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Development of kefir-based probiotic beverages with DNA protection and antioxidant activities using soybean hydrolyzed extract, colostrum and honey

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ABSTRACT

The aim of this study was to evaluate the use of different functional substrates (soybean hydrolyzed extract, colostrum and honey) to design novel probiotic beverages using kefir grains as starter culture. The fermentations were carried out at 30 °C for 24 h and physical-chemical composition and functional aspects were determined. It was found that fermentation processes with kefir grains increased the functional quality of all substrates evaluated. Honey-based kefir beverage had higher antioxidant activity and its microbial composition was assessed using molecular approaches (Rep-PCR and 16S rRNA gene sequencing). High levels of lactic acid bacteria and yeast populations (over 10^6 CFU/mL) were found in the product and were mainly composed of potential probiotic strains of *Lactobacillus statsumensis*, *Leuconostoc mesenteroides*, *Bacillus megaterium*, *Saccharomyces cerevisiae* and *Lachancea fermentati*. In addition, the honey-based kefir beverage showed protection effect on DNA damage and had a high sensory quality compared to traditional kefir beverage. The results demonstrated that honey could be an ideal alternative substrate for the production of functional cultured beverage, especially for vegans and lactose intolerant consumers.

1. Introduction

Probiotic food products are formulations containing sufficient numbers of selected live microorganisms (10^6–10^7 CFU/mL) that can beneficially modify the intestinal microbiota of the host (Rathore, Salmerón, & Pandiella, 2012). Kefir beverage is commonly manufactured by fermenting milk with kefir grains, which supports a complex microbial symbiotic mixture of lactic acid bacteria (e.g., *Lactobacillus*, *Lactococcus*, *Leuconostoc* and *Streptococcus*) and yeasts (e.g., *Kluyveromyces* and *Saccharomyces*) (Magalhães, de Melo Pereira, Campos, Dragone, & Schwan, 2011). Some of these different bacteria and yeasts found in kefir have been recognized as probiotics, e.g., *Leuconostoc mesenteroides* and *Saccharomyces cerevisiae* (Leite et al., 2015).

Kefir grains can be applied to ferment different substrates besides milk. These include cheese-whey, fruit juice and molasses or sugar syrups (Cui, Chen, Wang, & Han, 2013; Puerari, Magalhães, & Schwan, 2012). The development of alternative substrates used in production of fermented kefir beverage is an ideal way for the conversion of sugars to produce organic acids and alcohol. It is considered a simple and valuable biotechnology based method for maintaining and/or improving the safety, nutritional, sensory and shelf-life properties of fermented beverages (Prado, Parada, Pandey, & Soccol, 2008). Colostrum is a dairy substrate of great interest due to its positive functional properties (De Dea Lindner, Neviani, Santarelli, Soccol, & Yamaguishi, 2011). It is a complex biological fluid and a source of immunological compounds and nutrients, many proteins, immunoglobulins, non-protein nitrogen, fat, vitamins and minerals that can be used to treat or prevent infections of

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the gastrointestinal tract (Uruakpa, Ismond, & Akobundu, 2002). Additionally, soybean hydrolyzed extract and honey are both non-dairy matrixes with attractive color, good aroma and sweet sour mouthfeel, besides being a source of natural antioxidants and other functional benefits, such as hypolipidemic, anticholesterolemic, antiatherogenic and the effects of fructooligosaccharides presented in these substrates (Escriche, Kadar, Juan-Borrás, & Domenech, 2011; Pandey & Mishra, 2015). Soybean hydrolyzed extract and honey can serve as healthy alternatives for dairy probiotics to overcome problems as lactose intolerance, allergenic milk proteins and cholesterol contents (Soccol et al., 2012; Soccol & Prado, 2007).

The aim of this study was to evaluate the use of different functional products as raw material (soybean hydrolyzed extract, colostrum and honey) to design a probiotic beverage using kefir grains as starter culture. Functional characteristics and physic-chemical composition of these novel beverages were determined and compared to traditional milk kefir. In addition, the microflora, sensory quality and DNA protection effect of honey kefir beverage was evaluated due to its higher antioxidant activity. The bioprocess for the production of honey beverage fermented with kefir grains is part of a patented application process (No. BR 102014021724 0) authored by Soccol, Fiorda, Prado, & Bellettini, 2014.

2. Material and methods

2.1. Kefir grains and inoculum preparation

Kefir grains from Tibet (Province of Sigatse) and Mexico (Province of Guanajuato) were obtained from families that traditionally consumed kefir. The samples of Tibetan Kefir were preserved in sterilized milk (5%, w/v) and the samples of Mexican Kefir were preserved in brown sugary solution (10% w/v). To preserve the kefir grains the substrate was renewed daily for a period of seven days. The grains were then washed with sterile distilled water and subsequently used to inoculate different raw materials (soybean hydrolyzed extract, colostrum and honey).

