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A new functional kefir fermented beverage obtained from fruit and vegetable juice: Development and characterization

Jorge Luís Paredes, María Luisa Escudero-Gilete *, Isabel María Vicario

Food Colour and Quality Laboratory, Facultad de Farmacia, Universidad de Sevilla, 41012, Sevilla, Spain

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ABSTRACT

A juice containing a mixture of fruits and vegetables (70% apple, 9% strawberry, 12% carrot and beet 9%) was evaluated as a potential substrate for the production of a novel probiotic beverage made with kefir grains. The effects of the kefir grains amount (1–4%, w/v) and fermentation time (12, 24 and 48 h) on the beverage composition, sensory qualities and colour were investigated. The results indicated that the amount of kefir grains have a significant effect on the content of the organic acids (lactic, acetic, citric, succinic, and malic acid), CO_2 production, acidity, and viscosity and colour parameters (lightness (L^*), hue (h_{ab}) and Chroma (C^*_{ab})). Fermentation time also significantly affected all the parameters analyzed in the samples. The most suitable conditions to achieve the highest overall acceptability for the fermented beverage based on a mix of fruits and vegetable juice was: 2% (w/v) kefir inoculum during 24 h of fermentation time.

1. Introduction

Covid_19 pandemic has increased the consumers' interest in functional foods that provide health benefits related to immune system and stress, thereby driving the growth of the functional food market (The Business Research Company, 2021). Among these products are probiotics which exert a beneficial effect on the intestinal functions and may be able to prevent several diseases due to the living microorganisms (De Las Cagigas & Blanco, 2002). Kefir is an ancient fermented beverage associated with longevity in the Caucasus (Cevikbas et al., 1994), to which beneficial health effects such as reduction of symptoms of lactose intolerance, stimulation of the immune system, cholesterol-lowering, anti-mutagenic and anti-carcinogenic properties are attributed (Güzel-Seydim, Seydim, Greene, & Taş, 2006).

Kefir is a symbiotic medium of microorganisms characterized by presenting a mass composed of proteins, lipids and a soluble polysaccharide called kefiran where its microbiota, a spectrum of lactic acid bacteria, yeasts, and acetic bacteria, is found. The microbiota of kefir grains is formed by sugar-fermenting microorganism that produces lactic acid, alcohol, CO_2, B-complex vitamins, and other organic acids in their metabolism. The overall metabolic capacity of this consorcium is the most important characteristic of stable water Kefirs (Gulitz, Stadie, Wenning, Ehrmann, & Vogel, 2011; Stadie, Gulitz, Ehrmann, & Vogel, 2013).

Although dairy products are the best substrate for probiotics, there are some drawbacks related to milk composition like hypersensitivities (allergies or intolerance). In Europe, an average prevalence of milk protein allergy of 6–8% in children and 2% in adults is estimated (Álvarez Berciano & Álvarez Caro, 2008) while lactose intolerances affect one-third of the world population. Moreover, it is estimated that 600 million people in the world are vegetarian while 2%, 4% and 1% of the US, Swedish and German population are vegans. The vegetarian and vegan product industries are growing around 10% per year (Choudhary & Jadoun, 2014). These are some of the reasons why fermented non-dairy beverages have begun to play an important role in the diet of consumers who are hypersensitive to milk proteins (Álvarez Berciano & Álvarez Caro, 2008), lactose intolerant (FEN, 2013), vegetarians and vegans (Choudhary & Jadoun, 2014) also new studies on lactic acid fermentation of fruit juices are being conducted to bio-enriched in selenium (Crespo et al., 2021; Gaglio et al., 2021).

The juices of fruits and vegetables are rich in sugars that can be a fermentative substrate for kefir (Alves et al., 2021; Bueno et al., 2021). Many studies have linked consumption of fruits and vegetables with a reduction of the risk for several chronic diseases, such as cancer, cardiovascular diseases, cataracts, or immune dysfunction (Baines & Seal, 2012; Garcia, Guerin, Souidi, & Remize, 2020). These natural protective effects have been attributed to the antioxidant potential of several components, such as carotenoids, betalains, vitamins, polyphenols, and

* Corresponding author.
E-mail address: gilete@us.es (M.L. Escudero-Gilete).

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other phytochemicals. Some of these compounds, including betaines or carotenoids, have health claims authorized under Article 13(1)-Regulation (EC) No-1924/2006 by the European Food Safety Authority (EFSA). Thus, fermented vegetables and fruit juices are a promising approach for therapeutic foods (Noğay, 2019). Currently, the development of functional beverages based on fruit juices with probiotics has increased due to their health benefits and good acceptability by consumers of all ages (Tesfaye, Suarez-Lepe, Loira, Palomero, & Morata, 2019).

The work aimed to formulate a kefir beverage obtained by fermentation of commercial fruit and vegetable juice (Veggie) under different conditions, evaluate the influence of the fermentation conditions on the final composition and some bioactive components and finally evaluate its acceptance.

2. Materials and methods

2.1. Samples

The juice selected was an industrial product to assure a standard quality and minimize variations. A commercial juice from the same lot (Lot-120738) of Veggie brand, made of apple extract (70%), carrot (12%), beetroot (9%), and strawberry (9%), was used as base beverage. This juice was selected because of the beetroot in the composition, which, besides an attractive red colour, contains betaine a compound with a health claim authorized under Article 13(1)-Regulation (EC) No-1924/2006 "contributes to the normal metabolism of homocysteine" (Regulation (EU) 432/2012).

Kefir grains were obtained from a donor. The samples of Kefir grains were preserved in sterilized milk, renewed daily for two months. These grains were washed with sterile distilled water and subsequently used to inoculate the commercial juice.

Based on previous studies by Corona et al. (2016) and Sabokbar, Moosavi-Nasab, and Khodaiyan (2015), two different variables were considered: the kefir grains concentration and the fermentation time: four 1%, 2%, 3% and 4% w/v and three levels: 12, 24 and 48 h were considered respectively 12 different samples were prepared (Table 1). The whole experiment was done in triplicate under the same conditions.

Fermentation temperature was controlled at 26 °C. The fermented beverages were sampled to evaluate the physicochemical parameters. The samples for the sensory evaluation test were kept under refrigeration (4 °C) and in a modified atmosphere (with nitrogen addition) to prevent changes in the organoleptic properties. Fermented beverages were analyzed in triplicate using the commercial juice as the control sample.

2.2. Physicochemical determinations

pH, *total titratable acidity* and *soluble solid content* were performed according to the methodology proposed by the AOAC (2000).

Viscosity was calculated as per ASTM standard methods D 445 and D 2515 using a Cannon-Fenske viscometer at 25 °C. The absolute viscosity (η) was calculated as η = ctd, where c is the constant: 0,0084295 (at 50 °C), t (s) is the efflux time, and d (g/mL) is the sample density.

Density (g/mL). 25 mL of sample was weighed, and weight was divided by volume.

Table 1
Sample coding according to fermentation time and % of Kefir added.

Kefir concentration (% w/v)	Fermentation time (hours)		
	12	24	48
1%	1K12H	1K24H	1K48H
2%	2K12H	2K24H	2K48H
3%	3K12H	3K24H	3K48H
4%	4K12H	4K24H	4K48H

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

The content of *lactic, acetic, citric, malic and succinic acids* was determined by an HPLC method according to Zaky, Pensupa, Andrade-Eiroa, Tucker, and Du (2017) with modifications. Analyses were carried out in an Agilent 1100 chromatograph (Agilent Technology, Palo Alto, CA, USA) equipped with a diode-array detector, which was set to scan from 200 to 770 nm. The analysis conditions were: separation column Hi-plex-H (8 μm, 300 × 7.7 mm), $T^a = 30$ °C, mobile phase 5 mM sulphuric acid and 0.8 mL/min flow rate, injection volume = 50 μL.

Organic acids were identified by their retention time, UV–vis spectra, and comparison with external standards. They were quantified by external calibration with calibration curves constructed with external standards.

Total phenolic content was determined using Folin-Ciocalteu assay (Singleton & Rossi, 1965). The absorbance was read at 765 nm with a Hewlett-PackardUV–vis HP8453 spectrophotometer (Palo Alto, CA, USA). Gallic acid was employed as a calibration standard and results were expressed as gallic acid equivalents (mg GAE/L).

The *antioxidant activity* was analyzed according to *ABTS/persulphate assay*. The $ABTS^{\bullet+}$ radical was produced by the oxidation of 7 mM ABTS with potassium persulphate (2.45 mM) in water and allowed to stand in the dark at room temperature for 16 h before use. The $ABTS^{\bullet+}$ solution was diluted with phosphate-buffered saline (PBS) at pH 7.4 to give an absorbance of 0.7 ± 0.02 at 734 nm. 50 μL of sample was mixed with 2 mL of $ABTS^{\bullet+}$ diluted solution, vortexed for 30s, and the absorbance measured at 734 nm after 4 min of reaction at 30 °C.

