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Fermentation process for production of apple-based kefir vinegar: microbiological, chemical and sensory analysis

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ABSTRACT

The aim of this study was to develop a kefir apple-based vinegar and evaluate this fermentation process using new methodology with Biospeckle Laser. Brazilian kefir grains were inoculated in apple must for vinegar production. In this study, the microbial community present in kefir, and correspondent vinegar, was investigated using Matrix Assisted Laser Desorption/Ionization – Time of Flight Mass Spectrometry (MALDI-TOF MS) technique. *Saccharomyces cerevisiae*, *Lactobacillus paracasei*, *Lactobacillus plantarum*, *Acetobacter pasteurianus* and *Acetobacter syzygii* were the microbial species identified. *S. cerevisiae*, *L. plantarum*, *A. pasteurianus* and *A. syzygii* were found in smaller quantities at the beginning of the alcoholic fermentation, but were found throughout the alcoholic and acetic fermentation. Kefir grains were able to utilize apple must as substrate to produce ethanol, and acetic acid. Acetate, volatile alcohols and aldehydes in the vinegar-based kefir were also produced. The yield of acetic acid in the kefir vinegars was ~79%. The acetic acid concentration was ~41 g L^{-1}, reaching the required standard for the Brazilian legislation accepts it as vinegar (4.0% acetic acid). Kefir vinegar showed good acceptance in the sensory analysis. The technology proposed here is novel by the application of immobilized-cell biomass (kefir grains) providing a mixed inocula and eliminating the use of centrifuge at the end of the fermentative process. This step will save energy demand and investment. This is the first study to produce apple vinegar using kefir grains.

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Introduction

Apple (*Malus* spp.) is probably the oldest fruit known to man and is favored by millions of people around the globe.[10] In Brazil, some of these apple fruits are consumed by local populations, but the majority is wasted during harvest because of the high production per tree, the short shelf life of the fresh fruit, and the lack of use of these fruits as processed products.[1,22] Fruits wine and/or vinegar production, could be a good solution because they allow products to be maintained in alcohol or acetic acid.[1,9] Although the number of studies of fruit wines[1,5,7,21] and fruit vinegars[9,10,12] has recently increased, no study has focused on the production of apple kefir vinegar.

Kefir is a culture employed to produce beverages, such as the traditional Russian beverage also named "kefir" which is from milk, and has low alcohol content.[23,13–17] The kefir is a mixed culture of various yeast species of the genus *Kluyveromyces, Candida, Saccharomyces* and lactic acid from bacteria of the genus *Lactobacillus* combined in a matrix of proteins and polysaccharide 'kefiran', which are formed during cell growth under aerobic conditions.[23] The grains of kefir are irregularly shaped, with yellowish-white color, and hard granules, which resemble miniature cauliflower blossoms.[15] In Brazil, the grains of kefir are used in private household,[15] and they are added to different types of milk, such as cow, goat or sheep, coconut, rice and soy milk.[21,23] The grains are responsible for the fermentation that produce lactic acid, acetic acid, CO_2, alcohol (ethyl alcohol) and aromatic compounds. These compounds provide kefir's unique sensory characteristics: fizzy, acid taste, tart and refreshing flavor.[15] The beverage contains vitamins, minerals and essential amino acids that help the body with healing and maintenance functions and contains easily digestible complete proteins.[15]

An optical technique to evaluate the biological activity of kefir grains was used, the Laser Biospeckle. The technique defines when a laser beam is scattered by a biological sample; the scattered waves generated in the illuminated sample create the speckle pattern that changes its image in accordance with the changes in the monitored material. Thus, the surface appears to be covered with tiny bright dots that fluctuate in a seemingly random way as for a boiling liquid.[3,8]

The aim of this study was to develop an apple vinegar-based kefir and study this fermentation process using new method Biospeckle Laser to evaluate the biological activity of kefir grains.

Materials and methods

Brazilian milk kefir grains (Stock-culture of the Microbial Fermentation Laboratory of the Federal University of Lavras, Brazil) were used in the experiments. The apple fruits were obtained from Lavras city market, Minas Gerais State in Brazil. These apple fruits, which fail to meet the quality standards required for marketing, were washed in clean water to remove residues. The pulp was extracted using an automatic depulping machine (ITAMETAL 0.5 DS, Itabuna, BA, Brazil). The °Brix was analyzed, and the juice was divided into three 500 mL Erlenmeyer flasks to start batch fermentation.

The process of vinegar production

Alcoholic fermentation

The must was inoculated with kefir grains in a proportion of 10% (w/v). All fermentations were performed in triplicate. The flasks were incubated statically at a temperature of 28 °C and fermentation was monitored daily to observe the end of the fermentation (stability of the consumption of sugars – Brix). The fermentation must was filtered by kitassato filters (0.5 μm). The kefir grains were recovered. The alcoholic must was used for apple kefir vinegar production. Microbiological analysis (grains and must), measurement of Brix, reducing sugar analysis and pH [11] were carried out during fermentation.

To determine fermentation performance were calculated the substrate conversion factors in ethanol ($Y_{p/s}$), substrate conversion in glycerol ($Y_{g/s}$), substrate conversion in acetic acid ($Y_{ac/s}$), ethanol volumetric productivity of ethanol (Q_p), biomass productivity (P_x) and conversion efficiency (E_f). The total concentration of sugars was calculated considering the conversion for each mole of sucrose (342 g) in 1 mol of glucose (180 g) and 1 mol of fructose (180 g).

Acetic fermentation

The apple fermented must obtained in the previous section were acetified in 500 mL Erlenmeyer flasks, which were controlled temperature of 28 °C and agitation of 150 rpm. The acetic fermentation was conducted using the following treatments: (1) kefir grains 10% (w/v); (2) kefir grains 20% (w/v). During acetic fermentation, daily samples were taken (6 days) in triplicate for acidity analysis (pH meter) and alcohol (alcoholometer). The acetic fermentation was finish on the sixth day when the vinegar presented alcohol content below 1.0% (v/v).

The yield was calculated as the acetic acid produced in relation to the theoretical yield. The theoretical yield was calculated as the amount of ethanol converted to acetic acid, in which 1.0 g of ethanol yields 1.304 g of acetic acid.[5] The ethanol, methanol, higher alcohols were analyzed according to Magalhães et al.[15] using a Jasco chromatograph equipped with a refractive index (RI) detector (Jasco 830-RI, Madrid, Spain). A Chrompack column (30 cm × 6.5 mm) at 60 °C, using 5 mM sulphuric acid as the eluent, at a flow rate of 0.5 mL/min. Glycerol, organic acids (lactic, acetic, tartaric, malic, citric and succinic acid) and carbohydrates (glucose, sucrose and fructose) contents were quantified according to Puerari et al.[21] using a Jasco chromatograph equipped with a refractive index (RI) detector (Jasco 830-RI, Madrid, Spain) and UV–visible detector (Jasco 870-UV-visible). A Chrompack column (300 × 6.5 mm) at 60 °C, using 5 mM sulphuric acid as the eluent, at a flow rate of 0.5 mL/min and a sample volume of 20 L was used. All the samples were examined in triplicate.

Microbiological analysis

Yeast, lactic acid bacteria (LAB), and acetic acid bacteria (AAB) counts were performed on Yeast Extract Peptone Glucose [YEPG: 1% yeast extract, 2% peptone (Himedia, Mumbai, India),