	ns	***	ns	*	ns	ns	ns	
Tomato	VJ	4.1 ± 0.0	n.d.	n.d.	n.d.	n.d.	14.55 ± 0.64	4.45 ± 0.07	248.46 ± 12.98	74.30 ± 1.80	n.d.	28.74 ± 0.35	18.99 ± 1.21	3.85 ± 0.62	19.38 ± 1.30	11.40 ± 1.14	
	KLB	4.2 ± 0.1	1.48 ± 0.10	2.41 ± 0.32	1.25 ± 0.09	1.29 ± 0.11	6.70 ± 0.21	1.97 ± 0.06	268.31 ± 19.25	78.31 ± 0.14	n.d.	31.65 ± 0.59	21.92 ± 1.83	7.42 ± 0.99	23.14 ± 2.04	18.62 ± 0.98	5.55 ± 0.16
		ns	***	***	***	***	**	***	ns	*		***	**	***	**	***	

Mean values of five measurements for each replicate.

Abbreviations: VJ, vegetable juice after pasteurisation; KLB, kefir-like beverage; CO_2, carbon dioxide; TTA, total titratable acidity; SSC, soluble solid content; TP, total phenol (gallic acid equivalent mg/L); DPPH, 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity (%); TAC, total anthocyanin content (mg/L cyanidin-3-glucoside equivalents); L*, lightness; a*, redness; b*, yellowness; H°, hue angle; ΔE, colour differences; n.d., not detectable.

Significant differences among vegetable juices and fermented kefir-like beverages for each vegetable sample and each physico-chemical determination: ***, $p \leq 0.001$, **, $p \leq 0.01$; *, $p \leq 0.05$; ns, not significant.

2004; Kyung & Fleming, 1997; Lemar et al., 2007).

LAB and TMC reached loads similar to those detected for other sugary kefirs (Liu & Lin, 2000; Sabokbar & Khodaiyan, in press). The statistical differences between the levels of cocci and rod LAB were not significant as also observed by other authors (Magalhães, Pereira, Dias, & Schwan et al., 2010). In particular, Irigoyen, Arana, Castiella, Torre, and Ibanez (2005) reported cell densities of 10^8 CFU/mL for lactobacilli and lactococci after two days of fermentation of milk kefir. The presence of yeasts in KLBs, except that made from onion juice, was approximately in the same levels registered for several kefir products (Liu & Lin, 2000; Sabokbar & Khodaiyan, in press).

3.2. *Identification of the dominant microorganisms*

The results of our investigation confirmed the commercial starter preparation to contain LAB and yeasts at 10^9 CFU/g. Leuconostocs and lactococci belonged to a single species, specifically *Leuconostoc mesenteroides* (Acc. No. KT633927) and *Lactococcus lactis* (Acc. No. KT633921), whereas dominant lactobacilli were allotted into two species: *Lactobacillus kefiri* (Acc. No. KT633919) and *Lactobacillus fermentum* (KT633923). Identification of all yeasts was as *S. cerevisiae* (Bankit 1853683). All the species identified in the starter culture are commonly found associated to kefir products (Cheirsilp, Shoji, Shimizu, & Shioya, 2003; Nambou et al., 2014; Witthuhn, Schoeman, & Britz, 2005) and were isolated from KLBs. Thus, our study demonstrated the commercial starter preparation to be suitable for the fermentation of the vegetable juices used in this study.

3.3. *Physico-chemical parameters*

The results of the chemical determinations are shown in Table 2. Melon juice displayed the highest pH (6.4). The high value can explain the high microbial counts observed for the resulting KLB.

Ethanol content ranged between 0.09 and 3.00% v/v. Onion KLB registered the lowest concentration and is a clear consequence of the scarce growth of *Saccharomyces*, in particular *S. cerevisiae* primarily responsible for alcohol production (de Melo Pereira, Ramos, Galvão, Souza Dias, & Schwan, 2010). According to the Italian legislation (GURI, 2001), due to their ethanol content above 1.2% v/v, strawberry, melon, carrot and tomato KLBs produced in this study are alcoholic beverages. The presence of ethanol is important for a kefir product because it confers the typical light alcoholic flavour (Beshkova, Simova, Frengova, Simov, & Dimitrov, 2003) and, together with the CO_2 mainly deriving from yeast fermentation, provides the final product with the desirable exotic notes and yeasty aroma (Guzel-Seydim, Seydim, & Greene, 2000). In this study, estimation of CO_2 production was as reported by several authors for different fermented matrices (Liu & Shen, 2008; Lombardi et al. 2004; Varga, Klinke, Réczey, & Thomsen, 2004), through weight loss. There was well correlation of this indirect measure with yeast development.

Detection of lactic acid was at the highest concentration for carrot (4.81 g/L) and melon (4.80 g/L) KLBs. The presence of acetic acid in all KLBs confirmed the metabolic heterogeneity (homofermentative and heterofermentative species) of LAB active in kefir products. Carrot KLB displayed the highest concentration of acetic acid. Acetic acid contributes to provide a pleasant taste to kefir and plays a role in the inhibition of the undesirable (spoilage and/or pathogenic) microorganisms (Puerari, Magalhães, & Schwan, 2012).

