

Original article

Chemical composition and sensory analysis of cheese whey-based beverages using kefir grains as starter culture

Karina Teixeira Magalhães,^{1,2} Disney Ribeiro Dias,³ Gilberto Vinicius de Melo Pereira,¹ José Maria Oliveira,² Lucília Domingues,² José António Teixeira,² João Batista de Almeida e Silva⁴ & Rosane Freitas Schwan^{1*}

1 Department of Biology, Federal University of Lavras (UFLA), Campus Universitário, 37.200-000, Lavras, MG, Brazil

2 IBB – Institute for Biotechnology and Bioengineering, Centre of Biological Engineering, Universidade do Minho, Campus de Gualtar, 4710-057, Braga, Portugal

3 Department of Food Science, Federal University of Lavras (UFLA), 37.200-000, Lavras, MG, Brazil

4 Department of Biotechnology, Engineering School of Lorena, University of São Paulo (USP), 12.602-810, Lorena, SP, Brazil

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Summary The aim of the present work was to evaluate the use of the kefir grains as a starter culture for traditional milk kefir beverage and for cheese whey-based beverages production. Fermentation was performed by inoculating kefir grains in milk (ML), cheese whey (CW) and deproteinised cheese whey (DCW). Erlenmeyers containing kefir grains and different substrates were statically incubated for 72 h at 25 °C. Lactose, ethanol, lactic acid, acetic acid, acetaldehyde, ethyl acetate, isoamyl alcohol, isobutanol, 1-propanol, isopentyl alcohol and 1-hexanol were identified and quantified by high-performance liquid chromatography and GC-FID. The results showed that kefir grains were able to utilise lactose in 60 h from ML and 72 h from CW and DCW and produce similar amounts of ethanol (~12 g L⁻¹), lactic acid (~6 g L⁻¹) and acetic acid (~1.5 g L⁻¹) to those obtained during milk fermentation. Based on the chemical characteristics and acceptance in the sensory analysis, the kefir grains showed potential to be used for developing cheese whey-based beverages.

Keywords Cheese whey beverage, GC-FID, HPLC, kefir, lactic acid bacteria, sensory evaluation, yeasts.

Introduction

Cheese whey (CW) is the major by-product of the dairy industry and its disposal without expensive sewage treatments represents a major source of environmental pollution. Considerable efforts have been made over the past years to explore new outlets for CW utilisation and reduce environmental pollution (Magalhães *et al.*, 2010a). Besides potable ethanol production by lactose converting microorganisms (Guimarães *et al.*, 2010) and the production of distilled beverages (Dragone *et al.*, 2009) and kefir-like CW beverages (Magalhães *et al.*, 2010a) this by-product has also been suggested as an alternative for industrial residue utilisation which may reduce environmental pollution.

Traditionally kefir grains have been used in many countries, especially Eastern Europe, as a natural starter in the production of kefir, a unique self-carbonated dairy beverage. Kefir differs from other fermented milks in its starter, which exists in the form of grains (Simova *et al.*, 2002). Kefir grains contain lactic acid bacteria

(LAB) including *Lactobacillus*, *Lactococcus*, *Leuconostoc* and *Streptococcus* spp. and yeasts (*Kluyveromyces*, *Torula*, *Candida* and *Saccharomyces* spp.). Both the bacteria and yeast are surrounded by a polysaccharide matrix, called kefirin, which is a water-soluble branched glucogalactan (Magalhães *et al.*, 2010b).

The production of a functional beverage produced upon whey fermentation by kefir grains could be an interesting alternative for CW utilisation. CW fermentation by kefir microorganisms could decrease the high lactose content in CW, producing mainly lactic acid and other metabolites such as aroma compounds contributing to the flavour and texture and increasing carbohydrate solubility and sweetness of the end product. Manufacture of beverages through lactic fermentations can provide desirable sensory profiles and have already been considered an option to add value to CW (Pescuma *et al.*, 2008).