The results were expressed as Trolox-equivalent antioxidant capacity (TEAC; μmols of Trolox with the same antioxidant capacity as 1 L of the studied sample) by interpolating the absorbance on a Trolox calibration curve (30–1000 μM).

Colour was measured by diffuse reflectance with a spectrophotometer CM-5 (Konica Minolta Sensing Americas, Inc., NY). Each sample was placed in a tube cell of optical glass (CR-A506 Tube Cell Ø60/60 mm depth). The Illuminant D65 and the 10° observer were considered as references. The colour parameters corresponding to the uniform colour space CIELAB (L^*, a^*, b^*, C^*_{ab} and h_{ab}) (CIE, 1986) were obtained directly from the apparatus. Lightness (L^*) oscillates between 0 (black) and 100 (white). The coordinate a^* take positive values for reddish colours and negative values for greenish ones, and b^* takes positive values for yellowish colours and negative values for bluish ones. Additionally, two psychological parameters, hue (h_{ab}) and chroma (C_{ab}^*) are defined, which are related to a^* and b^* as follows: $C^*_{ab} = [(a^*)^2 + (b^*)^2]^{1/2}$; $h_{ab} = \arctan(b^*/a^*)$. C^*_{ab} is regarded as the quantitative attribute of colourfulness and enables to determine for each hue its degree of difference relative to grey colour with the same lightness. Hue angle (h_{ab}) takes values from 0° to 360° and is the qualitative attribute that allows any colour to be described as bluish, reddish, etc. This parameter allows any colour to be differentiated from a grey one with the same lightness. The colour differences (ΔE^*_{ab}) between two colours in the CIELAB space are calculated as the Euclidean distance between their locations in the three-dimensional space defined by L^*, a^* and b^*: $\Delta E^*_{ab} = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$

2.3. Sensory evaluation

The beverages were subjected to sensory evaluation. 10 mL aliquots of samples were served, in random order, in clear cups (30 mL volume) covered with Petri dishes and marked with three-digit random numbers. A glass of water was offered to the judges between samples to rinse their mouths. The assessments were carried out in individual booths (70 × 70 × 55 cm) at room temperature and under white light.

Two sensorial analyses were carried out to select the more preferred sample (Fig. 1). Either a trained or a consumer panel was used

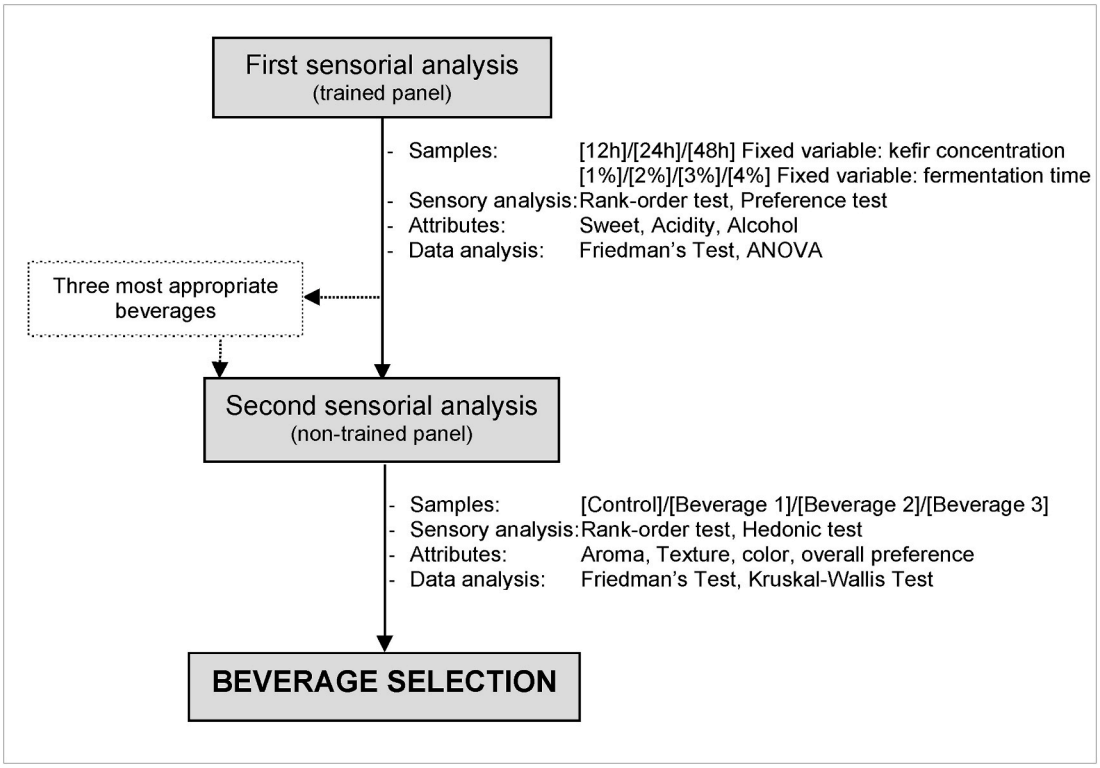

Fig. 1. Flow diagram of the sensory analyses leading to the more preferred beverage.

depending on the purpose of the analysis:

- The first sensory sessions were aimed at assessing the effect of fermentation time and kefir concentration on the sensory properties of the beverages and selecting the 3 most preferred products. A comprehensively trained 15-judge panel (9 women and 6 men, 23–55 years old) with long expertise in the sensory analysis of food was recruited among academic staff. Panellists were given 3 to 4 samples in random order in 7 different sessions. The effect of the fermentation time (4 sessions, 3 samples/session) and percentage of inoculation of kefir on the juice (3 sessions, 4 samples/session) were considered. Rank-order test and preference test were the two procedures used in each session. The panellist rank-ordered the samples according to sweetness, acidity, and alcohol; in addition, they had to indicate the most preferred sample.
- Once the three most appropriate beverage were singled out, they were submitted to a second sensory analysis with the objective of selecting the product with the best acceptance. The non-trained panel consisted of thirty tasters randomly recruited at the university campus (18 women and 12 men, between 21 and 54 years old). Hedonic test and rank-order test were the two procedures used. In the hedonic test, judges were asked to evaluate how much they liked the beverages using a 7-points hedonic scale (1 = 'Strongly disliked'; 2 = 'Moderately disliked'; 3 = 'Slightly disliked'; 4 = 'Indifferent'; 5 = 'Slightly liked'; 6 = 'Moderately liked' and 7 = 'Strongly liked') to determine if there were differences between products in judge's evaluation (Drake, 2007; Granato et al., 2010). Additionally, a rank-order test was carried out to evaluate the overall preference considering attributes such as aroma, texture and colour.

2.4. Statistical analysis

All experiments were done in triplicate and the data are presented as the mean and standard deviation of three independent experiments. The statistical analysis of data was performed by one-way analysis of variance (ANOVA), and statistically significant differences ($p < 0.05$) were determined using the Tukey multiple comparison test (Norman & Streiner, 1996).

Friedman's test (analysis of variance by ranks) was carried out when the variables were measured in terms of categories. As recommended by the ISO 8587, the Friedman test (Friedman, 1937) is for comparing three or more related samples and makes no assumptions about the underlying data distribution. The statistic of Friedman test for each sample was compared to critical values calculated according to Hollander and Wolfe (1973). When according to the Friedman's test statistically significant differences among samples were found, the pairs of differing samples were identified using an analogue of Fisher's least significant difference (Fisher, 1922).

Consumer data first underwent normality testing (Shapiro-Wilk test) and were subsequently analyzed using nonparametric tests (Kruskal-Wallis) to identify differences among samples.

To evaluate the influence of a selection of physicochemical parameters on the sensorial evaluation, the data were analyzed by multiple regression analysis.

All statistical analyses were performed using the program Statistica 8 for Windows (StatSoft, 2007).

3. Results and discussion

3.1. Physicochemical properties

Table 2 shows the results of the physicochemical analyses.

The metabolism rate of fermentable sugars increased with the fermentation time, decreasing the *content of soluble solids (°Brix)*. The highest decrease in °Brix was observed in the beverage fermented with 1% w/v kefir grain during 48 h (from 10.91 to 3.62 °Brix) and the lowest in the samples fermented during 12 h (from 10.91 to 10.27 °Brix) (Table 2). Corona et al. (2016) reported a sugar reduction (in °Brix) in kefir-fermented juices varying from 6.22 in melon and 4.77 in carrot to 3.48 in strawberry juices. Similar results were reported by Randazzo

Table 2
Physico-chemical analysis of samples (mean ± standard deviation) fermented with different kefir inoculum concentrations (1%, 2%, 3% and 4% w/v), during different fermentation times (12, 24 and 48 h).