A strict correlation between the decrease of solid soluble content and the increase of ethanol, lactic and acetic acids and CO_2 formation was found. For carrot, melon and strawberry KLBs, the total titratable acidity increased with fermentation, while there was characterization of the other KLBs, especially tomato, by lower values than the corresponding VJs.

In general, the total phenol content decreased after fermentation, with the most consistent reduction (49%) recorded for fennel KLB. However, carrot and tomato KLBs showed a negligible increase. There was positive correlation of the total phenol content to the antioxidant activity for all samples, before and after fermentation, a phenomenon also observed by Dani et al. (2007). Detection of anthocyanins was only in onion and strawberry juices and KLBs. A relevant antioxidant activity was registered especially for strawberry KLB. The radical scavenging activity is positively associated to the content in anthocyans (Gil, Tomás-Barberán, Hess-Pierce, Holcroft, & Kader, 2000).

Regarding colour parameters, generally significant variations were registered between juices and KLBs, except those of strawberry. The total colour difference was calculated for each sample and ranged between 2.94 (carrot) and 11.55 (fennel). Considering the just noticeable differences limit of 2.3 (Mahy, Eycken, & Oosterlinck, 1994), all samples changed their colour, on a human perception scale, after the fermentation process. Fennel and melon KLBs registered the most noticeable changes.

3.4. *Volatile organic compounds (VOCs) of vegetable kefir-like beverages*

A total of 134 different volatile organic compounds were detected by SPME GC–MS (Table 3). KLBs were characterized by higher aromatic complexities than the corresponding VJs, because there was detection of several VOCs only after fermentation. Furthermore, there was registration of some molecules present in VJs to higher levels in KLBs. There is strong influence of the sensory profile of a fermented matrix by the active microorganisms (Arrizon, Calderón, & Sandoval, 2006). In particular, the acids increased in carrot, melon and strawberry KLBs. The last product showed a consistent increase of hexanoic and octanoic acids. Both these organic acids might be defining for the sensory evaluation of the fermented products carrying a refreshing flavour, unique aroma and texture. However, their effect depends on their amount (Duarte et al., 2010).

The fermentation increased the number and the concentration of the alcoholic molecules. Isoamylalcohol increased especially in fennel, melon, strawberry and tomato KLBs. The increase of volatile higher alcohols and the corresponding esters is a common phenomenon during kefir fermentation (Magalhães et al., 2011). In particular, the concentration of isoamylalcohol and 1-hexanol registered in this study were below 20 μg/L, the maximum concentration exerting a positive influence on the flavour of fermented beverages (Dragone, Mussatto, Oliveira, & Teixeira, 2009; Magalhães et al., 2011). Among alcohols, glycerol is the main secondary product of alcoholic fermentation led by *S. cerevisiae* (Puerari et al., 2012), but in this study, its detection was at concentrations too low to confer body and texture to KLBs (Dias, Schwan, Freire, & Serôdio, 2007).

The esters increased with fermentation especially in fennel, melon, strawberry and tomato KLBs. The major esters were ethyl hexanoate, octanoate and decanoate that have strong relations with fruity/floral/green aromas and yeasts mainly produce them (Nambou et al., 2014). Moreover, esters generally have a low odour threshold in fermented alcoholic beverages such as beer and wine (Saerens, Delvaux, Verstrepen, & Thevelein, 2010).

Detection of sulphur compounds was only in onion juice before and after fermentation. This result is not surprising, since *Allium* species are known to contain these compounds. The high concentrations of sulphur compounds help to explain the low levels of yeast detected in onion KLB. Kyung and Fleming (1997) reported

Table 3
Analysis of the volatile organic compounds of vegetable juices and kefir-like beverages.