Recently, Magalhães *et al.* (2010a) set out to characterise kefir-associated microbiota by using two unrelated techniques, DNA analysis [by denaturing gradient gel electrophoresis (DGGE)] and optical microscopy (combining fluorescence staining with confocal laser microscopy). The composition of microbiota was related to *Lactobacillus kefirianofaciens* subsp. *kefirgranum*,

*Correspondent: Fax: +55 35 38291100;
e-mail: rschwan@dbi.ufla.br

Lactobacillus kefirifaciens subsp. *kefirifaciens*, an uncultured bacterium related to the genus *Lactobacillus*, *Kluyveromyces marxianus*, *Saccharomyces cerevisiae* and *Kazachstania unispora*. No differences were found in the community structure detected in the analysed beverage and Brazilian kefir grains, showing that microbiota of kefir grains is highly stable along the fermentations carried out in different substratum. However, this characterisation was restricted to the microbiota and until recently, we were not aware of any reports concerning the chemical and sensorial characterisation of these beverages. Therefore, the aim of the present work was to evaluate the use of the kefir grains as a starter culture for traditional milk kefir beverage and for CW-based beverages production, besides evaluating the biochemical changes, organic acids production and volatile compounds formation during fermentation process.

Materials and methods

CW-based and milk fermentation media

Three different substrates containing lactose concentration of 46 g L^{-1} were used as fermentation media: pasteurised full cows milk (ML), CW and deproteinised cheese whey (DCW). CW powder, obtained from a regional dairy industry (Lactogal, Porto, Portugal), was dissolved in sterile distilled water until the desired lactose concentration. DCW was made by autoclaving at $115 \text{ }^\circ\text{C}$ for 10 min the CW solution, followed by aseptic centrifugation (2220 g for 20 min) to remove cream. Confirmation of CW deproteinisation was accomplished for Kjeldahl method.

Kefir beverages production

Brazilian kefir grains were employed in the present study. The grains (12.5 g) were washed with sterile distilled water and inoculated in 250 mL of ML, CW and DCW. The milk is used commonly for kefir beverage, therefore was used to compare the fermentation with CW kefir beverages. Erlenmeyers containing kefir grains were statically incubated for 72 h at $25 \text{ }^\circ\text{C}$. Samples of the kefir beverages were aseptically taken every 12 h for analysis of organic acids, ethanol, sugars and volatile flavour substances. Determination of total reducing sugars was used to assess the substrate consumption. The pH of the fermented beverages was measured using a Micronal B474 pH meter. Two replicates were done in each fermentation batch.

Analytical methods

Chemicals

1-Hexanol and ethyl acetate were purchased from Aldrich Chemistry (Munich, Germany). 1-propanol,

2-methyl-1-propanol, 2-methyl-1-butanol, 3-methyl-1-butanol were purchased from Fluka Analyticals (Seelze, Germany). Ethyl acetate, Acetaldehyde lactose, were purchased from Sigma-Aldrich (St Louis, MO, USA) and acetic acid, lactic acid, ethanol, methanol were purchased from Merck (Darmstadt, Germany).

Organic acids, sugars and ethanol

Lactose and ethanol were quantified by high-performance liquid chromatography (HPLC), using a Jasco chromatograph equipped with a refractive index (RI) detector (Jasco 830-RI, Madrid, Spain). Lactic acid and acetic acid were also quantified by HPLC, using a Jasco chromatograph equipped with UV-Visible detector (Jasco 870-UV-visible). A Chrompack column ($300 \times 6.5 \text{ mm}$) at $60 \text{ }^\circ\text{C}$, using 5 mM sulphuric acid as the eluent, at a flow rate of 0.5 mL min^{-1} and a sample volume of $20 \text{ } \mu\text{L}$ was used.

