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Chemometric evaluation of the volatile profile of probiotic melon and probiotic cashew juice

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ABSTRACT

The aim of this study was to evaluate the influence of the lactic acid fermentation on volatile compounds of melon and cashew apple juices. The effect of the fermentation processing on the volatile profile of probiotic juices was assessed by HS-SPME/GC–MS coupled to chemometrics with 67.9% and 81.0% of the variance in the first principal component for melon and cashew juices, respectively. The *Lactobacillus casei* fermentation imparted a reduction of ethyl butanoate, ethyl-2-methylbutirate, and ethyl hexanoate for melon juice; and of ethyl acetate, ethyl-2-methyl butanoate, ethyl crotonate, ethyl isovalerate, benzaldehyde, and ethyl hexanoate for cashew juice. Measurements of the stability of these compounds and the formation of the component 3-methyl-2-butenyl in melon juice may be used as a volatile marker to follow the juice fermentation. These findings suggested that even though it is not a dairy product the lactic acid fermentation of fruits developed a volatile profile combining the fruit and lactic acid fermentation volatiles with mildly formation or degradation of aroma compounds.

1. Introduction

Humans have consumed fermented foods since ancient times. Fermentation is a way of preserving and modifying the taste of foodstuffs. Among fermented foods, alcoholic beverages are the most studied and the one with more available information. Fermented milk is also a widely consumed and studied food. Recently the use of probiotic cultures in fermented foods from vegetable origin such as fruit juice has increased. The microorganisms used for milk fermentation are usually lactic acid bacteria. Lactic acid bacteria are well adapted to milk and aside lactic acid production they are also able to produce several transformations on milk changing the taste, the texture, and the product aroma (Smid & Kleerebezem, 2014).

The most studied lactic acid bacteria (LAB) are *Lactobacillus bulgarius* and *Streptococus thermophillus*, which are industrially used to produce yogurt, the most known and consumed fermented dairy based product. However, the use of probiotic bacteria belonging to the genus *Lactobacillus* and *Bifidobacteria* has increased in non-dairy fermented products due to the additional benefits that these strains can promote in the human body due to its regular consumption. *Lactobacillus casei* was successfully applied to develop probiotic fruit juices (Alves, Messaoud, Desobry, Costa, & Rodrigues, 2016; Costa, Fonteles, Jesus, & Rodrigues, 2013; Fonteles et al., 2013; Pereira, Maciel, & Rodrigues, 2011).

Recently, the use of fruit juice as a vehicle for probiotic bacteria has been extensively studied (Costa et al., 2013; Fonteles et al., 2013; Pereira et al., 2011; Shah, Ding, Fallourd, & Leyer, 2010; Wang, Ng, Su, Tzeng, & Shyu, 2009). The use of fruit juice in substitution of milk allows the consumption of probiotic bacteria by consumers who cannot or avoid the consumption of dairy based products. The importance of the theme (probiotic fruit juice) is evident with the increased number of recent publications and patents. In the last five years, more than 100 publications and 46 patents were found for probiotic fruit juice (Source: Scopus database and Patentscope database, both accessed on May 2017). Also, there are at least three large companies with probiotic fruit juices on the market (Danone/ProViva, Tropicana, and GoodBelly). A probiotic commercial product must contain an enough number of an

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alive recognized probiotic strain (Fonteles et al., 2013; Pereira, Almeida, de Jesus, da Costa, & Rodrigues, 2013).

Despite the technological importance of lactic acid fermentation, the most published studies are focused on the final product quality such as acceptance, shelf life, texture, and other aspects. On the contrary of alcoholic beverages, which are obtained by yeast fermentation, where the volatile profile is well known and extensively studied, there are few published studies on lactic acid fermentation aroma production and all of them apply milk as raw material (Beshkova, Simova, Frengova, Simov, & Dimitrov, 2003; Kebede, Viljoen, Gadaga, Narvhus, & Lourens-Hattingh, 2007; Smid & Kleerebezem, 2014). LAB present fast growth and rapid acidification of the food matrix, which contributes to the food preservation and it is the primary reason for using these LAB in food processing. However, besides lactic acid production, several secondary changes are also promoted by LAB fermentation such as texture and nutritional changes as well as aroma production. All these changes are strongly dependent on the metabolic activity of the fermenting bacteria along with the chemical composition of the food matrix. In fact, the aroma production in lactic acid fermentation can be due the microbial metabolic activity and other factors (non-metabolic activity) such as extracellular enzyme activity of aroma precursor or cell lysis (Smid & Kleerebezem, 2014).

Aroma compounds are volatile organic substances widely distributed in fruit juices. The evaluation of these compounds is a challenging to gas chromatography (CG) methods due to the overlapping signals and the high number of compounds, usually of the most significant to taste and flavor (Augusto, Valente, Tada, & Rivellino, 2000; Ikegami, Tomomatsu, Takubo, Horie, & Tanaka, 2008). Due to the highly complex GC–MS datasets usually obtained from fruit juices and the inherent similarity between the chromatograms (same matrices), chemometric techniques have become essential to analyze the chemical variability and to detect slightly and almost imperceptible composition changes (Alves Filho et al., 2016; Costa et al., 2010). Chemometric tools have been used as an additional method for data exploration, such as exploratory analysis which enables the determination of the natural clusters; consequential recognition of samples; determination of the data information content; and verification of variables that better define the groups. For this purpose, exploratory methods can be applied using the unsupervised pattern recognition techniques as Hierarchical Cluster Analysis (HCA) and Principal Component Analysis (PCA) (Choze et al., 2013; Silva, Alves Filho, Choze, Lião, & Alcantara, 2012).