Fermentation Time	0H	12H				24H				48H			
%Kefir Inoculum	C	1%	2%	3%	4%	1%	2%	3%	4%	1%	2%	3%	4%
SSC (°Brix)	10.91 ± 0.21 1a	10.27 ± 0.13 2b	9.79 ± 0.11 2c	10.10 ± 0.12 2b	9.83 ± 0.10 2c	9.93 ± 0.04 3b	9.87 ± 0.04 2b	8.69 ± 0.04 3c	9.19 ± 0.36 3c	3.62 ± 0.04 4b	3.81 ± 0.19 3bc	4.13 ± 0.18 4c	4.06 ± 0.16 4c
CO_2 (g/100 mL)	n.d 1a	0.45 ± 0.07 2bc	0.52 ± 0.02 2b	0.46 ± 0.03 2c	1.16 ± 0.03 2d	1.23 ± 0.02 3b	0.70 ± 0.01 3c	0.68 ± 0.02 3c	1.35 ± 0.02 3d	4.09 ± 0.01 4b	4.28 ± 0.04 4c	4.29 ± 0.03 4c	4.31 ± 0.03 4c
pH	3.80 ± 0.04 1a	3.65 ± 0.01 2b	3.60 ± 0.01 2c	3.62 ± 0.02 2bc	3.53 ± 0.03 2d	3.70 ± 0.03 2b	3.67 ± 0.03 3bc	3.65 ± 0.01 2bc	3.64 ± 0.01 3c	3.48 ± 0.02 3b	3.41 ± 0.01 4c	3.40 ± 0.03 3c	3.39 ± 0.02 4c
TTA (g/L Malic Acid)	4.00 ± 0.08 1a	4.18 ± 0.04 2b	4.40 ± 0.10 2c	5.19 ± 0.05 2d	5.27 ± 0.04 2d	4.78 ± 0.08 3b	5.36 ± 0.13 3c	5.64 ± 0.07 3d	6.28 ± 0.27 3e	5.49 ± 0.07 4b	6.12 ± 0.14 4c	6.95 ± 0.29 4d	6.86 ± 0.10 4d
Viscosity (CP)	2.78 ± 0.30 1a	3.47 ± 0.13 2b	2.97 ± 0.17 1acd	2.92 ± 0.02 2c	2.80 ± 0.02 1d	3.40 ± 0.03 2b	2.55 ± 0.01 2c	2.64 ± 0.01 3d	2.78 ± 0.01 1e	2.66 ± 0.04 3b	2.52 ± 0.01 2c	2.53 ± 0.11 3bc	2.52 ± 0.04 2c
Density (g/mL)	1.0287 ± 0.0025 1a	1.0283 ± 0.0006 12a	1.0280 ± 0.0017 12ab	1.0247 ± 0.0021 2b	1.0267 ± 0.0015 1ab	1.0230 ± 0.0035 2b	1.0263 ± 0.0012 2b	1.0257 ± 0.0012 2b	1.0267 ± 0.0021 1ab	1.0137 ± 0.0006 3b	1.0087 ± 0.0067 3b	1.0140 ± 0.0020 3b	1.0130 ± 0.0069 2b
TP (mg/L)	1051.97 ± 14.47 1a	1046.69 ± 42.20 1a	1081.83 ± 44.29 1a	1276.22 ± 34.11 2b	1228.22 ± 33.80 2b	1155.28 ± 24.88 2b	1381.03 ± 39.30 2c	1065.17 ± 31.16 1a	1151.44 ± 36.95 2b	1046.25 ± 26.29 1a	1167.89 ± 27.24 3b	1061.50 ± 13.39 1a	853.39 ± 30.20 3c
ABTS (umoles/L)	684.33 ± 7.51 1a	443.24 ± 7.61 2b	370.82 ± 59.93 2bcd	344.42 ± 5.50 2c	421.61 ± 6.09 2d	341.55 ± 7.79 3b	420.37 ± 9.95 2c	419.35 ± 6.03 3c	391.39 ± 10.42 3d	427.87 ± 7.53 2b	490.69 ± 5.06 3c	444.21 ± 5.52 4d	447.19 ± 4.71 4d
Lactic Acid (mg/L)	n.d 1a	289.61 ± 15.68 2b	631.00 ± 26.67 2c	899.49 ± 20.04 2d	1479.92 ± 22.85 2e	515.45 ± 20.72 3b	1099.94 ± 23.95 3c	1609.50 ± 71.12 3d	2042.28 ± 9.75 3e	559.11 ± 60.65 3b	1094.25 ± 71.62 3c	2739.09 ± 70.75 4d	3250.47 ± 76.87 4e
Acetic Acid (mg/L)	n.d 1a	n.d 1a	n.d 1a	62.07 ± 6.65 2d	85.40 ± 20.24 2d	n.d 1a	37.01 ± 3.79 2b	96.39 ± 8.02 3c	98.32 ± 15.66 2c	n.d 1a	60.73 ± 4.52 3b	248.49 ± 25.14 4c	266.81 ± 16.79 3c
Citric Acid (mg/L)	899.86 ± 68.53 1a	682.86 ± 12.34 2b	614.80 ± 39.26 2c	542.84 ± 19.61 2d	498.22 ± 18.64 2e	607.55 ± 23.45 3b	543.96 ± 10.76 3c	499.33 ± 22.84 2d	496.54 ± 27.85 2cd	594.17 ± 21.78 3b	601.42 ± 36.45 23bc	567.95 ± 49.34 2bc	532.80 ± 27.21 2c
Succinic Acid (mg/L)	234.43 ± 33.66 1a	249.39 ± 11.25 1b	352.99 ± 16.44 2c	279.29 ± 14.45 1a	201.32 ± 22.73 2d	364.73 ± 21.33 2b	273.95 ± 11.55 1a	287.84 ± 15.14 1a	267.54 ± 30.90 1a	380.75 ± 24.26 2b	264.34 ± 12.82 1a	369.01 ± 31.61 2b	379.69 ± 12.82 3b
Malic Acid (mg/L)	832.37 ± 55.27 1a	874.41 ± 29.00 2b	871.86 ± 17.76 2b	1005.01 ± 90.37 2b	1190.39 ± 39.91 2c	1421.63 ± 66.89 3b	1105.66 ± 47.48 3c	1449.03 ± 33.99 3b	1210.14 ± 37.56 2d	1552.87 ± 63.45 3b	2244.70 ± 64.14 4c	1728.69 ± 17.24 4d	1759.90 ± 26.76 3d
L*	29.52 ± 0.07 1a	28.12 ± 0.01 2b	28.36 ± 0.04 2c	29.27 ± 0.07 2d	28.56 ± 0.06 2e	29.15 ± 0.09 3b	30.52 ± 0.05 3c	30.16 ± 0.46 1acd	30.36 ± 0.05 3d	31.77 ± 0.05 4b	31.27 ± 0.40 4bc	31.68 ± 0.10 3b	30.92 ± 0.03 4c
C^*_{ab}	17.64 ± 0.06 1a	13.51 ± 0.02 2b	14.37 ± 0.10 2c	17.04 ± 0.38 2d	14.85 ± 0.05 2e	16.72 ± 0.27 3b	19.42 ± 0.12 3c	19.03 ± 0.17 3d	19.15 ± 0.60 3cd	22.53 ± 0.05 4b	22.63 ± 0.53 4bc	22.78 ± 0.13 4c	20.62 ± 0.01 4d
h_{ab}	14.49 ± 0.07 1a	10.88 ± 0.16 2b	10.92 ± 0.21 2b	13.64 ± 0.24 2c	11.02 ± 0.25 2b	13.30 ± 0.07 3b	14.87 ± 0.12 3c	13.40 ± 0.03 2b	13.33 ± 0.23 3b	13.28 ± 0.03 3b	13.07 ± 0.01 4c	12.56 ± 0.01 3d	12.41 ± 0.02 4e
DE^*_{ab}	0	4.52 ± 0.10	3.66 ± 0.03	0.75 ± 0.28	3.16 ± 0.08	1.12 ± 0.36	1.99 ± 0.18	1.56 ± 0.26	1.73 ± 0.14	5.35 ± 0.14	5.27 ± 0.43	5.56 ± 0.16	3.30 ± 0.11

Mean values of three measurements for each replicate.
C: control beverage; SSC: soluble solid content; CO_2: carbon dioxide; TTA: total titratable acidity; TP: total phenol (gallic acid equivalent mg/L); ABTS: Trolox equivalent antioxidant activity (TEAC: Trolox-equivalent antioxidant capacity, μmoles de Trolox/L); L*: lightness; h_{ab}: hue; C^*_{ab}: chroma; n.d.: not detectable.
Different letters in the same file indicate significant differences between samples with different concentration inoculum of kefir for each time fermentation time ($p < 0.05$).
Different numbers in the same file indicate significant differences between samples with different fermentation times for each concentration of kefir ($p < 0.05$).

et al. (2016) for grape pomegranate and quince juices (6.46, 6.36 and 5.8 °Brix reductions). The high sucrose content of the fruit-vegetable juice probably stimulates the growth of *Saccharomyces* species, which can hydrolyze sucrose into glucose and fructose by the enzyme invertase, making this carbon source available to lactic acid bacteria (Fiorda et al., 2017).