Chemical compound (μg/L)	Carrot		Fennel		Melon		Onion		Strawberry		Tomato	
	VJ	KLB	VJ	KLB	VJ	KLB	VJ	KLB	VJ	KLB	VJ	KLB
Acids												
Acetic acid	35.31 ± 1.65	1239.85 ± 57.87	11.84 ± 0.09	236.38 ± 15.94	35.96 ± 1.55	711.86 ± 55.67	n.d.	n.d.	14.06 ± 0.32	230.27 ± 1.23	15.39 ± 0.59	490.37 ± 23.23
Propionic acid	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	3.85 ± 0.87	n.d.	n.d.
Isobutyric acid	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	32.12 ± 1.23	n.d.	n.d.
Hexanoic acid	n.d.	n.d.	n.d.	56.20 ± 0.56	n.d.	89.45 ± 2.43	n.d.	n.d.	17.13 ± 2.34	294.22 ± 8.72	32.27 ± 1.11	66.78 ± 4.89
Octanoic acid	n.d.	16.72 ± 0.67	n.d.	119.85 ± 9.67	n.d.	124.77 ± 2.25	n.d.	n.d.	n.d.	289.75 ± 11.34	4.77 ± 0.47	106.53 ± 4.79
Decanoic acid	0.56 ± 0.01	1.95 ± 0.06	n.d.	32.57 ± 1.34	n.d.	57.05 ± 3.43	n.d.	n.d.	n.d.	92.02 ± 4.10	n.d.	16.56 ± 0.51
Total	35.87 ± 1.66	1258.5 ± 58.60	11.84 ± 0.09	445.00 ± 27.51	35.96 ± 1.55	983.14 ± 63.78	n.d.	n.d.	31.19 ± 2.66	942.23 ± 27.49	52.43 ± 2.17	680.24 ± 33.42
Alcohols												
Isobutanol	n.d.	n.d.	n.d.	n.d.	n.d.	138.84 ± 11.39	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Isoamylalcohol	n.d.	194.83 ± 12.76	n.d.	522.07 ± 4.78	0.95 ± 0.02	2210.61 ± 54.67	n.d.	n.d.	n.d.	2468.84 ± 167.54	n.d.	675.81 ± 25.37
1-pentanol	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	5.32 ± 0.59	27.88 ± 2.54
1-hexanol	1.10 ± 0.07	63.55 ± 0.43	66.23 ± 0.67	28.82 ± 1.21	n.d.	25.99 ± 1.4	n.d.	11.80 ± 0.44	2.56 ± 0.11	527.40 ± 7.41	1142.32 ± 21.50	1648.35 ± 150.23
cis-3-hexen-1-ol	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	96.75 ± 0.49	96.77 ± 5.01
trans-2-hexenol	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1.95 ± 0.03	n.d.	121.40 ± 0.65	n.d.
1-octen-3-ol	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	6.19 ± 0.29	15.40 ± 0.70
5-hepten-2-ol, 6-methyl	n.d.	5.01 ± 0.34	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	22.04 ± 1.50	337.01 ± 11.94
2-ethylhexanol	2.47 ± 0.07	13.73 ± 0.09	n.d.	n.d.	3.32 ± 0.04	9.32 ± 0.03	n.d.	n.d.	6.25 ± 0.08	10.00 ± 0.05	2.18 ± 0.04	16.10 ± 0.99
4-hepten-1-ol	n.d.	n.d.	n.d.	n.d.	n.d.	3.70 ± 0.01	n.d.	n.d.	n.d.	3.98 ± 0.05	n.d.	6.03 ± 0.81
2,3-butanediol	n.d.	142.88 ± 2.99	19.74 ± 1.45	8.43 ± 0.23	n.d.	934.63 ± 33.67	n.d.	n.d.	n.d.	3.65 ± 0.04	n.d.	264.08 ± 19.97
1-octanol	2.65 ± 0.76	31.19 ± 2.46	n.d.	46.07 ± 3.54	n.d.	39.18 ± 2.45	n.d.	n.d.	n.d.	25.33 ± 1.56	71.88 ± 4.16	170.61 ± 12.13
Fenchyl alcohol	n.d.	n.d.	968.53 ± 77.82	2033.62 ± 87.22	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Furfuryl alcohol	0.69 ± 0.01	18.75 ± 1.11	3.32 ± 0.22	58.54 ± 4.56	10.85 ± 0.88	n.d.	n.d.	n.d.	4.77 ± 0.03	20.40 ± 2.22	1.58 ± 0.21	25.57 ± 0.04
Benzyl alcohol	n.d.	n.d.	n.d.	4.47 ± 0.22	1.36 ± 0.04	175.46 ± 12.54	n.d.	n.d.	0.24 ± 0.00	6.08 ± 0.45	n.d.	12.52 ± 1.51
Phenylethylalcohol	2.35 ± 0.03	54.34 ± 0.56	3.86 ± 0.11	68.80 ± 4.31	n.d.	393.97 ± 26.43	n.d.	n.d.	n.d.	215.72 ± 4.65	n.d.	255.27 ± 15.55