Volatile flavour substances

In order to identify the volatile compounds, the kefir beverages were analysed directly without any previous treatment according to Fraile *et al.* (2000). A Chrompack CP-9000 gas chromatograph equipped with a Split/Splitless injector, a flame ionisation detector, and a capillary column ($50 \text{ m} \times 0.25 \text{ mm i.d.}$, $0.2 \text{ } \mu\text{m}$ film thickness; Chrompack) coated with CP-Wax 57 CB was used. The temperature of the injector and detector was set to $250 \text{ }^\circ\text{C}$. The oven temperature was held at $50 \text{ }^\circ\text{C}$ for 5 min, then programmed to run from $50 \text{ }^\circ\text{C}$ to $220 \text{ }^\circ\text{C}$ at $3 \text{ }^\circ\text{C min}^{-1}$ and then held at $220 \text{ }^\circ\text{C}$ for 10 min. Helium was used as the carrier gas at 125 kPa, with a split vent of 15 mL min^{-1} . Injections of $1 \text{ } \mu\text{L}$ were made in the splitless mode (vent time, 15 s); 4-nonanol (internal standard) was added to the sample to a final concentration of 122.05 mg L^{-1} . The volatile compounds were identified by comparing the retention times of the samples with those of standard compounds. Quantification of volatile compounds was performed with Varian Star Chromatography Workstation software (Version 6.41) and expressed as 4-nonanol equivalents, after determining the detector response factor for each compound.

Sensory evaluation

The final kefir beverages (ML, CW and DCW) were evaluated by twenty-five untrained tasters, males and females, 25–35 years of age (students of the Centre of Biological Engineering, University of Minho, Campus Gualtar, Braga, Portugal). Randomised, refrigerated ($10 \text{ }^\circ\text{C}$) samples of 10 mL were served (containing 1.0 mg of sucrose) in clear, tulip-shaped glasses with a volume of 50 mL; these were marked with three digit random numbers and covered with Petri dishes. Distilled water was provided for rinsing of the palate during the testing. Tasters were asked to indicate how much they liked or disliked each product on a 9-point hedonic

scale (9 = like extremely; 1 = dislike extremely) according to colour, odour, aroma, appearance, taste and overall acceptability characteristics.

Statistical analysis

Statistical analysis was carried out with Statistica software version 9.0 (StatSoft Inc., Tulsa, OK, USA). Principal component Analysis (PCA) was used to summarise the information in a reduced number of principal components. A one-way ANOVA was performed for chemical parameters, concentration of volatile compounds values to determine significant differences ($P < 0.05$) by using the Duncan's multiple range test using the SPSS version 10.0.

Results and discussion

Microbial metabolites

Milk and CW-based kefir beverages were monitored during the 72 h fermentation period by determining the acidity. During the 72 h of incubation, pH values of the fermented milk kefir and whey-based beverages ranged from 6.1 to 3.9, not finding significant differences for all the substrates ($P < 0.05$) (*data not shown*). These pH values were similar to those previously reported for kefir beverage (Magalhães *et al.*, 2010b). pH is an important factor that can strongly affect the quality of a beverage (Sharma *et al.*, 2009). Furthermore, pH values of the fermentation broth significantly influence the fermentation time of lactose and the levels of volatile compounds, reflecting possible variations in the sensory characteristics of the final product (Athanasiadis *et al.*, 2004).

High performance liquid chromatography (HPLC) was used to analyse organic acids, ethanol and sugars in the produced kefir beverages. The Figs 1–3 show the

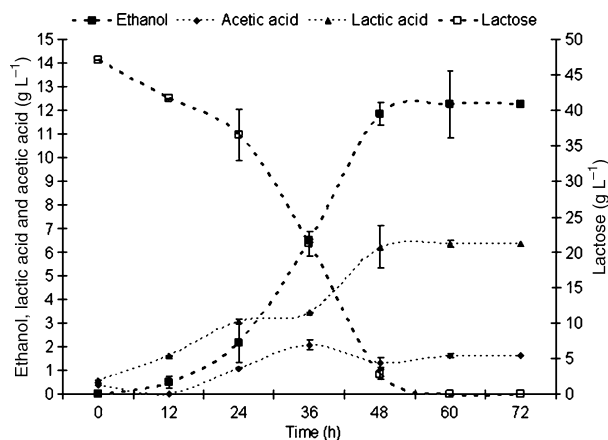


Figure 1 Chemical parameters of the fermentation process of milk kefir beverage.