Accordingly, *Carbon dioxide (CO_2) production* was also related to the fermentation times and significant differences ($p \leq 0.05$) among them were detected. The highest CO_2 production (4.31 g CO_2/100 mL) was found in the beverage fermented with 4% w/v of kefir grains for 48 h. This behaviour is related to the decrease of soluble solids content during the fermentation time. Corona et al. (2016) and Randazzo et al. (2016), on similar fermentation conditions in melon and pomegranate juices, reported lower amounts of CO_2 production (3.39 g CO_2/100 mL and 3.21 g CO_2/100 mL respectively). CO_2 is the major fermentation product of yeasts that contributes to the desirable exotic notes and yeast flavor (Güzel-Seydim, Seydim, Greene, & Bodine, 2000).

Significant differences ($p < 0.05$) in pH *and Titulable Total Acidity (TTA)* among samples with different fermentation times were detected. The highest increase in TTA and the lower pH value was observed for the sample fermented 48 h with 3% w/v of kefir grains which had 6.95 g of malic acid/L and pH 3,4, this is in accordance with previously published results in kefir fermented fig juice (Corona et al., 2016). The production of organic acids in fermented foods reduces the pH value and increases total titratable acidity (Puerari, Magalhaytild, Guedes, & Schwan, 2015). According to Anton et al. (2016), low pH values prevent the growth of most waste and pathogenic organisms, also create a suitable environment for the growth of yeasts and probiotic lactic acid bacteria.

The *organic acids* analyzed were lactic, acetic, citric, succinic, and malic acid (Table 2). The concentration of **lactic acid** increased significantly ($p < 0.05$) with the fermentation time and the content of kefir grains. Therefore, at a fermentation time of 48 h and 4% w/v of kefir grains, the lactic acid reached the highest concentration with a value of 3250.5 mg/L. The production of lactic acid during fermentation is attributed to the metabolism of lactic acid bacteria. According to Puerari, Magalhães-Guedes, and Schwan (2015), lactic acid is the result of homofermentative metabolism, and it is of great importance due to the inhibitory effect on pathogenic microorganisms (Texeira, Pereira, Ribeiro, & Freitas, 2010).

The first traces of **acetic acid** were detected after 12 h of fermentation in samples with 3% kefir w/v (62.1 mg/L). For concentrations of kefir above 2% w/v, the production of acetic acid was directly proportional to the fermentation time. Therefore, the maximum concentration of acetic acid was detected in kefir at 4% w/v, after 48 h of fermentation (266.8 mg/L.) Bellow 1% w/v of kefir, acetic acid production was not detected in accordance with Bensmira and Jiang (2011) and Texeira et al. (2010) who reported that the average concentration of acetic acid was practically zero during the first 18–24 h of fermentation of peanut milk and whey in kefir. When the lactic and acetic acid bacteria present in the kefir microflora use the heterofermentative route, an increase in the amount of acetic acid and, in smaller proportion, of succinic, formic acid and carbon dioxide, among others are detected (Texeira et al., 2010). In this case, likely the preferred metabolic route of fermentation of the kefir microflora in the commercial product was homofermentative.

The **citric acid** decreased as the concentration of kefir grains increased and the effect of the fermentation time followed this trend only in 1% w/v kefir. Samples with 3 and 4% w/v kefir during the first 24 h of fermentation decreased the concentration of citric acid to a value of 499.3 and 496.5 mg/L respectively, representing the lowest registered values; followed by the sample with 4% w/v kefir during 48 h of fermentation (532.8 m/L). Similarly, Sabokbar et al. (2015) reported that the level of citric acid decreased during the fermentation of apple juice and whey with kefir, and Bensmira and Jiang (2011) showed that the level of citric acid decreased due to some lactic acid bacteria which prefer citric acid as a substrate to produce acetoin and diacetyl.

Succinic acid showed an oscillating tendency and, except for 2% w/v kefir, succinic acid increased its concentration with the fermentation time (from 12 to 48 h). The values of succinic acid for the sample with 1% w/v ranged between 249.4 and 380.8 mg/L, being the last one the highest concentration at 48 h. This is in accordance with results reported by Texeira et al. (2010).

For **malic acid,** it was observed an increase in concentration with increasing fermentation times. However, this tendency was not found for increasing concentrations of kefir. Therefore, after 48 h of fermentation, the highest malic acid concentration was obtained for the sample fermented with kefir at 2% w/v (2244.7 mg/L). Sabokbar et al. (2015), reported a reduction in malic acid concentration in a mixture of apple juice and whey fermented with kefir.

However, the increase in malic acid observed could be due to the presence of *Saccharomyces cerevisiae* in the microbiota of the selected kefir since it is one of the microorganisms capable of producing malic acid (Chi, Wang, Wang, Khan, & Chi, 2016).

The results shown in Table 2 indicate that the *viscosity* increased only in the first 12 h of fermentation. At that point, the viscosity tends to decrease as the fermentation time increases. Consequently, the highest viscosity value was observed in the sample at 1% w/v of kefir grains during the first 12 h of fermentation with a value of 3.47 CP. On the other hand, the variation of the content of kefir grain also had a significant effect on the viscosity.

The increase in viscosity in the first 12 h of fermentation is related to lactobacilli which reform the internal structure of the beverages improving the consistency of kefir and causing greater resistance of the inner layer of the drink and consequently a higher viscosity (Irigoyen, Arana, Castiella, Torre, & Ibáñez, 2005). However, Degeest, Mozzi, and De Vuyst (2002) stated that glycohydrolases may hydrolyze exopolysaccharide (EPS) material in their monomers explaining the decrease in viscosity detected afterwards. Glycohydrolases can decrease the viscosity of the polymers produced by *Lactobacillus rhamnosus*, as well as release some reducing sugars (Pham, Dupont, Roy, Lapointe, & Cerning, 2000).

As shown in Table 2 the *density* of the juices decreases as the fermentation time increases, this decrease was significant ($p < 0.05$) in some cases, except for the samples between 12 and 24 h of fermentation. These results may be related to the metabolization sugars which are fermented into lactic acid, acetic acid, alcohol and carbon dioxide, and other compounds which may cause a change in the density of the beverage.

Total phenols (TP) content of the samples changed significantly during the different times of fermentation (Table 2). The sample with 4% w/v of kéfir grains and 48 h of fermentation was the one with the highest reduction in TP concentration (decrease: 19%). The sample at 2% w/v of kefir grains in 24 h of fermentation reached the highest concentration of TP (increase: 31%). Randazzo et al. (2016) reported a decrease in TP in kiwi, pomegranate, strawberry, apple, grape and quince juices in similar fermentation conditions as in this research (4% w/v kéfir, 48 h of fermentation). According to McCue and Shetty (2005) the decrease in TP content could be the result of the degradation of phenolic structures as possible mechanisms of antimicrobial detoxification of yeasts and bacteria.

The fermentation time had a significant effect on the *antioxidant activity (AA)* values of the samples ($p < 0.05$). The results reported in Table 2 show a decrease between 28% (490.69 μmol TE/L) and 37% (427.87 μmol TE/L) after 48 h of fermentation on the AA compared to the control beverage (684.33 μmol TE/L). Randazzo et al. (2016) reported similar results, with a decrease of AA in pomegranate, grape, apple, kiwi and fig juice, only the quince juice showed a slight increase in AA. According to McCue and Shetty (2005) there is an inverse relation between total phenolic content and the antioxidant activity of the kefir fermented beverage, as observed in the present investigation.

3.2. Colour analysis

The location of the samples within the (a*b*) plane is shown in Fig. 2. All the samples are clustered in the red area of the diagram, with values of a* and b* around 14–22 and 2–4 CIELAB units, respectively. Colour parameters L*, C^*_{ab}, and h_{ab} were significantly affected by the fermentation time (Table 2). The results show a significant decrease of values ($p < 0.05$) in colourimetrics parameters L*, C^*_{ab} and h_{ab} during the first 12 h of fermentation with respect to the control beverage (0.8–4.7%, 3.5–23.4% and 5.8–24.9%, respectively). However, after 48 h of fermentation, the samples significantly increased their L* and C^*_{ab} values with respect to the control beverage (4.7–7.6% and 16.8–29.1% respectively). The hue values (h_{ab}) of the samples tend to stabilize as time increases. That is, the fermentation time provides lighter and more reddish beverages.