6,10-dimethyl 5,9-undecadien-2-ol	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	84.96 ± 3.34
Caryophyllene alcohol	0.71 ± 0.04	10.86 ± 0.34	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Glycerol	n.d.	n.d.	69.98 ± 1.98	11.81 ± 0.98	5.51 ± 0.04	n.d.	n.d.	n.d.	0.99 ± 0.00	8.18 ± 0.45	2.43 ± 0.21	45.78 ± 2.82
Total	9.97 ± 0.98	535.13 ± 21.08	1131.66 ± 82.25	2782.63 ± 107.05	21.99 ± 1.02	3931.70 ± 142.59	n.d.	11.80 ± 0.44	16.76 ± 0.25	3289.58 ± 184.42	1472.09 ± 29.64	3682.14 ± 252.95
Aldehydes												
Hexanal	3.68 ± 0.75	3.49 ± 0.87	9.32 ± 0.98	n.d.	17.41 ± 1.22	n.d.	n.d.	n.d.	9.59 ± 0.02	n.d.	164.72 ± 2.73	41.22 ± 1.08
2-methyl-2-penten-1-al	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1905.23 ± 22.41	978.32 ± 37.78	n.d.	n.d.	n.d.	n.d.
1-octanal	1.70 ± 0.02	4.30 ± 0.06	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	14.13 ± 0.25	34.21 ± 4.34
Nonanal	3.65 ± 0.04	21.45 ± 0.10	n.d.	6.16 ± 2.56	38.22 ± 0.76	63.77 ± 3.54	6.86 ± 0.34	9.20 ± 0.23	6.51 ± 0.03	52.22 ± 2.31	11.12 ± 0.38	102.68 ± 7.73
trans-2-octenal	1.55 ± 0.21	3.94 ± 0.12	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.56 ± 0.04	7.67 ± 0.45	68.53 ± 1.22	8.40 ± 0.32
4-methylbenzaldehyde	8.06 ± 0.16	61.25 ± 0.34	n.d.	n.d.	n.d.	n.d.	48.77 ± 3.54	180.13 ± 2.45	n.d.	n.d.	n.d.	n.d.
Decanal	n.d.	9.94 ± 1.12	n.d.	n.d.	n.d.	14.07 ± 1.11	n.d.	n.d.	1.86 ± 0.01	36.25 ± 0.92	2.46 ± 0.01	71.02 ± 4.90
Benzaldehyde	2.15 ± 0.36	6.43 ± 1.09	8.44 ± 0.76	12.52 ± 0.91	4.64 ± 0.23	18.22 ± 0.98	n.d.	n.d.	11.42 ± 0.04	45.38 ± 0.89	4.06 ± 0.19	15.88 ± 1.07
β-cyclocitral	4.71 ± 1.00	63.62 ± 3.98	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	17.57 ± 0.37	27.01 ± 3.29
Phenylethanal	n.d.	n.d.	n.d.	n.d.	n.d.	147.29 ± 2.23	n.d.	n.d.	n.d.	164.22 ± 22.63	38.43 ± 0.45	n.d.
Geraniol	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	23.48 ± 0.84	19.06 ± 3.20
Total	25.50 ± 2.54	174.42 ± 7.68	17.76 ± 1.74	18.68 ± 3.47	60.27 ± 2.21	243.35 ± 7.86	1960.86 ± 26.29	1167.65 ± 40.46	29.94 ± 0.14	305.74 ± 27.20	344.51 ± 6.44	319.48 ± 25.93
Several functional groups												
6-methoxymellein	2.66 ± 0.16	17.99 ± 0.45	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
2-pentylfuran	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	106.11 ± 3.20	19.58 ± 0.64
2-isobutylthiazole	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	221.77 ± 1.40	264.71 ± 11.39
Furfuraldehyde	1.75 ± 0.01	18.90 ± 1.34	n.d.	126.60 ± 1.23	14.94 ± 0.99	n.d.	n.d.	n.d.	9.71 ± 0.44	79.66 ± 0.67	5.66 ± 0.01	54.33 ± 0.44
5-methylfurfural	n.d.	n.d.	5.45 ± 0.38	21.70 ± 1.99	7.33 ± 0.43	n.d.	n.d.	n.d.	2.50 ± 0.12	10.16 ± 0.23	n.d.	n.d.
5-hydrxoymethylfurfural	2.85 ± 0.09	46.80 ± 1.65	20.41 ± 0.98	344.95 ± 19.76	66.50 ± 2.76	3.25 ± 0.01	n.d.	n.d.	30.96 ± 1.22	165.71 ± 4.56	8.92 ± 0.32	226.38 ± 8.23
Total	7.26 ± 0.26	83.69 ± 3.44	25.86 ± 1.31	493.25 ± 22.98	88.77 ± 4.18	3.25 ± 0.01	n.d.	n.d.	43.17 ± 1.78	255.53 ± 5.46	342.46 ± 4.93	565.00 ± 20.70
Esters												