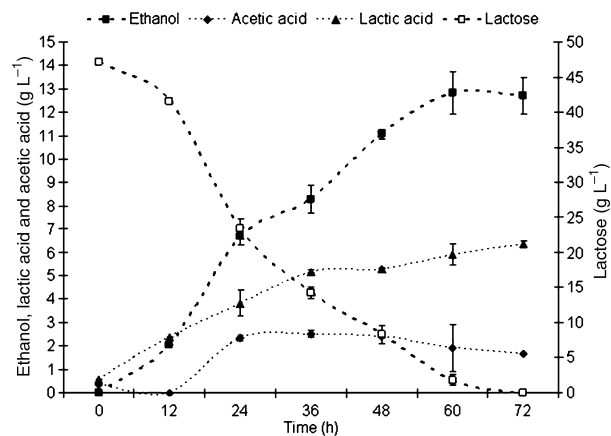


Figure 2 Chemical parameters of the fermentation process of cheese whey kefir beverage.

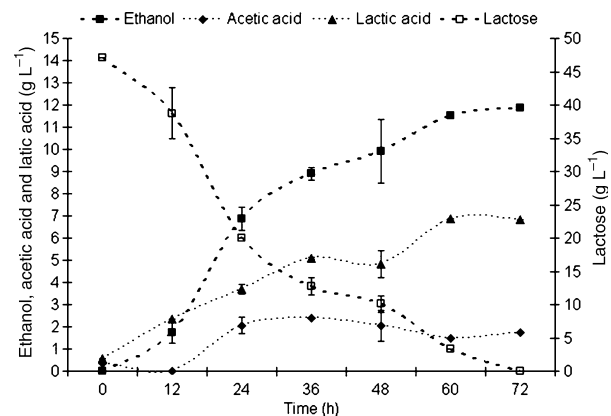


Figure 3 Chemical parameters of the fermentation process of deproteinised cheese whey kefir beverage.

concentration of sugars, organic acids and ethanol obtained by ML, CW and DCW fermentation. The production process of organic acids and alcohol was followed by the lactose consumption in kefir beverages (ML, CW and DCW). The total lactose consumption was observed in 60 h in the ML fermentation and 72 h in the CW and DCW fermentation (Figs 1–3). This likely reflects an adaptation period of the microbial community to the whole and deproteinised CW as kefir grains are fermented in milk commonly. Lactose readily degraded to galactose and glucose by Group N streptococci, *Lactobacillus* and by some strains of *Kluyveromyces* (Güzel-Seydim *et al.*, 2000).

In the present work, the lactic acid content increased during the 72 h of fermentation process in kefir beverages, reaching maximum value of 6.35 g L^{-1} , 6.34 g L^{-1} and 6.81 g L^{-1} in ML, CW and DCW kefir beverages,

respectively (Figs 1–3). The fermentation of lactose by LAB present in kefir culture can be associated with the increase in lactic acid production through the hydrolysis of sugars released from the glycomacropeptide of casein as well as the glycoproteins associated with the fat globule membrane (Rynne *et al.*, 2007). Acetic acid was also formed during the fermentation process of kefir beverages (ML, CW and DCW), reaching maximum value of $\sim 1.5 \text{ g L}^{-1}$ (Figs 1–3). The acetic acid was formed probably by heterolactic bacteria, previously identified in Brazilian kefir beverages (Magalhães *et al.*, 2010b). These results are of great importance since lactic acid and acetic acid provides pleasant taste and inhibits the development of undesirable or pathogenic microorganisms, due to the substrate acidity increase (Magalhães *et al.*, 2010b).

Ethanol concentration increased during the kefir fermentation process in all three kefir beverages, reaching maximum concentration of 12.26, 12.72 and 11.86 g L^{-1} in ML, CW and DCW kefir beverages, respectively (Figs 1–3). *Saccharomyces cerevisiae*, previously identified in kefir beverages (Magalhães *et al.*, 2010a), which exhibits strong fermentative metabolism and tolerance to ethanol, is primarily responsible for the alcohol production (Pereira *et al.*, 2010). However, some bacteria from the genus *Lactobacillus* also have the ability to produce ethanol, since they have alcohol-dehydrogenase activity, an enzyme able to convert acetaldehyde to ethanol (Magalhães *et al.*, 2010b). The content of alcohol should be enough to give kefir the flavour of a light alcoholic beverage that is typical of traditional (ancient) kefir of the Caucasus and the yeast aroma ensures the specificity of this type of fermented beverage (Beshkova *et al.*, 2003).