The variation of kefir grain concentrations only had a significant effect ($p < 0.05$) on the values of L* and C^*_{ab} after the first 12 h of fermentation, and for h_{ab} values after 48 h of fermentation.

Randazzo et al. (2016) state that the lightness reduction and the reddish increase could be explained by the browning processes that occur during the fermentation of these beverages. This phenomenon is due to the activation of oxidase enzymes, such as polyphenol oxidase, when the environments are not completely anaerobic (Corona et al., 2016). The above confirms the variation in L*, C^*_{ab} and h_{ab} observed in the samples due to the fermentation time. The colour differences between the control (raw juice) and the fermented samples were determined. It was found that the beverages fermented during 48 h presented the highest colour difference values ($\Delta E^*_{ab} = 3.30–5.56$ CIELAB units) which could be perceived by the human eye, according to Martínez, Melgosa, Pérez, Hita, and Negueruela (2001), which indicate that ΔE^*_{ab} values above 2.7 CIELAB can be clearly detected by a non-trained human eye.

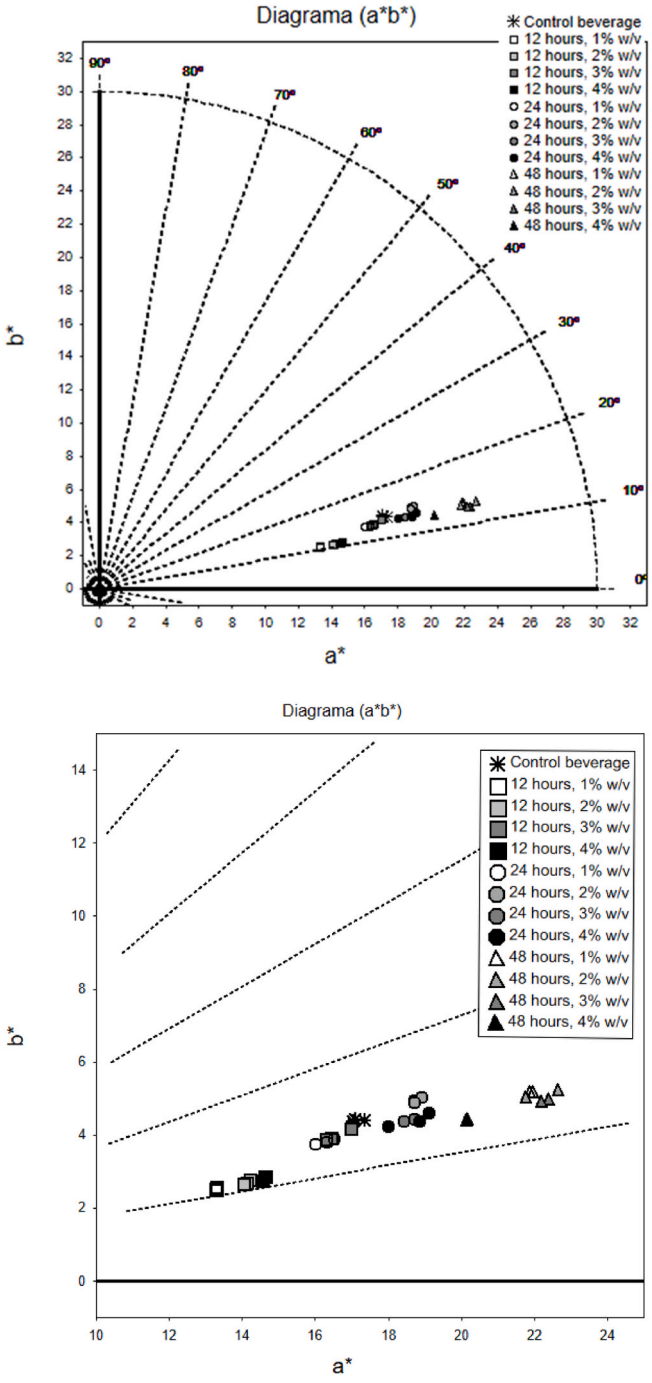

Fig. 2. Location of the samples within the (a*b*) plane.

3.3. Sensorial analysis

Results from the two sensorial analyses are described in this section: the aim of the first one was to choose the three best beverages from 12 samples consisting of beverages with three levels of fermentation time and four levels of kefir grain concentration, and the second one was to select the best beverage amongst the three samples chosen in the previous sensorial analysis.

3.3.1. Sensory analysis to assess the effect of fermentation time and kefir concentration on sensorial properties of the beverages

The sample rank-sum was calculated, and the friedman test was used

Table 3
Sensory analysis to assess the effect of fermentation time on sensorial properties of beverages. Summary of the judges' answers in the rank-order tests and the Friedman's test results.

Fixed variable (nº judges/ session)	Attribute	Rank sums Samples[a] [12h]/ [24h]/[48h]	F (Friedman)	$F_{critical}$ ($\alpha = 0.05$)	$F_{critical}$ ($\alpha = 0.01$)
1% w/v Kefir (15)	Sweet	$[37]^a/[35]^a/[18]^b$	14.53**	6.40	8.93
	Acidity	[25]/[29]/[36]	4.13		
	Alcohol	[26]/[28,5]/[35,5]	3.23		
2% w/v kefir (15)	Sweet	$[29]^a/[43]^b/[18]^c$	20.93**		
	Acidity	$[29]^{ab}/[23]^a/[38]^b$	7.60*		
	Alcohol	$[32]^{ab}/[22]^a/[36]^b$	6.93*		
3% w/v kefir (15)	Sweet	$[38]^a/[35]^a/[17]^b$	17.20**		
	Acidity	[28.5]/[27.5]/[34]	1.63		
	Alcohol	[29]/[25]/[36]	4.13		
4% w/v kefir (15)	Sweet	$[35.5]^a/[37.5]^a/[17]^b$	17.03**		
	Acidity	[28]/[28]/[34]	1.60		
	Alcohol	[29.5]/[25.5]/[35]	3.03		

[a] Different superscripts in the same row indicate significant differences, *$p < 0.05$ and **$p < 0.01$.

to verify significant differences among the samples. Tables 3–5 present the Friedman test statistic for each attribute (perception of sweetness, acidity, and alcohol). When significant differences among samples were found, the pairs of differing samples were computed according to the minimal significant differences of Fisher (Tables 4–6).

Tables 3 and 4 shows the results of evaluating the effects of fermentation time on the sensory properties. The Friedman's test applied to the ranking test revealed that, regardless of the concentration of kefir inoculum, the samples with different fermentation times (12, 24 and 48 h) were significantly different in terms of sweetness perception ($F_{1\%} = 14.5$, $F_{2\%} = 20.9$, $F_{3\%} = 17.2$, $F_{4\%} = 17.0$; $F_{critical} = 8.9$, $p < 0.01$). The judges have a significantly greater perception of sweetness in samples of 12 and 24 h of fermentation time than in samples of 48 h. The sample corresponding to a fermentation time of 24 h and a concentration of kefir of 2% w/v presented significantly the highest score in terms of sweetness perception (judges score = 2.9). The results of the rank-order test related to the perception of acidity and alcohol in the products allowed to determine that the judges perceived greater acidity and alcohol in the samples with 48 h of fermentation. Significant differences were found only between samples containing 2% w/v kefir and different fermentation times (acidity: $F = 7.6$ and alcohol: $F = 6.9$; $F_{critical} = 6.4$, $p < 0.05$). The sample with fermentation time of 48 h and a concentration of kefir of 2% w/v presented significantly the highest score in the acidity and alcohol perception (judges score = 2.5 and 2.4, respectively). This result is in accordance with the production of organic acids as it can be observed in Table 2, significant differences were found ($p < 0.05$) for samples inoculated at 2% w/v of kefir and different fermentation times. On the other hand, the sample with the significantly lowest perception of alcohol by the tasters was the sample at 2% w/v kefir fermented for 24 h (judges score = 1.5).