(*continued on next page*)

Table 3 (*continued*)

Chemical compound (μg/L)	Carrot		Fennel		Melon		Onion		Strawberry		Tomato	
	VJ	KLB	VJ	KLB	VJ	KLB	VJ	KLB	VJ	KLB	VJ	KLB
Isoamylacetate	3.68 ± 0.21	7.45 ± 0.46	12.58 ± 1.11	7.50 ± 0.22	204.86 ± 9.43	956.35 ± 37.56	n.d.	n.d.	1.28 ± 0.03	67.58 ± 0.55	n.d.	112.48 ± 3.31
Methylhexanoate	n.d.	n.d.	n.d.	n.d.	5.14 ± 0.29	17.50 ± 2.34	n.d.	n.d.	8.38 ± 0.44	28.02 ± 0.54	n.d.	n.d.
Ethyl hexanoate	n.d.	n.d.	n.d.	79.11 ± 1.38	7.65 ± 0.57	944.53 ± 8.65	n.d.	n.d.	2.10 ± 0.10	2217.0 ± 117.33	4.61 ± 0.01	386.90 ± 27.05
Hexyl acetate	n.d.	n.d.	n.d.	n.d.	39.25 ± 2.56	84.20 ± 4.65	n.d.	n.d.	2.35 ± 0.20	2265.0 ± 116.51	12.02 ± 0.77	1067.01 ± 81.82
cis-3-hexenyl acetate	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.42 ± 0.01	38.73 ± 6.77
Ethyl heptanoate	n.d.	5.56 ± 0.35	n.d.	4.13 ± 0.11	n.d.	9.34 ± 0.76	n.d.	n.d.	n.d.	96.59 ± 1.61	n.d.	20.70 ± 1.18
Ethyl lactate	0.80 ± 0.01	18.41 ± 0.09	n.d.	9.92 ± 0.32	n.d.	17.47 ± 0.05	n.d.	n.d.	n.d.	n.d.	n.d.	83.86 ± 3.88
1-Heptyl acetate	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	139.89 ± 6.55	n.d.	n.d.
Methyloctanoate	n.d.	4.22 ± 0.34	n.d.	10.15 ± 0.87	n.d.	26.10 ± 0.09	n.d.	n.d.	n.d.	32.57 ± 1.61	n.d.	43.53 ± 2.06
Ethyl octanoate	100.35 ± 7.56	174.90 ± 9.98	303.35 ± 22.45	962.43 ± 13.45	25.00 ± 1.45	6271.7 ± 156.88	6.28 ± 0.08	6.79 ± 0.03	10.78 ± 1.34	13579.50 ± 550.11	26.03 ± 0.70	2564.51 ± 63.27
Isoamyl hexanoate	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	60.24 ± 2.45	n.d.	n.d.
Octyl acetate	n.d.	n.d.	n.d.	n.d.	n.d.	68.02 ± 9.78	n.d.	n.d.	n.d.	62.34 ± 3.65	n.d.	25.12 ± 1.33
Ethyl nonanoate	n.d.	7.26 ± 0.45	273.47 ± 21.56	40.40 ± 2.76	n.d.	51.81 ± 3.77	n.d.	n.d.	n.d.	109.96 ± 7.23	n.d.	11.26 ± 1.01
Isobutyloctanoate	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	23.13 ± 1.66	n.d.	n.d.
Isoamyl lactate	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	8.35 ± 0.07
Methyl decanoate	n.d.	n.d.	n.d.	n.d.	n.d.	21.20 ± 1.33	n.d.	n.d.	n.d.	15.59 ± 1.11	n.d.	24.02 ± 1.35
γ-butyrolactone	n.d.	n.d.	n.d.	9.60 ± 0.45	n.d.	n.d.	n.d.	n.d.	1.19 ± 0.09	5.11 ± 0.09	n.d.	n.d.
Ethyldecanoate	71.08 ± 3.28	109.87 ± 1.23	154.23 ± 9.45	346.78 ± 22.76	7.39 ± 0.49	3923.17 ± 231.99	n.d.	n.d.	3.30 ± 0.03	5087.62 ± 43.56	18.46 ± 0.01	1380.96 ± 93.47
Isoamyl octanoate	2.49 ± 0.16	n.d.	n.d.	n.d.	n.d.	14.00 ± 0.91	n.d.	n.d.	n.d.	79.97 ± 1.54	0.74 ± 0.02	15.06 ± 0.34
Ethyl-9-decenoate	15.54 ± 1.02	10.04 ± 0.79	n.d.	7.50 ± 0.45	n.d.	178.85 ± 1.02	n.d.	n.d.	0.62 ± 0.01	2156.25 ± 101.33	n.d.	45.51 ± 3.09
Phenylmethyl acetate	n.d.	n.d.	n.d.	n.d.	3.27 ± 0.32	81.55 ± 2.22	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
2(5H)-furanone	n.d.	n.d.	n.d.	18.81 ± 1.87	4.96 ± 0.11	n.d.	n.d.	n.d.	2.31 ± 0.02	11.44 ± 0.43	1.72 ± 0.03	n.d.
Methyl salicylate	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	6.56 ± 0.45	n.d.	5.76 ± 0.04