Aroma-related compounds

LAB present in kefir grains starter cultures produce a plethora of enzymes that contribute to the formation of volatiles via proteolysis, lipolysis and carbohydrate degradation during ripening. Such enzymes are peptidases which are involved in the transformation of casein into free amino acids which are further degraded to volatile aroma compounds. Other enzymes are esterases and lipases that hydrolyse triglycerides of dairy of fat in free fatty acids (Dragone *et al.*, 2009). GC/FID analysis was employed to determine volatile compounds in kefir beverages (ML, CW and DCW) during 72 h fermentation process. The Table 1 shows the results of the following aroma forming compounds produced in the kefir beverages. Isoamyl alcohol (3-methyl-1-butanol), isobutanol (2-methyl-1-propanol), 1-propanol, isopentyl alcohol (2-methyl-1-butanol) and 1-hexanol were the alcohols found in the kefir beverages (ML, CW and DCW) (Table 1). The identified ester is represented for ethyl acetate, while

amongst the aldehyde group, acetaldehyde was found in kefir beverages. According to some authors (Apostolopoulou *et al.*, 2005), ethyl esters (mainly ethyl acetate), alcohols with three or more carbon units, and acetaldehyde, are the major agents responsible for the flavour of fermented beverages.

Ethyl acetate has a significant effect on the organoleptic characteristics of fermented beverages. The presence of this ester results in a pleasant aroma with fruity properties, but can turn vinegary at levels above 150 mg L^{-1} , adding spoilage notes to the beverage (Falqué *et al.*, 2001). Thus, the ethyl acetate concentration in kefir beverages (8.18 , 8.27 and 8.38 mg L^{-1} in ML, CW and DCW, respectively) was found at a level suitable to confer a pleasant flavour. Normally, increased ethyl acetate concentrations are indicative of long term storage of the raw material and probable acetic bacterial spoilage. 1-propanol can also be an indicator of bacterial spoilage. The low final concentration of 1-propanol in kefir beverages (1.97 mg L^{-1} for ML, 2.44 mg L^{-1} for CW and 2.38 mg L^{-1} for DCW) can be compared with levels of other beverages, such as whiskies and cider brandies (Apostolopoulou *et al.*, 2005). The concentration of 2-methyl-1-propanol in kefir beverages (Table 1) can also be well compared with levels in other beverages (Apostolopoulou *et al.*, 2005; Dragone *et al.*, 2009).

Amyl alcohols (3-methyl-1-butanol and 2-methyl-1-butanol) are formed during fermentation by deamination and decarboxylation reactions from isoleucine and leucine, respectively (Dragone *et al.*, 2009). Such compounds constitute quantitatively the greater fraction of the alcohols in most fermented beverages (Soufleros *et al.*, 2004). In the kefir beverages produced in our study they were found in the final concentration of 8.80 mg L^{-1} for 2-methyl-1-butanol and 5.80 mg L^{-1} for 3-methyl-1-butanol (Table 1). Increased concentration of amyl alcohols can contribute negatively to the aroma of the beverages (Falqué *et al.*, 2001). The 1-hexanol alcohol was also found in kefir beverages. This alcohol has a positive influence on the aroma of the fermented beverage when it occurs in concentrations up to 20 mg L^{-1} . On the contrary, increased concentration of 1-hexanol, seriously impairs the organoleptic characteristics of the beverage (Falqué *et al.*, 2001). The low 1-hexanol final concentration found in kefir beverages (0.5 mg L^{-1} for ML, 0.57 mg L^{-1} for CW and 0.58 mg L^{-1} for DCW) can be considered to affect positively the flavour of the product. Methanol was not found in kefir beverages, that is benefic since a highly toxic effect has been reported for this compound (maximum legal limit 1000 g hL^{-1} of 100% vol. ethanol – Council Regulation (EEC) No. 1576/89, 1989) (Geroyiannaki *et al.*, 2007). The absence of methanol in kefir beverages is probably due to the lack of pectin in milk and CW.