Tables 5 and 6 shows the results of the sensory analysis to assess the effect of kefir concentration on the sensory properties. The Friedman's test applied to the ranking test revealed that, in the cases with 12 and 48 h of fermentation times like fixed variable, the samples with different kefir concentrations (1, 2, 3 and 4% w/v) were significantly different in terms of sweetness perception ($F_{12h} = 8.4$, $F_{48h} = 8.8$; $F_{critical} = 7.8$, $p < 0.05$). The judges have significantly greater perception of sweetness in samples with 1% w/v of kefir than in samples with 2, 3 and 4% w/v of kefir. The sample corresponding to kefir of 1% w/v and a fermentation time of 48 h was scored significantly as the highest in terms of sweetness perception (judges score = 3.5); and the samples with the lowest perception of sweetness by the tasters were the ones corresponding to 4% w/v kefir with 12, 24 and 48 h of fermentation time (judges score = 2.1, 2.1 and 1.9 respectively). The results of the rank-order test related to the perception of the acidity allow to determine that the judges perceived greater acidity in the samples with 4% w/v kefir. Significant differences were found only between samples fermented for 24 h and containing different kefir concentrations ($F = 19$; $F_{critical} = 11.34$, $p < 0.01$). The sample with 4% w/v of kefir and fermented for 24 h was scored significantly as the highest in the acidity perception (judges score = 3.5). In the case of the alcohol perception, according to Friedman's test results, samples were not statistically different.

In each session judges were asked to answer "What sample do you prefer?". One point was assigned to the favorite sample and 0 points to the others. All the points were summed up and divided by the number of judges to obtain the final score of each sample. The results of the preference test (Fig. 3) indicate that the tasters had a greater preference for the samples that have been fermented for 24 h at 1%, 2% and 3% w/v kefir. Therefore, these results show that the judges have preference for a product with an acidity between 4.78 and 5.64 g/L of malic acid, pH between 3.65 and 3.7, refractive index between 8.69 and 9.93 °Brix. In addition, the judges preferred a product with low alcohol perception.

3.3.2. Sensory analysis to select the product with the best acceptance

Only the beverages preferred by the judges in the previous assay, were included: samples with 1%, 2% and 3% w/v kefir and 24 h of fermentation, and the control beverage.

Table 7 presents the Friedman test statistic for each attribute (aroma, texture and colour). When significant differences among samples were found, the pairs of differing samples were computed according to the minimal significant differences of Fisher. The Friedman's test applied to the ranking test revealed that the samples were significantly different in

Table 4
Pair comparison test using minimal significant differences of Fisher. Difference between the rank sums in the formulations studied. This value compares the perceived differences in attributes between two fermentation times.

Fixed variable (nº judges/ session)	Attribute	12–24 h	12–48 h	24–48 h	$LSD_{0.05}$	$LSD_{0.01}$
1% w/v Kefir (15)	Sweet	2	19**	17**	10.74	14.11
	Acidity	4	11*	7		
	Alcohol	2.5	9.5	7		
2% w/v kefir (15)	Sweet	14*	11*	25**		
	Acidity	6	9	15**		
	Alcohol	10	4	14*		
3% w/v kefir (15)	Sweet	3	21**	18**		
	Acidity	1	5.5	6.5		
	Alcohol	4	7	11*		
4% w/v kefir (15)	Sweet	2	18.5**	20.5**		
	Acidity	0	6	6		
	Alcohol	4	5.5	9.5		

LSD: Least significant difference.
Significant difference between two fermentation times, *$p < 0.05$ and **$p < 0.01$.

Table 5
Sensory analysis to assess the effect of kefir concentration on sensorial properties of beverages. Summary of the judges' answers in the rank-order tests and the Friedman's test results.

Fixed variable (nº judges/ session)	Attribute	Rank sums Samples* [1%]/ [2%]/ [3%]/[4%]	F (Friedman)	$F_{critical}$ ($\alpha = 0.05$)	$F_{critical}$ ($\alpha = 0.01$)
12h fermentation time (15)	Sweet	[50]^a/ [34]^b/ [34]^b/[32]^b	8.44*	7.81	11.34
	Acidity	[35]/ [35.5]/ [31.5]/[48]	6.26		
	Alcohol	[33]/ [43.5]/ [37.5]/[36]	2.34		
24h fermentation time (15)	Sweet	[38]/[47]/ [34]/[31]	5.80		
	Acidity	[26]^a/ [28]^a/ [44]^b/[52]^b	19.00**		
	Alcohol	[31.5]/ [32.5]/ [46.5]/ [39.5]	5.84		
48h fermentation time (15)	Sweet	[52.5]^a/ [29.5]^b/ [37.5]^b/ [28.5]^b	8.80*		
	Acidity	[28]/ [42.5]/ [39.5]/[40]	5.02		
	Alcohol	[26.5]/ [44.5]/ [37.5]/ [41.5]	7.44		

(1) Different superscripts in the same row indicate significant differences, *$p < 0.05$ and **$p < 0.01$.

Table 6
Pair comparison test using minimal significant differences of Fisher. Difference between the rank sums in the formulations studied. This value compares the perceived differences in attributes between two kefir concentrations.

Fixed variable (nº judges/session)	Attribute	1–2%	1–3%	1–4%	2–3%	2–4%	3–4%	$LSD_{0.05}$	$LSD_{0.01}$
12h fermentation time (15)	Sweet	16*	16*	18*	0	2	2	13.86	18.22
	Acidity	0.5	3.5	13	4	12.5	16.5*		
	Alcohol	10.5	4.5	2	6	7.5	1.5		
24h fermentation time (15)	Sweet	9	4	7	13	16*	3		
	Acidity	2	18*	26**	16*	24**	8		
	Alcohol	1	15*	8	14*	7	7		
48h fermentation time (15)	Sweet	23**	15*	24**	8	1	9		
	Acidity	14.5*	11.5	12	3	2.5	0.5		
	Alcohol	18*	11	15*	7	3	4		

LSD: Least significant difference.
Significant difference between the two kefir concentrations, *$p < 0.05$ and **$p < 0.01$.

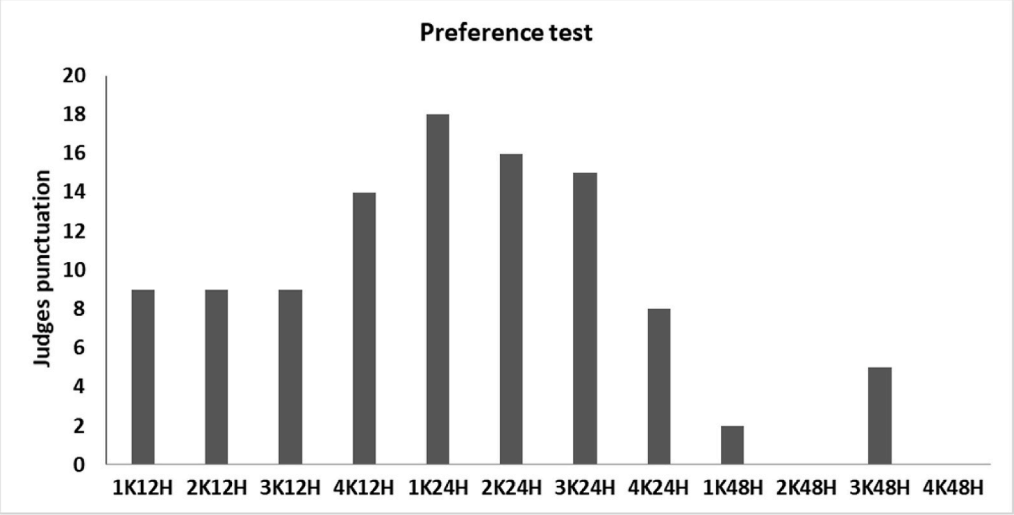

Fig. 3. Summary of the judges' answers in the preference test.

Table 7
Sensory analysis to assess the consumer's preference on beverages. Summary of the judges' answers in the rank-order tests and the Friedman's test results.

nº judges/ session	Attribute	Rank sums Samples[a] [1K24H]/ [2K24H]/ [3K24H]/[C]	F (Friedman)	$F_{critical}$ (α = 0.05)	$F_{critical}$ (α = 0.01)
30	Aroma	$[92.5]^a$/ $[74.5]^b$/ $[70.5]^b$/$[62.5]^b$	9.66*	7.81	11.34
	Texture	$[77]^{ab}$/$[91]^b$/ $[68.5]^a$/$[63.5]^a$	8.69*		
	Color	[81]/[71.5]/ [63.5]/[84]	5.23		

[a] Different superscripts in the same row indicate significant differences, *$p < 0.05$. Pair comparison test using minimal significant differences of Fisher.

terms of aroma and texture preference ($F = 9.66$ and $F = 8.69$ respectively, $F_{critical} = 7.81$, $p < 0.05$). The judges had significantly greater preference for the aroma and texture of the samples corresponding to a fermentation time of 24 h and kefir concentration of 1% and 2% w/v (judges score for aroma = 3.1 and texture = 3.0). Regarding the colour attribute, there were not significant differences ($p < 0.05$) among the judges' preferences.