Phenylethylacetate	2.29 ± 0.18	4.37 ± 0.35	n.d.	n.d.	n.d.	203.49 ± 13.43	n.d.	n.d.	n.d.	92.57 ± 1.88	n.d.	39.49 ± 1.71
Ethyldodecanoate	9.97 ± 0.91	26.54 ± 3.04	67.78 ± 1.54	61.71 ± 0.92	n.d.	453.47 ± 11.09	n.d.	n.d.	2.36 ± 0.04	463.62 ± 11.33	6.02 ± 0.18	199.54 ± 15.61
Isoamyl decanoate	n.d.	n.d.	n.d.	n.d.	n.d.	6.63 ± 0.22	n.d.	n.d.	n.d.	20.79 ± 0.19	n.d.	n.d.
Ethyl dihydro-cinnamate	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	6.08 ± 0.99	n.d.	n.d.
Neryl propionate	61.43 ± 4.80	144.54 ± 11.47	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1.82 ± 0.04	n.d.
Ethyl tetradecanoate	1.27 ± 0.14	0.99 ± 0.02	3.33 ± 0.76	10.29 ± 0.34	n.d.	23.06 ± 0.78	n.d.	n.d.	0.67 ± 0.00	19.54 ± 1.33	2.00 ± 0.01	5.49 ± 0.18
Ethyl cinnamate	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	208.43 ± 8.66	n.d.	n.d.
2-Phenylethyl hexanoate	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1.84 ± 0.03
Elemicin	0.51 ± 0.26	2.73 ± 0.01	1.63 ± 0.11	3.07 ± 0.01	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Ethyl hexadecanoate	n.d.	n.d.	n.d.	n.d.	n.d.	36.42 ± 0.77	n.d.	n.d.	n.d.	n.d.	2.63 ± 0.29	32.16 ± 1.98
Phenylethyl octanoate	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1.91 ± 0.01	n.d.	n.d.
Coumaran	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	118.87 ± 5.77	n.d.	n.d.
Total	269.41 ± 18.53	516.88 ± 28.58	816.37 ± 56.98	1571.40 ± 45.91	297.52 ± 15.22	13,388.86 ± 488.29	6.28 ± 0.08	6.79 ± 0.03	35.34 ± 2.30	26976.17 ± 988.47	76.47 ± 2.07	6112.28 ± 309.55
Hydrocarbons												
Undecane	2.18 ± 0.26	20.92 ± 0.88	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Decane	n.d.	6.49 ± 0.33	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
1,3,8-p-menthatriene	5.58 ± 0.36	10.10 ± 0.98	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Total	7.76 ± 0.62	37.51 ± 2.19	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Ketones												
6-methyl-5-heptene-2-one	4.08 ± 0.83	24.22 ± 3.01	n.d.	n.d.	2.89 ± 0.01	12.91 ± 0.81	n.d.	n.d.	n.d.	8.76 ± 0.08	520.50 ± 5.62	266.79 ± 8.95
Acetoin	n.d.	26.50 ± 2.25	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	3.15 ± 0.04	24.14 ± 0.22
α-ionone	7.33 ± 1.23	55.30 ± 0.99	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	34.04 ± 0.34	n.d.
Geranylacetone	7.26 ± 0.23	95.15 ± 2.09	8.19 ± 0.43	20.06 ± 0.72	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	207.03 ± 11.55	220.53 ± 7.70
β-ionone	1.97 ± 0.60	31.44 ± 2.43	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	15.79 ± 0.42	32.17 ± 1.72
Hydroxyacetone	1.07 ± 0.02	9.16 ± 1.98	7.62 ± 0.45	50.78 ± 1.18	19.48 ± 0.99	n.d.	n.d.	n.d.	7.42 ± 0.01	39.94 ± 0.43	n.d.	n.d.
1-(3-ethylphenyl) ethanone	4.41 ± 0.03	7.27 ± 0.91	10.44 ± 0.23	14.72 ± 1.77	4.44 ± 0.22	7.52 ± 0.88	13.14 ± 0.98	10.41 ± 0.03	2.03 ± 0.01	10.19 ± 0.31	2.46 ± 0.08	9.10 ± 0.09
Total	26.12 ± 2.94	249.04 ± 13.66	26.25 ± 1.11	85.56 ± 3.67	26.81 ± 1.22	20.43 ± 1.69	13.14 ± 0.98	10.41 ± 0.03	9.45 ± 0.02	58.89 ± 0.82	782.97 ± 18.06	552.73 ± 22.67