As the fermentation process and the microbial metabolism impart changes in the volatile compounds of the food matrix, the aim of this study was to evaluate the influence of lactic acid fermentation process in the volatile compounds of melon and cashew juices. The fermentation processing was already optimized by our research group using *Lactobacillus casei* NRRL B-442 (Fonteles, Costa, Jesus, & Rodrigues, 2012; Fonteles et al., 2013; Pereira, Almeida, Lima, Costa, & Rodrigues, 2014; Pereira et al., 2011), and it is not the focus of the present study. Therefore, HS-SPME/GC–MS combined with chemometrics regarding the chemical changes due to the fruit juice fermentation was used to determine the pattern distribution of samples and to identify the most affected compounds due to the fermentation. A recent study using MRS and L. *casei* was published by (Gallegos, Arce, Jordano, Arce, & Medina, 2017). However, the aroma profile obtained was different from ours, attesting the effect of food matrix on the aroma profile. To the best of our knowledge, no previous studies on the aroma profile of fermented fruit juices by *Lactobacillus casei* were published.

2. Materials and methods

2.1. Materials

2.1.1. Fruit juice preparation

The cashew fruits (*Anacardium occidentale* L.) were obtained from Embrapa Pacajus Experimental Station (Pacajus, Ceará - Brazil). After the nut removal, the juices were obtained by pressing the cashew in an expeller pressing (INCOMAP, Fortaleza, Ceará, Brazil). The juices were then clarified with gelatin (1% *v*/v) to remove tannins (Pereira et al., 2014; Silveira, Fontes, Guilherme, Fernandes, & Rodrigues, 2012). Frozen cantaloupe melon pulps were obtained at the local market, and the juices were prepared by diluting the pulp to 3° Brix in potable water as recommended by the manufacturer.

2.1.2. Microorganism

In the present study, a lyophilized strain of *Lactobacillus casei* NRRL B-442 donated by ARS Culture Collection (Peoria, Illinois – USA) was used (Fonteles et al., 2012; Pereira et al., 2011).

2.1.3. Juices fermentation

The juice fermentation was carried out twice (replicate) as previously optimized (Fonteles et al., 2012). The stock culture of L. *casei* B-442, was propagated in 100 mL of MRS broth at 37 °C for 12 h to obtain an initial cell concentration of approximately 9.0 log counting forming units per milliliter (log CFU.mL^{-1}). Fermentation was carried out statically in 500 mL Erlenmeyer flasks containing 100 mL of the desired juice. Cantaloupe melon juice was fermented at the optimum conditions: initial pH 6.1 at 31 °C for 8 h as determined by Fonteles et al. (2012). Cashew juice was fermented at pH 6.4 and 30 °C for 16 h as determined by Pereira et al. (2011). The initial pH was adjusted to the required value with NaOH 3.0 M (cashew juice) or citric acid 0.1 M (cantaloupe melon juice). After the juice fermentation, the microbial cells were removed by centrifugation at 11.806*g* for 10 min at 4 °C using a Sigma 6 K-15 centrifuge (Sigma Centrifuges, Germany). After the centrifugation, the samples without the bacterial cells were stored frozen (− 20 °C) until the analysis.

2.2. Sample preparation and HS-SPME/GC–MS analysis

An aliquot of 8.0 mL of melon or cashew juices samples was added to 20 mL vial and equilibrated at 60 °C for 10 min with constant stirring at 500 rpm. An automatic SPME holder (Supelco, Bellafonte, PA, USA) with a DVB/CAR/PDMS (50/30 μm) of 2 cm length fiber was used to capture the volatile compounds. The SPME fiber was exposed to the sample headspace at constant depth for 25 min. The temperature was kept at 60 °C throughout the extraction of the volatile compounds without agitation. After the extraction, the volatiles were directly desorbed on the GC liner and maintained at 250 °C for 3 min for fiber reconditioning. Samples were analyzed in a GC–MS Varian/Agilent 450GC-240MS connected to an ion trap detector operating in the EI mode at 70 eV and 200 °C with a scan mass range of 40 to 400 *m/z* at a sampling rate of 3.0 μscans. The carrier gas was helium at 1.0 mL.min^{-1}. The injector and the interface temperatures were 250 °C in splitless mode. The temperature ramp was: 40 °C for 2 min, increased to 200 °C at 5 °C.min^{-1}. The temperature was held at 200 °C for 5 min and then increased to 260 °C at 30 °C.min^{-1}. The final temperature (260 °C) was held for 5 min. Chromatographic separations were performed using 5% phenyl-methyl column (30 m × 0.25 mm ID × 0.25 μm film). Individual components were identified by comparing their linear retention indexes (LRI) with a C_8-C_{30} *n*-alkanes series (Van den Dool & Kratz, 1963). The mass spectra were compared with the NIST mass spectra library and with those available in the literature (Beaulieu, 2005; Beaulieu & Grimm, 2001; Chaparro-Torres, Bueso, & Fernández-Trujillo, 2015; Garruti, Franco, Silva, Janzantti, & Alves, 2003, 2006). All the analyses were performed in triplicate.

2.3. Chemometric analysis

Chemometric analyses were performed using the non-fermented juices (codified as “nFerm”) and the fermented juices (codified as “Ferm”). All the chromatographic data were converted to the ASCII (American Standard Code for Information Interchange) files and