Table 1 Concentration of volatiles compounds present in kefir beverages spirit by GC-FID

Kefir beverages fermentation	Compounds volatile						
	Acetaldehyde	Ethyl acetate	1-Propanol	2-Methyl-1-propanol (isobutyl alcohol)	2-Methyl-1-butanol (isopentyl alcohol)	3-Methyl-1-butanol (isoamyl alcohol)	1-Hexanol
Milk (mg L⁻¹)							
0 h	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
12 h	2.16 ± 0.02 acde	n.d.	n.d.	n.d.	n.d.	0.08 ± 0.04 bcde	n.d.
24 h	2.17 ± 1.12 acde	2.88 ± 1.22 bcde	n.d.	5.14 ± 1.56 de	3.97 ± 1.26 de	3.46 ± 1.58 bcde	n.d.
36 h	2.68 ± 0.75 acde	3.71 ± 0.86 bcde	n.d.	5.49 ± 1.96 de	4.20 ± 1.67 de	4.24 ± 0.47 bcde	n.d.
48 h	3.38 ± 0.65 acde	3.77 ± 1.30 bcde	n.d.	6.82 ± 0.35 de	4.44 ± 0.90 de	4.40 ± 1.79 bcde	n.d.
60 h	5.08 ± 0.82 acde	8.18 ± 1.67 bcde	1.97 ± 0.29 ab	10.89 ± 2.15 de	8.65 ± 1.94 de	5.89 ± 1.73 bcde	0.50 ± 0.02 abc
72 h	5.08 ± 0.82 acde	8.18 ± 1.67 bcde	1.97 ± 0.29 ab	10.89 ± 2.15 de	8.65 ± 1.94 de	5.89 ± 1.73 bcde	0.50 ± 0.02 abc
Cheese whey (mg L⁻¹)							
0 h	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
12 h	1.56 ± 0.01 bcde	1.06 ± 0.08 cde	0.15 ± 0.07 abcd	n.d.	n.d.	n.d.	n.d.
24 h	2.92 ± 0.16 bcde	2.35 ± 0.46 cde	2.54 ± 1.22 abcd	3.99 ± 1.10 bcde	3.84 ± 0.14 de	5.62 ± 0.26 de	n.d.
36 h	3.16 ± 0.95 bcde	2.56 ± 1.98 cde	2.46 ± 0.57 abcd	4.43 ± 1.76 bcde	4.15 ± 2.14 de	5.45 ± 0.40 de	n.d.
48 h	3.23 ± 0.98 bcde	2.56 ± 1.84 cde	2.15 ± 0.78 abcd	4.21 ± 0.30 bcde	4.07 ± 0.07 de	5.73 ± 0.05 de	n.d.
60 h	5.97 ± 0.11 bcde	6.42 ± 0.19 cde	2.37 ± 0.13 abcd	8.88 ± 2.03 bcde	8.15 ± 0.95 de	5.75 ± 0.71 de	0.57 ± 0.02 abc
72 h	5.98 ± 0.17 bcde	8.27 ± 0.37 cde	2.44 ± 0.18 abcd	10.51 ± 0.41 bcde	8.88 ± 0.23 de	5.91 ± 0.16 de	0.57 ± 0.06 abc
Deproteinised cheese whey (mg L⁻¹)							
0 h	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
12 h	1.39 ± 0.11 bcde	0.98 ± 0.03 bcde	0.10 ± 0.01 abcd	n.d.	0.13 ± 0.04 e	0.27 ± 0.24 bcde	n.d.
24 h	2.81 ± 0.01 bcde	2.32 ± 0.50 bcde	2.34 ± 0.93 abcd	n.d.	3.62 ± 0.17 e	5.40 ± 0.57 bcde	n.d.
36 h	2.80 ± 0.04 bcde	2.56 ± 0.57 bcde	2.31 ± 0.04 abcd	3.61 ± 0.55 bce	3.70 ± 0.42 e	5.37 ± 0.52 bcde	n.d.
48 h	3.12 ± 0.82 bcde	2.75 ± 0.35 bcde	2.35 ± 0.5 abcd	3.94 ± 1.07 bce	4.01 ± 0.02 e	5.80 ± 0.15 bcde	n.d.
60 h	5.95 ± 0.08 bcde	6.30 ± 0.36 bcde	2.39 ± 0.16 abcd	4.04 ± 0.07 bce	7.74 ± 0.37 e	5.83 ± 0.74 bcde	0.59 ± 0.01 abc
72 h	5.98 ± 0.61 bcde	8.38 ± 0.25 bcde	2.38 ± 0.25 abcd	10.60 ± 0.28 bce	8.89 ± 0.16 e	5.82 ± 0.02 bcde	0.58 ± 0.04 abc
Odour threshold (mg L ⁻¹)	25 ⁿ §	12.3 ^m †	266 ⁿ 750 ^o §	-	7 ^m * ‡	7 ^m * ‡	0.592 ⁿ * ‡
Descriptors	Whey ⁿ	Whey ⁿ	Whey ⁿ	-	Cheese ^m , Whey ⁿ	Cheese ^m	Whey ⁿ