Phenols												
Phenol	4.14 ± 0.37	33.76 ± 1.45	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.84 ± 0.01	4.87 ± 0.75
p-cresol	1.20 ± 0.11	1.34 ± 0.01	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Eugenol	0.49 ± 0.02	5.89 ± 0.08	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Tymol	n.d.	1.93 ± 0.23	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	2.56 ± 0.10	n.d.	n.d.
Total	5.83 ± 0.50	42.92 ± 1.77	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	2.56 ± 0.10	0.84 ± 0.01	4.87 ± 0.75
Sulphur compounds												
Methyl disulfide	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	222.79 ± 9.32	232.79 ± 2.35	n.d.	n.d.	n.d.	n.d.
2,4-dimethylthiophene	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	16.61 ± 1.11	15.52 ± 0.22	n.d.	n.d.	n.d.	n.d.
Methyl propyl disulfide	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	42.81 ± 0.45	116.03 ± 5.54	n.d.	n.d.	n.d.	n.d.
3,4-dimethylthiophene	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	921.42 ± 5.61	706.74 ± 6.99	n.d.	n.d.	n.d.	n.d.
2,5-dimethylthiophene	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	181.11 ± 14.45	158.00 ± 2.34	n.d.	n.d.	n.d.	n.d.
1,3-dithiane	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	3147.54 ± 271.11	2692.06 ± 176.67	n.d.	n.d.	n.d.	n.d.
Dimethyl trisulfide	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	5686.36 ± 321.23	7369.83 ± 53.67	n.d.	n.d.	n.d.	n.d.
Diallyl disulphide	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	76.54 ± 4.01	75.23 ± 0.99	n.d.	n.d.	n.d.	n.d.
Total	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	10295.18 ± 627.39	11366.20 ± 248.77	n.d.	n.d.	n.d.	n.d.
Aromatic hydrocarbons												
Styrene	n.d.	6.66 ± 0.43	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	2545.58 ± 41.11	n.d.	1.69 ± 0.01
p-cymene	120.20 ± 4.98	196.31 ± 12.89	1009.28 ± 39.51	735.27 ± 13.54	0.70 ± 0.01	n.d.	n.d.	n.d.	n.d.	n.d.	1.76 ± 0.02	n.d.
2,5-Dimethylstyrene	46.42 ± 2.63	153.22 ± 11.37	6.43 ± 0.23	6.33 ± 0.23	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Total	166.62 ± 7.61	356.19 ± 24.69	1015.71 ± 39.74	741.60 ± 13.77	0.70 ± 0.01	n.d.	n.d.	n.d.	n.d.	2545.58 ± 41.11	1.76 ± 0.02	1.69 ± 0.01
Terpenes and terpenoids												
Camphene	2.69 ± 0.76	8.76 ± 0.23	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
β-pinene	20.04 ± 3.09	26.95 ± 0.21	11.66 ± 0.80	6.71 ± 0.23	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
α-phellandrene	111.85 ± 4.11	77.77 ± 3.87	8.15 ± 0.23	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Linalool	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	28.43 ± 1.44	388.76 ± 13.45	n.d.	n.d.
β-myrcene	175.07 ± 8.68	270.50 ± 11.65	73.67 ± 0.90	68.60 ± 2.45	n.d.	n.d.	n.d.	n.d.	n.d.	1586.53 ± 57.51	1.81 ± 0.03	n.d.
α-terpinene	41.44 ± 1.44	101.39 ± 11.48	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
D-limonene	100.25 ± 3.52	176.49 ± 11.21	3321.36 ± 88.45	4271.66 ± 83.56	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	8.09 ± 0.04	28.26 ± 0 0.33
Terpinen-4-ol	3.61 ± 0.14	345.68 ± 10.43	n.d.	69.61 ± 2.23	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
α-terpineol	1.39 ± 0.19	40.67 ± 2.78	n.d.	15.43 ± 0.99	n.d.	n.d.	n.d.	n.d.	n.d.	7.50 ± 0.32	n.d.	n.d.
Estragole	12.15 ± 1.68	6.05 ± 0.43	396.49 ± 25.56	760.24 ± 33.45	0.80 ± 0.03	8.19 ± 1.23	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
β-phellandrene	14.13 ± 1.13	17.14 ± 0.23	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
γ-terpinene	110.87 ± 2.06	609.83 ± 14.49	634.46 ± 24.13	559.09 ± 45.90	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
3-carene	20.00 ± 1.56	40.87 ± 3.78	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
α-terpinolene	606.71 ± 25.03	1563.34 ± 99.32	n.d.	145.16 ± 11.33	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	7.62 ± 0.03	n.d.
Citronellol	n.d.	n.d.	1.75 ± 0.01	7.60 ± 2.34	n.d.	n.d.	n.d.	n.d.	n.d.	3.42 ± 0.99	6.30 ± 0.02	35.81 ± 11.64
Geraniol	6.24 ± 0.47	35.66 ± 0.47	n.d.	2.48 ± 0.05	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	3.18 ± 0.04	14.06 ± 0.16
trans-carveol	n.d.	n.d.	44.03 ± 9.98	4.50 ± 0.08	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
β-caryophyllene	679.39 ± 20.23	3778.30 ± 84.69	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	56.65 ± 2.43	n.d.
Nerolidol	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	41.58 ± 0.65	288.13 ± 10.23	n.d.	n.d.
β-farnesene	19.00 ± 0.31	91.33 ± 5.89	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Anethol	40.84 ± 1.17	155.58 ± 11.43	6092.43 ± 93.78	16628.19 ± 345.65	n.d.	80.01 ± 1.37	66.98 ± 3.45	97.42 ± 0.67	18.73 ± 0.43	99.10 ± 2.76	14.46 ± 0.82	83.54 ± 12.50
β-himachalene	18.92 ± 0.14	12.89 ± 0.98	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
α-zingiberene	2.53 ± 0.04	26.13 ± 0.67	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
α-himachalene	123.90 ± 1.97	375.35 ± 14.76	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Myristicin	48.46 ± 1.89	146.63 ± 11.00	99.14 ± 11.12	139.02 ± 22.45	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
α-cedrene	15.15 ± 0.81	25.27 ± 3.54	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	15.26 ± 1.44	n.d.	n.d.
δ-guaiene	864.86 ± 15.62	2699.14 ± 9.22	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	2.16 ± 1.36	40.20 ± 0.09	52.83 ± 3.45	n.d.
Curcumene	115.61 ± 3.74	135.79 ± 11.11	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.32 ± 0.02	5.67 ± 0.01	5.22 ± 0.04	n.d.
γ-bisabone	83.16 ± 3.38	334.59 ± 23.89	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	4.03 ± 0.09	4.72 ± 0.44	n.d.
Total	3238.26 ± 103.36	11102.10 ± 347.76	10683.14 ± 254.96	22678.29 ± 550.71	0.80 ± 0.03	88.20 ± 2.60	66.98 ± 3.45	97.42 ± 0.67	91.22 ± 3.90	2438.60 ± 86.89	160.88 ± 7.34	161.67 ± 24.63