2.2. Must preparation

2.2.1. Soybean hydrolyzed extract

Mature soybean seeds were obtained from local market in Curitiba, Paraná - Brazil. The seeds were thoroughly washed and soaked overnight at 25 °C with 10 times their weight of distilled water. Using a blender, the soybean seeds were homogenized at low speed for 1 min. Soybean hydrolyzed extract was obtained from the resulting slurry by the removal of an insoluble residue (soybean pulp fiber) by filtration. The soybean hydrolyzed extract was heated at 96 °C for 40 min and then cooled to room temperature (25 °C).

2.2.2. Honey media

Honey was obtained from local market in Curitiba, Paraná - Brazil. Honey-based media was prepared by mixing honey with sterile distilled water in proportion to obtain a must of 40 °Brix, therefore, was used the Equation (1).

$$W_{honey} \times {}^{o}Brix_{honey} = W_{must} \times 40^{o}Brix \quad (1)$$

Where W_{honey} is the necessary amount of honey and W_{must} is the amount of must is desired to produce. After determining the required amount of honey, the amount of water being added was estimate by Equation (2). The honey must was pasteurized at 63 °C/30 min before use.

$$W_{water} = W_{must} - W_{honey} \quad (2)$$

2.2.3. Bovine colostrum

Bovine colostrum was collected within the first 12 h after calving from three healthy cows (breed "jersey") kept under veterinary supervision at a dairy farm localized in the city of Castro (24° 47′ 28″ S and 50° 00′ 43″ W) Southern of Brazil. The colostrum was defatted by centrifugation (3000 × g/20 min/2 °C), pasteurized at 63 °C/30 min and divided into aliquots that were kept frozen at −20 °C until use (De Dea Lindner et al., 2011).

2.3. Production of kefir beverage

The Tibetan kefir grains were inoculated into soybean hydrolyzed extract, colostrum and cow milk substrates, while Mexican kefir grains were inoculated into honey must. The selection of raw material and its respective kefir inoculum (Tibetan or Mexican) was based on preliminary tests carried out with biomass growth (data not shown). Wet weight cells of 100 g were transferred into 2 L of fermentation substratum. A batch aerobic fermentation was carried out in static conditions at 30 °C for 24 h. The pH kinetic of the fermented kefir beverages was determined using a pH meter and measured after 0, 12, 24, 36 and 48 h. Even thought the fermentation time was 24 h, the pH was measured until 48 h in order to determine the change in pH over the period of fermentation time.

2.4. Physical-chemical characterization of kefir beverages

2.4.1. Volatile flavor compounds

Aroma compounds of kefir beverages produced after 24 h of fermentation were measured by headspace analysis in a gas chromatograph (Shimadzu model 17A) equipped with a flame ionization detector at 230 °C. Aroma compounds were identified by comparing the peak retention times against those of authentic standards purchased from Sigma. The operation conditions were as follows: a 30 m × 0.32 mm HP-5 capillary column, column temperature of 40–150 °C at a rate of 20 °C/min, injector temperature at 230 °C. Individual volatiles were expressed as μmol/L of headspace, as ethanol equivalent (Pereira et al., 2014).

2.4.2. HPLC analyses

Sugars (glucose and lactose), ethanol and lactic acid were quantified by high-performance liquid chromatography (HPLC). The kefir beverages were separated by centrifugation at 6000 × g and filtered through 0.22-μm pore size filter (Millipore Corp., Billerica, MA). The filtered samples were injected (50 μL) into HPLC system equipped with an HPX-87H column (300 by 7.8 mm; Bio-Rad Laboratories, California) connected to a refractive index (RI) detector (HPG1362A; Hewlett–Packard Company). The column was eluted with a degassed mobile phase containing 5 mM H_2SO_4 at 60 °C at a flow rate of 0.6 mL/min (Prado et al., 2015).

2.4.3. Functional aspects

The functional aspects (antioxidant activity and exopolysaccharides production) were performed in samples at the start of the fermentation (0 h) and after 24 h of fermentation.

2.4.3.1. DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging assay. The DPPH radical scavenging activity was measured in kefir beverages (0 and 24 h of fermentation) according to the procedure described by Rufino et al. (2010). A DPPH· solution (80 μM) was freshly prepared in 95% methanol. A volume of 250 μL of this solution was allowed to react with 35 μL sample and the absorbance was measured at 515 nm, for 30 min. The capability to scavenge the DPPH radical was calculated using the following