Data are average values of duplicate ± standard deviation. n.d. - not detected.

*Olfactory perception threshold in hydro-alcoholic solution.

† Olfactory threshold in model wine.

‡ Olfactory perception threshold in water.

§ Olfactory difference threshold in beer.

ⁿEscudero *et al.* (2004).

^mDragone *et al.* (2009).

a-e = The averages of the columns with different letter are significantly different ($P < 0.05$).

Low molecular mass carbonyl compounds such as aldehydes and ketones are normally found in fermented beverages as by-products of yeasts fermentation, intermediates in the formation of fusel oil and as a result of alcohol oxidation at various stages of beverage production. Nevertheless, their presence is not desirable because some of them are responsible for unpleasant organoleptic properties (Dragone *et al.*, 2009). In the present study, acetaldehyde was the only carbonylated compound identified amongst the major volatile compounds, but its concentration value (5.08 mg L^{-1} for ML and 5.98 mg L^{-1} for CW and DCW) was low when compared to other beverages, such as tsipouro or grappa (Apostolopoulou *et al.*, 2005). This low concentration value is interesting, because elevated acetaldehyde concentrations give a pungent irritating odour to the beverage, and can be health hazards (Geroyiannaki *et al.*, 2007).

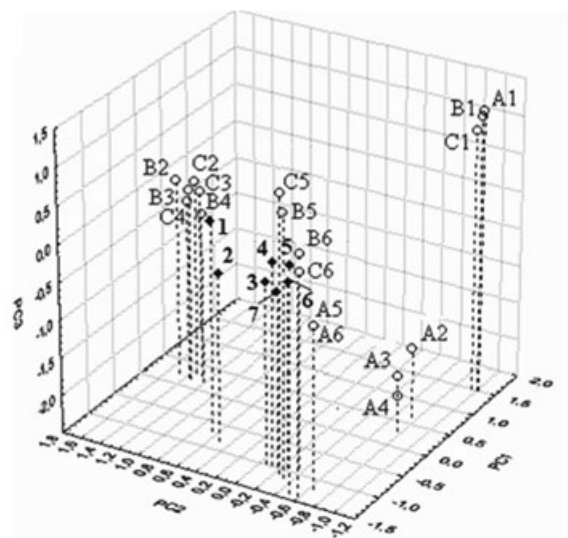
The results obtained for the volatile compounds (Table 1) were submitted to PCA to obtain a more simplified view of the relationships amongst the volatile compounds analysed (Fig. 4). The first principal component accounted for 76.85% of the total variation, while PC2 and PC3 explained 13.29% and 7.86% of the total variation, respectively. A plot of the results shows the formation of four groups (Fig. 4). Two of the groups are located on the first factor (*X*-axis positive, *Y*-axis

negative and *Z*-axis positive or negative), and includes A1, B1, C1 and A2, A3, A4 samples. These groups corresponded to the early times of fermentation and were far plotted of the 1-propanol, isoamyl alcohol and isopentyl alcohol point, suggesting that these volatiles compounds made significant contributions to separation between these. The third group is closely related on the second or third part of the axis (*X*-axis positive, *Y*-axis positive and *Z*-axis positive or negative), and includes the samples B2, B3, B4, C2, C3, and C4. These times of fermentation were mainly associated with larger concentrations of isoamyl alcohol and 1-propanol. The following group is also closely related on the second or third part of the axis (*X*-axis negative, *Y*-axis negative and *Z*-axis positive or negative) and includes the samples of end fermentation (A5, A6, B5, B6, C5 and C6). The final concentration of volatiles revealed only little variation amongst in the samples of beverages produced (ML, CW and DCW), confirming results (Table 1).