The chemicals are grouped per chemical class.

Results indicate mean values of three measurements ± S.D.

1-heptanol was used as internal standard.

Abbreviations: VJ, vegetable juice; KLB, kefir-like beverage; n.d. not detected.

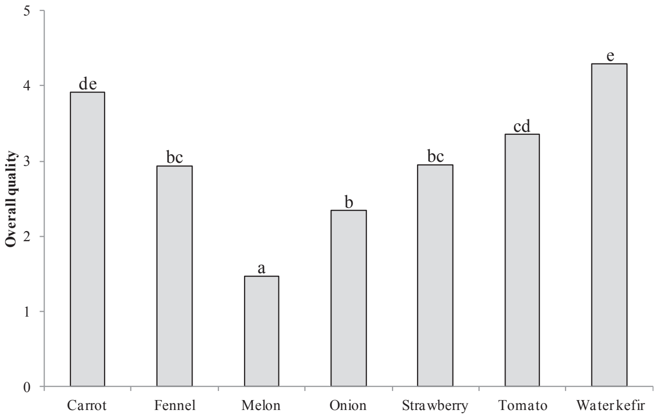

Fig. 1. Sensory evaluation of vegetable-based kefir-like beverages. Bars with the same letter are not statistically different at P < 0.05 (Tukey–Kramer's multiple range test).

that the sulphur compounds derived from cabbage showed inhibitory activities against yeasts. They found that 20 ppm of dimethyl trisulfide inhibited several strains of different species, such as *S. cerevisiae* Y6, *Torulopsis etchellsii* Y24, *Hansenula mrakii* Y27 and *Pichia membranefaciens* Y20. The same authors found that dimethyl disulphide retarded the growth of *S. cerevisiae*. Other compounds of this chemical class, such as diallyl trisulfide, diallyl tetrasulfide and dimethyl trisulfide, inhibit several yeasts at concentrations ranging between 2 and 45 ppm (Kim et al., 2004). The amount of dimethyl trisulfide detected in onion KLB in this work is more than 160 folds higher than the minimum inhibitory concentration reported by Kim et al. (2004). The mechanisms of action of diallyl disulphide has been studied against *Candida*; the compound is able to trigger cell death most probably by eliciting oxidative stress as a consequence of thiol depletion and impaired mitochondrial function (Lemar et al., 2007).

Detection of terpene and terpenol compounds was in fennel and carrot KLB at very high concentrations compared to those of the corresponding VJs. Anethol increased in all samples, but consistently in fennel KLB. Strawberry KLB showed an increase of β-myrcene.

3.5. Overall quality

Fifteen untrained tasters were asked to judge the overall quality of the KLBs and Fig. 1 shows the results. A water kefir was prepared with the same microbial mixture according to the producer's instructions and used as control kefir for panellists. Only carrot KLB showed an overall quality evaluation comparable with that of water kefir. However, tomato KLB did not significantly differ from carrot KLB.

4. Conclusions

Taking into account the increasing complexity of the needs of different typologies of consumers, including vegan vegetarian and subjects with intolerance/allergy to dairy products, we applied an integrated technological approach in this work to obtain kefir-like beverages from an updated selection of vegetable substrates, using commercial water kefir microorganisms. Analysis were performed to address their microbial composition, physico-chemical characteristics and sensory profile, in order to evaluate the preservation of vegetable and kefir synergistic properties that exert benefits to the human health in the final beverages and test their appreciation by potential consumers.

We developed new-functional non-dairy beverages whose heterogeneous microbial characteristics reflected the same codominance of LAB and yeasts typical of traditional milk or water kefirs. In addition, physico-chemical and organoleptic properties of some vegetable-based KLBs, especially carrot KLB, well met the expectations and tastes of panellists. The beverages produced in this work may help to link the gap between the actual and an ideal and innovative consumption of vegetables, recommended in human diet. Characterization of some KLBs was by the presence of molecules with antioxidant activity giving an additional benefit to the experimental products, suggesting their production at large scale as healthy products, satisfying a wider range of consumers and showing a new way of vegetable administration. The new products might represent important foods providing live microorganisms to vegan people with a limited availability of fermented products.

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