Fig. 4 shows the results of the preference hedonic test. The Kruskal-Wallis test was applied to identify differences among samples. The product at 2% w/v of kefir [2K24H] got the highest score (5.37). This score was significantly higher than the one of the other analized beverages, including the control sample (the original juice). Besides, this sample stands out from the other samples analized on attributes such as low acidity, high perception of sweetness, low alcohol perception and better preference about texture. Consequently, this product has a higher probability of having a better acceptance by consumers.

3.4. Explorative multivariate analysis of physicochemical and sensory data

To assess the influence of the most relevant physicochemical parameters on the sensory evaluation, a multiple regression analysis was applied. The sensory attributes and general preference were considered as dependent variables, and the physicochemical parameters, as independent ones. The results are summarized in Table 8. The variables selected by the forward stepwise analyses, explained more than 87% of the sensorial analysis variations of the beverages analyzed, and the F of each regression was statistically significant ($p < 0.05$). High correlations ($R^2 > 0,96$) among the selected physicochemical parameters and sensory attributes were obtained for sweetness, aroma, texture, colour and preference.

°Brix, showed a significant ($p < 0.05$) and positive correlation with sweetness (b = 1211), aroma (b = 0,244) and colour (b = 1888), and was also negatively related to alcohol perception. CO_2 was significant ($p < 0.05$) and positively correlated with aroma (b = 1046), texture (b = 1392), colour (b = 0,335) and preference (b = 1237). pH and TTA were related to acidity, alcohol perception, texture, and preference while viscosity and density were selected variables to predict alcohol, aroma,

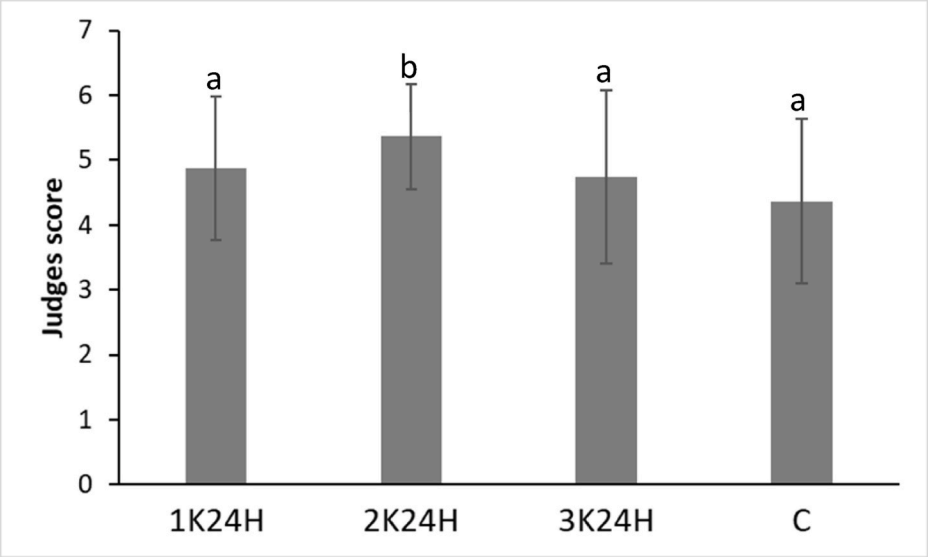

Fig. 4. Mean values preference scores given by non-trained panel (Bar with different letters indicate significant differences between samples, $p < 0.05$).

Table 8
Summary of the regression analyses carried out considering sensory attributes as dependent variables and the physicochemical parameters as independent variables.

Dependent variable	R^2	Independent variables selected by the forward stepwise analyses[a]
Sweet	0.96	*°Brix*, C^*_{ab}, L*, h_{ab}
Acidity	0.88	°Brix, *L**, C^*_{ab}, CO_2, TTA
Alcohol	0.87	pH, Viscosity, °Brix, L*, TTA, CO_2
Aroma	0.99	*CO_2*, TTA, *°Brix*, C^*_{ab}, Density
Texture	0.99	*CO_2*, *h_{ab}*, *C^*_{ab}*, pH, *L**, *TTA*, Viscosity
Colour	0.99	TTA, *CO_2*, *°Brix*, C^*_{ab}, *Viscosity*, *L**, Density, h_{ab}, pH
Preference	0.99	TTA, °Brix, *CO_2*, *h_{ab}*, *C^*_{ab}*, pH, *L**, Viscosity

TTA: total titratable acidity.
[a] The variables in italics present significant correlation coefficients ($p < 0.05$).

texture, colour and preference. It is interesting to note the relevance of colour parameters (h_{ab}, C^*_{ab}, L*) in the sensory attributes evaluated. Chroma, hue and lightness of the beverages showed a significant and positive correlation (b = 0,568; 0,865; 0,178) with the preference. Preference was also affected by °Brix, TTA and viscosity.

4. Conclusions

Fermentation of the comercial fruits and vegetable juice promoted considerable changes such as: a decrease in sugar content, and increase in acidity, total phenols, carbon dioxide and organic acids (lactic acid, acetic acid, and succinic acid). Fermentation also affected sensory attributes like colour, with an increase in lightness (L*) and chroma (C^*_{ab}) values; and a decrease in density, antioxidant activity, citric acid and hue (h_{ab}) values, these changes were related to the level of kefir grains inoculum and the fermentation time. These changes in the physico chemical properties which could also have a beneficial health effect, were best sensory evaluated in the sample fermented for 24 h and containing 2% w/v of kefir. This new functional non-dairy beverage can meet the needs of some consumers including vegan, vegetarian, and people with intolerance/allergy to dairy products besides those with cardiovascular disease which could benefit of the betaine effect on homocysteine, a component of beetroot.

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CRediT authorship contribution statement

Jorge Luís Paredes: Methodology, Formal analysis, Writing – original draft. **María Luisa Escudero-Gilete:** Conceptualization, Methodology, Data curation, Writing – review & editing, Project administration. **Isabel María Vicario:** Resources, Visualization, Writing – review & editing, Project administration.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

Álvarez Berciano, F., & Álvarez Caro, F. (2008). Reacciones adversas a alimentos e historia natural de la alergia alimentaria en la infancia. *Boletín de la Sociedad de Pediatría de Asturias, Cantabria, Castilla y León, 48*, 21–36.

Alves, V., Scapini, T., Camargo, A. F., Bonatto, C., Stefanski, F. S., de Jesus, E. P., et al. (2021). Development of fermented beverage with water kefir in water-soluble coconut extract (*Cocos nucifera* L.) with inulin addition. *LWT – Food Science and Technology, 145*, 111364.

Anton, D., Raudsepp, P., Roasto, M., Meremäe, K., Kuusik, S., Toomik, P., et al. (2016). Comparative study of microbiological, chemical and sensory properties of kefirs produced in Estonia, Latvia and Lithuania. *Journal of Dairy Research, 83*, 89–95. https://doi.org/10.1017/S0022029915000710

AOAC. (2000). *Official methods of analysis* (17th ed.). Gaithersburg, MD, USA: The Association of Official Analytical Chemists.

Baines, D., & Seal, R. (2012). *Natural food additives, ingredients, and flavourings* (1st ed.). Woodhead Publishing.

Bensmira, M., & Jiang, B. (2011). Organic acids formation during the production of a novel peanut-milk kefir beverage. *British Journal of Dairy Sciences, 2*, 18–22.

Bueno, R. S., Ressutte, J. B., Hata, N. N. Y., Henrique-Bana, F. C., Guergoletto, K. B., de Oliveira, A. G., et al. (2021). Quality and shelf life assessment of a new beverage produced from water kefir grains and red pitaya. *LWT – Food Science and Technology, 140*, 110770. https://doi.org/10.1016/j.lwt.2020.110770

Cevikbas, A., Yemni, E., Ezzedenn, F. W., Yardimici, T., Cevikbas, U., & Stohs, S. J. (1994). Antitumoural antibacterial and antifungal activities of kefir and kefir grain. *Phytotherapy Research, 8*, 78–82. https://doi.org/10.1002/ptr.2650080205

Chi, Z., Wang, Z. P., Wang, G. Y., Khan, I., & Chi, Z. M. (2016). Microbial biosynthesis and secretion of l-malic acid and its applications. *Critical Reviews in Biotechnology, 36*, 99–107. https://doi.org/10.3109/07388551.2014.924474

Choudhary, S. K., & Jadoun, R. S. (2014). Current research issue, trend & applications of powder mixed dielectric electric discharge machining (PM-EDM): A review. *International Journal of Engineering Sciences & Research Technology, 3*, 335–358.

Commission Internationale de l'Eclairage (CIE). (1986). *Colourimetry* (2nd ed.). Vienna: Commission Internationale de l'Eclairage. Publication CIE No. 15.2.