Our results indicated a significant contribution of kefir culture in volatile compounds as the composition of organic acids, esters, acetaldehyde and alcohols. The compounds identified in milk and CW-based kefir beverages are similar to those present in other beverages, like sake (Teramoto *et al.*, 2002), mouro (Soufleros *et al.*, 2004), or mescal (León-Rodríguez *et al.*, 2006) produced from *Agave salmiana*, for example. Dragone *et al.* (2009) also found similar compounds in CW alcoholic beverage.

Sensory analysis

The kefir beverages were subjected to sensory analysis to assess its acceptance. (Table 2). For all attributes assessed the beverages showed good acceptance (at least 5 points or 50% of acceptance). The likeness in sensory analysis found amongst these three beverages analysed here might be the result of the similar chemical and volatile compounds compositions of these final products (Figs 1–3 and Table 1).



[[1 = 1-Propanol; 2 = isoamyl alcohol; 3 = isopentyl alcohol; 4 = acetaldehyde; 5 = 1-Hexanol; 6 = Ethyl acetate; 7 = isobutyl alcohol.]. Samples A, B e C correspond to fermentation of ML, CW and DCW respectively. A1, B1 e C1 12 hours of fermentation; A2, B2 and C2, 24 hours of fermentation; A3, B3 and C3, 36 hours of fermentation; A4, B4 and C4, 48 hours of fermentation; A5, B5 and C5 60 hours of fermentation; A6, B6 and C6 72 hours of fermentation.]

Figure 4 Principal Component Analysis (PCA) 3D plot of concentration of volatiles compounds of kefir beverages.

Table 2 Sensory colour, appearance, taste and overall acceptability scores on a 9-point hedonic scale (9 = like extremely; 1 = dislike extremely) of kefir beverages

Kefir beverages	Sensory properties				Overall acceptability
	Colour	Aroma	Appearance	Taste	
Milk	6.02	6.11	5.82	6.17	6.16
Cheese whey	5.78	6.01	5.69	6.08	6.01
Deproteinised cheese whey	5.51	5.92	5.54	5.82	5.89

The traditional milk kefir beverage is commonly manufactured in different countries and is known for its organoleptic characteristics assessed. The potential for use of CW as a medium for manufacturing products with a sensory profile similar to that of fermented milk beverages was demonstrated by the results of this study, i.e. there was no evidence that, when using the kefir grains, adversely effect the substrate (CW or milk) on volatile compounds and sensory (Figs 1–3 and Table 1).

Assadi *et al.*, (2008) tested the technological potential of various ratios of lactic bacteria, yeasts and acetic acid bacteria isolated from kefir grains starter culture for CW fermentation. The best results were obtained when all the starter cultures were combined, i.e. bacterial mixed yeast fermentation. The potential of starter culture in production of healthy beverage from CW was found and the beverage produced good organoleptic quality and presented taste of artificial butter milk and naturally carbonated. These results confirm the good acceptable of the beverages produced in this study by kefir grains containing mixed bacterial and yeast cultures.

Conclusions

Kefir grains were able to reduce the lactose concentration in CW producing volatile compounds for good quality of the beverages. GC-FID revealed the presence of volatile compounds in the kefir beverages. Most of these compounds are similar to those reported for other fermented beverages, although the concentration values are different. Higher alcohols (mainly isobutyl alcohol and isopentyl alcohol) and ethyl esters (mainly ethyl acetate) were the most dominant compounds present, contributing thus for the greatest proportion of the total aroma. The results of this study indicate that novel beverages of acceptable organoleptic character can be produced by CW-based fermentation by kefir grains.

The proposed technology in this study is environmentally significant due to the fact that a very polluting liquid industrial waste is employed to produce products of nutritional value, including the use of probiotic kefir grains as alternative. The one key point for industrial application of the proposed technology is the promotion of fermentation by kefir of granular biomass which provides the possibility of eliminating the use of centrifugal separators that have a high energy demand and require high industrial investment.

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