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Development of new non-dairy beverages from Mediterranean fruit juices fermented with water kefir microorganisms

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ABSTRACT

The aim of this work was to explore the use of several Mediterranean fruit juices as fermentable substrates to develop new non-dairy fermented beverages. Microbiological, chemical and sensory features of kefir-like beverages obtained after the fermentation of juices extracted from fruits cultivated in Sicily (southern Italy) with water kefir microorganisms were investigated. Results indicated that both lactic acid bacteria and yeasts were able to develop in the fruit juices tested, but the highest levels were registered with prickly pear fruit juice. All fruit juices underwent a lactic fermentation, since a lactic acid content was detected in the resulting kefir-like beverages. Except kiwifruit and quince based kefirs, total titratable acidity increased for the other experimental products. A general decrease of the soluble solid content and an increase of the number of volatile organic compounds (VOCs) was also observed after fermentation. As expected, the fermentation increased the concentration of alcohols. The main fermentation in KLBs resulted to be yeast-based. Kiwifruit and pomegranate juices possessed a high antioxidant activity. Esters compounds were present at higher amount after the fermentation, especially in grape, pomegranate and quince. Aldehydes showed an opposite trend. Changes in colour attributes were registered as noticeable at human perception scale. The overall quality evaluation indicated that, among the Mediterranean fruit juices tested, apple and grape beverages were the products mostly appreciated by the tasters. Therefore, these findings support the possibility to develop fruit-based kefir-like beverages with high added value and functional properties.

1. Introduction

Functional foods influence positively one or more biological function in the human body, improving the state of health and wellness, and reducing the risk of developing diseases (Diplock et al., 1999). This food category includes all products containing probiotic microorganisms defined as “live microorganisms which when administered in adequate amounts confer a health benefit on the host” (Araya et al., 2002). The idea that alimentation might prevent human diseases is very old; “Let food be thy medicine and medicine be thy food” is a quote by Hippocrates 400 years B.C. (Otles and Cagindi, 2012).

Yogurt is undoubtedly the fermented milk product best known and consumed in the world. However, kefir represents another important fermented milk. It became very popular during the 20th century because researchers investigated on its contribution to better health (Shavit, 2008). Kefir was used for the treatment of tuberculosis, cancer and gastrointestinal disorders when modern medical treatments were not available and it is also associated with longevity in Caucasus, mountain region where it originated (Cevikbas et al., 1994; Zourari and Anifantakis, 1988). Nowadays, there is a renewed interest for this product (Shavit, 2008).

Water kefir is a non-dairy kefir prepared with a sucrose solution with or without fruit extracts (Schneedorf, 2012) fermented by kefir grains, consisting of a consortium of yeasts, mainly *Kluyveromyces*, *Candida* and *Saccharomyces*, and lactic acid bacteria (LAB), including the genera *Lactobacillus*, *Lactococcus*, *Leuconostoc* and *Streptococcus*. All these microorganisms are embedded in a resilient water-soluble branched glucogalactan matrix named kefiran (Rodrigues et al., 2005; Gulitz et al., 2011; Magalhães et al., 2010). Several of the different bacteria and yeasts that can be found in

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kefir are recognized as probiotics (Latorre-García et al., 2007; de LeBlanc et al., 2006; Zhou et al., 2009a, 2009b).

When grains are applied to ferment fruit juice, molasses or sugary solution, it is referred to as sugary kefir, water kefir or tibico (tibico's tepache) (Koutinas et al., 2009; Magalhães et al., 2010). Indeed, fruit juices contain water, sugar, proteins, amino acids, vitamins and minerals being a suitable and rich medium for microbial growth (Dias et al., 2003; Schwan, 1998) that can be used to prepare fermented beverages, like kefir, wine and other products (Duarte et al., 2010). Moreover, the fermentation of these substrates makes appreciated kefir beverages with acidic taste, refreshing, slightly carbonated, low alcoholic and acetic content (Grønnevik et al., 2011; Miguel et al., 2011).

Since the consumption of vegetables and fruits is strongly advised by many Governments to reduce the risk of several diseases and functional declines associated with aging (Temple, 2000; Willett, 1994, 1995), their fermentation might widen the choice for the consumption of these products. Over the years, new and diverse methods for processing fruits have been studied in an effort to minimize production losses, increasing farmers' income, and to introduce new products to the market (Duarte et al., 2010). The development of fruit juice-based fermented beverage with kefir may be perceived by consumers as healthy (Puerari et al., 2012).

Due to the numerous positive effects of kefir as well as vegetables and fruits on the human health, this work was aimed to evaluate the characteristics of kefir-like beverages obtained after the fermentation of juices extracted from fruits cultivated in Sicily (southern Italy) with water kefir microorganisms, in order to develop new non-dairy fermented beverages and to valorise the agricultural productions of this Mediterranean region.