Corona, O., Randazzo, W., Miceli, A., Guarcello, R., Francesca, N., Erten, H., et al. (2016). Characterization of kefir-like beverages produced from vegetable juices. *Lebensmittel-Wissenschaft und -Technologie- Food Science and Technology, 66*, 572–581. https://doi.org/10.1016/j.lwt.2015.11.014

Crespo, L., Gaglio, R., Martínez, F. G., Martín, G. M., Franciosi, E., Madrid-Albarrán, Y., et al. (2021). Bioaccumulation of selenium-by fruit origin lactic acid bacteria in tropical fermented fruit juices. *Lebensmittel-Wissenschaft und -Technologie- Food Science and Technology, 151*, 112103. https://doi.org/10.1016/j.lwt.2021.112103

De Las Cagigas Reig, A. L., & Blanco Anesto, J. (2002). Prebióticos y probióticos, una relación beneficiosa. *Revista Cubana de Alimentación y Nutrición, 16*, 63–68.

Degeest, B., Mozzi, F., & De Vuyst, L. (2002). Effect of medium composition and temperature and pH changes on exopolysaccharide yields and stability during *Streptococcus thermophilus* LY03 fermentations. *International Journal of Food Microbiology, 79*, 161–174. https://doi.org/10.1016/S0168-1605(02)00116-2

Drake, M. A. (2007). Invited review: Sensory analysis of dairy foods. *Journal of Dairy Science, 90*, 4925–4937. https://doi.org/10.3168/jds.2007-0332

Española de la Nutrición, F. (2013). *Libro Blanco de la Nutrición en España*. Fundación Española de la Nutrición (FEN).

Fiorda, F. A., de Melo Pereira, G. V., Thomaz-Soccol, V., Rakshit, S. K., Binder Pagnoncelli, M. G., Porto de Souza Vandenberghe, L., et al. (2017). Microbiological, biochemical, and functional aspects of sugary kefir fermentation - a review. *Food Microbiology, 66*, 86–95. https://doi.org/10.1016/j.fm.2017.04.004

Fisher, R. A. (1922). On the interpretation of $\chi 2$ from contingency tables, and the calculation of P. *Journal of the Royal Statistical Society, 85*, 87–94. https://doi.org/10.2307/2340521

Friedman, M. (1937). The use of ranks to avoid the assumption of normality implicitin the analysis of variance. *Journal of the American Statistical Association, 32*, 675–701. https://doi.org/10.1080/01621459.1937.10503522

Gaglio, R., Pescuma, M., Madrid-Albarrán, Y., Franciosi, E., Moschetti, G., Francesca, N., et al. (2021). Selenium bio-enrichment of Mediterranean fruit juices through lactic acid fermentation. *International Journal of Food Microbiology, 354*, 109248. https://doi.org/10.1016/j.ijfoodmicro.2021.109248

Garcia, C., Guerin, M., Souidi, K., & Remize, F. (2020). Lactic fermented fruit or vegetable juices: Past, present and future. *Beverages, 6*, 8. https://doi.org/10.3390/beverages6010008

Gulitz, A., Stadie, J., Wenning, M., Ehrmann, M. A., & Vogel, R. F. (2011). The microbial diversity of water kefir. *International Journal of Food Microbiology, 151*(3), 284–288. https://doi.org/10.1016/j.ijfoodmicro.2011.09.016

Güzel-Seydim, Z. B., Seydim, A. C., Greene, A. K., & Bodine, A. B. (2000). Determination of organic acids and volatile flavor substances in kefir during fermentation. *Journal of Food Composition and Analysis, 13*, 35–43. https://doi.org/10.1006/jfca.1999.0842

Güzel-Seydim, Z. B., Seydim, A. C., Greene, A. K., & Taş, T. (2006). Determination of antimutagenic properties of acetone extracted fermented milks and changes in their total fatty acid profiles including conjugated linoleic acids. *International Journal of Dairy Technology, 59*, 209–215. https://doi.org/10.1111/j.1471-0307.2006.00265.x

Hollander, M., & Wolfe, D. A. (1973). *Nonparametric statistical methods*. New York: Wiley.

Irigoyen, A., Arana, I., Castiella, M., Torre, P., & Ibáñez, F. C. (2005). Microbiological, physicochemical, and sensory characteristics of kefir during storage. *Food Chemistry, 90*, 613–620. https://doi.org/10.1016/j.foodchem.2004.04.021

Martínez, J. A., Melgosa, M., Pérez, M. M., Hita, E., & Negueruela, A. I. (2001). Visual and instrumental colour evaluation in red wines. *Food Science and Technology International, 7*, 439–444. https://doi.org/10.1106/VFAT-5REN-1WK2-5JGQ

McCue, P., & Shetty, K. (2005). Phenolic antioxidant mobilization during yogurt production from soymilk using Kefir cultures. *Process Biochemistry, 40*, 1791–1797. https://doi.org/10.1016/j.procbio.2004.06.067

Noğay, N. H. (2019). Kefir beverage and its effects on health. *Milk-Based Beverages, 9*, 273–296. https://doi.org/10.1016/B978-0-12-815504-2.00008-6

Norman, G. R., & Streiner, D. L. (1996). *Bioestadística. Mosby/doyma libros S.A*. Madrid: Times Mirror International Publishers.

Pham, P. L., Dupont, I., Roy, D., Lapointe, G., & Cerning, J. (2000). Production of exopolysaccharide by *Lactobacillus rhamnosus* R and analysis of its enzymatic degradation during prolonged fermentation. *Applied and Environmental Microbiology, 66*, 2302–2310. https://doi.org/10.1128/AEM.66.6.2302-2310.2000

Puerari, C., Magalhães-Guedes, K. T., & Schwan, R. F. (2015). Physicochemical and microbiological characterization of chicha, a rice-based fermented beverage produced by Umutina Brazilian Amerindians. *Food Microbiology, 46*, 210–217. https://doi.org/10.1016/j.fm.2014.08.009

Randazzo, W., Corona, O., Guarcello, R., Francesca, N., Germanà, M. A., Erten, H., et al. (2016). Development of new non-dairy beverages from Mediterranean fruit juices fermented with water kefir microorganisms. *Food Microbiology, 54*, 40–51. https://doi.org/10.1016/j.fm.2015.10.018

Regulation (EC) No 1924. (2006). *of the European Parliament and of the Council of 20 December 2006 on nutrition and health claims made on foods*.

Regulation (EU) No 432/2012 Of 16 May 2012 establishing a list of permitted health claims made on foods, other than those referring to the reduction of disease risk and to children's development and health.

Sabokbar, N., Moosavi-Nasab, M., & Khodaiyan, F. (2015). Preparation and characterization of an apple juice and whey based novel beverage fermented using kefir grains. *Food Science and Biotechnology, 24*, 2095–2104. https://doi.org/10.1007/s10068-015-0278-6

Singleton, V. L., & Rossi, J. A. (1965). Colourimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Viticulture, 16*, 144–158.

Stadie, J., Gulitz, A., Ehrmann, M. A., & Vogel, R. F. (2013). Metabolic activity and symbiotic interactions of lactic acid bacteria and yeasts isolated from water kefir. *Food Microbiology, 35*, 92–98. https://doi.org/10.1016/j.fm.2013.03.009

StatSoft Inc. (2007). *STATISTICA (data analysis software system), 8.0*. Tulsa, OK.

Tesfaye, W., Suarez-Lepe, J. A., Loira, I., Palomero, F., & Morata, A. (2019). Dairy and nondairy-based beverages as a vehicle for probiotics, prebiotics, and symbiotics: Alternatives to health versus disease binomial approach through food. *Milk-Based Beverages, 9*, 473–520. https://doi.org/10.1016/B978-0-12-815504-2.00014-1

Texeira, K., Pereira, G. V. M., Ribeiro, D., & Freitas, R. (2010). Microbial communities and chemical changes during fermentation of sugary Brazilian kefir. *World Journal of Microbiology and Biotechnology, 26*, 1241–1250. https://doi.org/10.1007/s11274-009-0294-x

The Business Research Company. (2021). *Functional food global market opportunities and strategies to 2030: COVID-19 growth and change*.

Zaky, A. S., Pensupa, N., Andrade-Eiroa, A., Tucker, G. A., & Du, C. (2017). A new HPLC method for simultaneously measuring chloride, sugars, organic acids and alcohols in food samples. *Journal of Food Composition and Analysis, 56*, 25–33. https://doi.org/10.1016/j.jfca.2016.12.010

Zilio, F., Tosi, E., Lombardi, A., & Delfini, C. (2004). Contributo alla valorizzazionedel vino Valpolicella D.O.C. mediante l'isolamento, la ca-ratterizzazione ed il successivoimpiego di lieviti specifici. Contributo alla valorizzazione del vino Valpolicella D.O.C. ratterizzazione ed il successivo impiego di lieviti specifici. *Vignevini, 7/8*, 1–5.