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Characterization of kefir-like beverages produced from vegetable juices

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ABSTRACT

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 back-slopping 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.