2. Materials and methods

2.1. Production of fruit kefir

In this study, apple (*Malus domestica* Borkh, cv Gala), quince (*Cydonia oblonga* Mill., cv Del Portogallo), grape (*Vitis vinifera* L., white-berry cv Italia), kiwifruit (*Actinidia chinensis* Pl., cv Hayward), prickly pear (*Opuntia ficus-indica* L., cv Sanguigna) and pomegranate (*Punica granatum* L., cv Dente di cavallo) juices were subjected to fermentation. All fruits were peeled before being processed, except grape. The characteristics of the juices, just after fruit squeezing, are reported in Tables 1–3. Fruit juices (FJ) were subjected to pasteurization at 75 °C for 5 min and cooled at room temperature before processing.

Beverages were produced by backslopping: the freeze-dried microbial mixture (0.125 g) was first activated in fruit juices (50 mL) at 25 °C for 72 h to develop the inoculants (Ins); each In was then added (4%, v/v) to 1 L of the corresponding juice and the fermentation was statically performed at 25 °C for 48 h.

The fermentation was carried out with a commercial water kefir microbial preparation (BioNova snc, Villanova sull'Arda, Italy) containing approximately 10^9 CFU/g of LAB (*Lactobacillus*, *Lactococcus* and *Leuconostoc*) and *Saccharomyces* spp., as declared by the producer, which were identified as *Lactobacillus fermentum* (Acc. No. KT633923), *Lactobacillus kefiri* (Acc. No. KT633919), *Lactococcus lactis* (Acc. No. KT633921), *Leuconostoc mesenteroides* (Acc. No. KT633927) and *Saccharomyces cerevisiae* (Acc. No. KT724951) by Corona et al. (in press). Kefir-like beverage (KLB) productions were carried out in triplicate.

It should be emphasized that such an extensive 72 h fermentation period, designed to simulate backslopping, can result in strain ratios different from that of the originating freeze-dried starter. Thus, one would expect in the Ins a selective survival/growth of the acid-resistant strains, particularly the yeasts.

2.2. Microbiological analyses

FJs, Ins and KLBs were microbiologically investigated for several microbial populations. Decimal dilutions of samples, subjected to agitation by means of an orbital shaker (150 rpm for 1 min), were prepared in Ringer's solution (Sigma-Aldrich, Milan, Italy). Since no high-shear homogenization of the sample was carried out in order to break cell chains of lactic acid bacteria, the CFUs might be slightly underestimated (Champagne et al., 2011). Cell suspensions were plated and incubated as follows: total mesophilic count (TMC) spread plated on plate count agar (PCA), incubated aerobically at 30 °C for 72 h; *Enterobacteriaceae* pour plated on double-layered violet red bile glucose agar (VRBGA), incubated aerobically at 37 °C for 24 h; pseudomonads spread plated on *Pseudomonas* agar base (PAB) supplemented with 10 mg/mL cetrimide fucidin, incubated aerobically at 20 °C for 48 h; rod LAB pour plated 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 pour plated on M17 agar, incubated anaerobically at 30 °C for 48 h; yeasts spread plated on dichloran rose Bengal chloramphenicol (DRBC) agar, incubated aerobically at 25 °C for 48 h. Count plates were carried out in duplicate for each independent production.

2.3. Monitoring of dominant strains

LAB and yeast colonies (almost four for each morphology observed) developed on the agar media from the highest dilutions of the cell suspensions of the freeze-dried commercial starter preparation and KLBs were isolated, purified to homogeneity by successive sub-culturing on the same agar media used for plate counts, checked microscopically, transferred in broth media and subjected to strain differentiation.

DNA from broth cultures, developed overnight at the optimal temperatures, was extracted by InstaGene Matrix kit (Bio-Rad, Hercules, CA, USA) following the manufacturer's instruction and used for PCRs. The differentiation of the bacterial isolates was performed by random amplification of polymorphic DNA (RAPD)-PCR analysis. Strain typing was carried out in 25-μL reaction mix using the single primers M13, AB111, and AB106 as previously described by Settanni et al. (2012). Yeasts were subjected to the interdelta sequence analysis (ISA), as described by Legras and Karst (2003).

The PCR products and the molecular size marker GeneRuler 100 base pair (bp) Plus DNA ladder (M Medical Srl, Milan, Italy) were separated by electrophoresis on 1.5% (w/v) agarose (Gibco BRL, Cergy Pontoise, France) gels. The gels were stained with the SYBR® safe DNA gel stain (Molecular probes, Eugene, OR, USA) and visualised by UV trans-illumination. The polymorphic profiles were analyzed using Gelcompare II software version 6.5 (Applied-Maths, Sint-Marten-Latem, Belgium). The monitoring of the dominant strains after fermentation was obtained by profile comparison.

2.4. Physico-chemical determinations

FJ and KLB samples were subjected to several determinations. Analyses of pH and soluble solids were performed according to the methodology reported by the Association of Official Analytical Chemistry (AOAC, 2000). Measurements of pH were determined electrometrically using the pH meter BASIC 20+ (Crison Instrument S.A., Barcelona, Spain). Soluble solid content (SSC) was measured using a digital refractometer (MTD-045nD, Three-In-One Enterprises CO. Ltd., Taiwan) and reported as °Brix. Total titratable acidity (TTA) was determined by titration of the samples with 0.1 N NaOH to an end point of pH 8.1 and expressed as g/L of citric acid. Total phenolic compounds (TPs) were analysed according to the Folin-