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## Potential application of four types of tropical fruits in lactic fermentation



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### ABSTRACT

Fruit juice has been used for the production of lactose-free or low-lactose beverages. This study evaluated the potential of four types of tropical fruit (carambola, guava, mango and pitaya) for the production of fermented lactic beverages with and without whey addition. Pasteurized fruit juices were used at (% v/v): 100% fruit juice, 100% whey and 50:50% of juice: whey. The fermentations were conducted with a lactic culture (*L. casei*, *S. thermophilus*, and *L. bulgaricus*) at 37 °C for 72 h. The final characteristics obtained were pH 3.0–4.0, soluble solids concentration (SS) 5.0–7.0 °Brix (g/100 g), and acidity 0.2–1.0 %w/v. A validation test obtained 4.0–4.8 (pH), 6.0 °Brix (SS), and 0.30–0.50 %w/v (acidity) at 24 h. The lactic bacteria had better growth ( $10^7$ – $10^8$  CFU/mL) and the highest sensory acceptance rates (70% for taste, 75% for flavor and 90% for color) utilizing mango or guava. Carambola and pitaya caused a partial inhibition to the cellular growth ( $10^3$ – $10^4$  CFU/mL) and resulted in beverages with lower sensory acceptance. In all cases, formulations containing only fruit juice were lactose-free and better sensory acceptance than the ones with of whey.

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## 1. Introduction

Lactic fermentation of food has several advantages and it is utilized mainly for the production of different dairy products. In this process, the lactose is fermented by acid-lactic bacteria which reduce the concentration of this sugar in the fermentation medium, promote the acidification and the formation of secondary metabolites. The production of lactic acid and other metabolites is responsible for the characteristics of lactic fermented products, such as flavor, taste, increase of shelf life and reduction of the lactose content (Fox & McSweeney, 1998).

The lactic fermentation is usually associated with milk or dairy products; however, it can be applied successfully to other substrates, such as fruit pulp or juice, due to the high fermentable sugar contents. Moreover, fruits and vegetables naturally contain acid lactic or acetic bacteria in their microbiota (DiCagno, Codda, De Angelis, & Gobbetti, 2013; Nyanga et al., 2007; Sagdic, Ozturk, Yapar, & Yetim, 2014). The application of fruit as substrate for lactic fermentation also has the advantage of the incorporation of flavors and nutrients specific to each type of fruit, resulting in products with different sensory and physico-chemical characteristics. Furthermore, the fruit based fermented products are lactose free, which meet the needs of consumers with lactose intolerance.

There are reports in the literature of some studies with fruits and vegetables juices applied successfully to the lactic fermentation process, such as: pomegranate (*Punica granatum* L.) (Filannino et al.,

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2013), cantaloupe melon (*Cucumis melo* L.) (Fonteles et al., 2013; 2012), cashew apple (*Anacardium occidentale*) (Pereira, Maciel, & Rodrigues, 2011), carrot, cabbage, beetroot and onion (Gardner, Savard, Obermeier, Caldwell, & Champagne, 2001; Yoon, Woodams, & Hang, 2006, 2005; 2004). Kourkoutas, Xolias, Kallis, Bezirtoglou, and Kanellaki (2005) utilized fruit as supports for the growth of acid-lactic bacteria and DiCagno, Minervini, Rizzello, De Angelis, & Gobbetti, 2011 obtained fermented fruit smoothies. However, it is known that the survival of lactic bacteria in fruit substrates tends to be more complex than in the dairy ones. This is due mainly to the natural acidity of fruit, the high level of polyphenols and the absence of lactose, which may interfere in the survival of certain sorts of microorganisms as well as in their growth (DiCagno et al., 2011; Fonteles et al., 2013).

Based on the promising results reported on the literature for the lactic fermentation of fruit, the aim of this study was to evaluate the potential of four types of tropical fruit (carambola - *Averrhoa carambola* L.; guava - *Psidium guajava*; mango - *Magifera indica* L. var. Tommy Atkins; and red bark pitaya - *Hylocereus undatus*) for the production of fermented lactic beverages. Additionally, whey, a sub-product largely utilized in dairy products, was also investigated in association with the tropical fruits studied. The fermented lactic beverages were evaluated regarding the concentration of soluble solids (SS), cellular viability, pH and titratable acidity along the fermentation time and the sensory acceptance of the final product.

## 2. Materials and methods

### 2.1. Fruit juice preparation

For this study, four types of tropical fruits were chosen: carambola (*Averrhoa carambola* L.), guava (*Psidium guajava*), mango (*Magifera indica* L. var. Tommy Atkins), and red bark pitaya with white pulp (*Hylocereus undatus*). The fruits were purchased in a local supermarket (Campinas, São Paulo, Brazil). They were all ripe, undamaged and visually adequate for consumption (good color, odor and texture). For the preparation of the pulps, the fruits were washed, peeled and crushed in a blender. The initial SS of pulps were adjusted by the dilution with distilled water, until the obtainment of juice with 7–8 °Brix (g/100 g of sugar). Thereafter, the juice samples were pasteurized in batches at 80 °C for 5 min (Nagpal, Kumar, & Kumar, 2012) and bottled hot in glass flasks of 500 mL. After bottling, the flasks were closed hermetically and, after cooled to room temperature, were frozen at –18 °C (de Paula Valim, Aguiar-Oliveira, Kamimura, Alves, & Maldonado, 2016).

### 2.2. Whey preparation

Fresh milk (15.0 L) was obtained from a local dairy processor (Campinas, São Paulo, Brazil). The coagulation was conducted by the addition of a lactic culture (170 g of industrialized yogurt resulting in an inoculation of  $1.10^6$  CFU/mL) and 5.0 mL of liquid rennet (as indicated by the producer). After 1 h of coagulation at room temperature (25 °C), the coagulated mass was separated from the whey by filtration. The whey was pasteurized and stored under the same conditions as for the fruit juice.

### 2.3. Lactic acid fermentation

The fermentations were performed with three substrate volume compositions: 100% fruit juice; a mixture of 50:50% fruit juice and whey and 100% whey, totaling nine different formulations. All formulations were evaluated in triplicate utilizing 500 mL Erlenmeyer flasks previously sterilized. To each flask, 300 mL of substrate were added and inoculated with a lyophilized lactic culture

containing *Lactobacillus casei*, *Lactobacillus bulgaricus* and *Streptococcus thermophilus* (Rhodia Foods®) in quantity enough to reach the initial counting of  $1.10^6$  CFU/mL. The flasks were kept at 37 °C without agitation for a period of 72 h. Samples of each fermentation were collected at 24, 48 and 72 h for the quantification of the concentration of soluble solids (SS), pH, and titratable acidity and to evaluate the microbial growth. The fermented beverages obtained at the end of the fermentation were utilized for the sensory evaluation.

### 2.4. Physico-chemical, microbiological and sensory analyses

The concentration of soluble solids (SS) was directly measured with a refractometer (Atago - Master-53M) and the pH was measured with a pHmeter (Hanna - HI 3222-01). The acidity was measured by titration with NaOH 0.1 mol/L and the end point was determined by monitoring the pH within the range 7.8–8.2 using the pHmeter. The microbial growth was measured qualitatively by Gram stain method in order to identify the type of microorganisms as well as their growth in the fermentation medium. The slides were prepared as described by Barile (1983) and analyzed with a microscope (Bioval - L-2000B-PL) with a 1000x oil-immersion lens.

The fermented beverages obtained were stored in a refrigerator at 4 °C for 24 h and then submitted to the sensory evaluation by 32 non trained panelists, by means of a consumer affective test. A 9 points verbal hedonic scale (1 = dislike it very much and 9 = like it very much) was utilized to evaluate the attributes taste; flavor and color and a 9 points ideality scale (1 = much lower than the ideal and 9 = much higher than the ideal) was utilized to evaluate the attributes acidity and sweetness. From the data obtained, the average and the deviation standard for each attribute were calculated and the analysis of variance (ANOVA) and the Tukey test of average were applied to verify if there were significant differences among samples.

The sensory evaluation was performed in two sessions. The non-sugared fermented beverages of mango and pitaya (with and without whey) were evaluated in the first session. The sugared [10% (g/100 mL) of sucrose] fermented beverages of guava and carambola (with and without whey) were evaluated in the second session. In both sessions, the fermented beverage obtained using just whey was included in the sensorial analysis. The addition of sucrose to beverages from guava and carambola was made due to the high acidity verified in the sensory evaluation of the first session. This study was conducted according to the ethical standards established by the Brazilian legislation for research involving human beings and is registered in the Brazilian Ministry of Health in the Brazil Platform (CAAE: 50655215.6.0000.5425).

### 2.5. Validation experiment

A validation experiment was performed with the same formulations of the first experiment, but the concentration of soluble solids (SS) for all formulations was standardized (6 °Brix) by dilution with distilled water. The fermentations were conducted using  $1.10^6$  CFU/mL of inoculum, at 37 °C and without agitation. The fermentation time was limited to 24 h because the major changes in fermentation, observed in the previous experiments, occurred during this period. Samples were collected at the beginning and at the end of fermentation for the physico-chemical analyses. The cellular growth was measured through the colony forming units (CFU). Serial decimal dilutions of each sample were plated in triplicate onto Man, Rugosa and Sharpe (MRS) Agar plates (Kasvi®, Brazil) and incubated at 37 °C for 48 h. Plates with number of colonies from 30 to 300 were enumerated and the results were expressed as CFU/mL (counting forming units) (Fonteles, Costa, De

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