LWT - Food Science and Technology 62 (2015) 838–846

Contents lists available at ScienceDirect

LWT - Food Science and Technology

journal homepage: www.elsevier.com/locate/lwt

# Probiotic clarified apple juice with oligofructose or sucralose as sugar substitutes: Sensory profile and acceptability

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ARTICLE INFO

*Article history:*
Received 14 April 2014
Received in revised form
1 August 2014
Accepted 5 August 2014
Available online 13 August 2014

*Keywords:*
*Lactobacillus casei* 01
Prebiotic
QDA
Sweetener
Fruit juice

ABSTRACT

The effect of the addition of oligofructose or sucralose as sugar substitutes and *Lactobacillus paracasei* ssp. *paracasei* as a probiotic on the sensory profile and acceptance of clarified apple juice were evaluated. The influence of the sensory attributes on the acceptability was also determined. Juices with oligofructose (20 g/L) showed less sweetness than those with sucrose (20 g/L), while those with sucralose (0.03 g/L) had a lighter colour. Oligofructose or sucralose contributed to the increased acceptance (taste and overall impression) of the pure juices, making it similar to that of the sucrose-product. There were no differences in the acceptance of the appearance, aroma or texture. The probiotic increased the turbidity of the juice, however, it did not alter the acceptance (appearance, aroma, flavour, texture and overall impression). The acceptance was driven positively by sweet taste, sweet aroma and bitter aftertaste, and negatively by apple flavour, apple aroma, darker colour and sour taste, verified by PLS. It was possible to develop a synbiotic apple juice that showed a similar sensory profile (except presence of particles and turbidity) and acceptance to that of the sucrose-added juice by adding *L. paracasei* as a probiotic culture and oligofructose as a sugar substitute and prebiotic.



## 1. Introduction

Live microorganisms that confer beneficial effects on the host when administered in adequate amounts are named probiotics (FAO/WHO, 2002). Probiotic cultures are generally added to fermented milks and yogurts. However, with the increasing number of vegetarian consumers, the demand for non-dairy probiotic products has increased (Vasudha & Mishra, 2013). Furthermore, fermented milk products are inadequate for the growing number of people with lactose intolerance, milk protein allergies, and high cholesterol (Granato, Branco, Nazzaro, Cruz, & Faria, 2010; Martins et al., 2013).

Fruit juices have been considered suitable for the addition of probiotic cultures because they already contain beneficial nutrients; have flavour profiles considered enjoyable by people of all ages, are considered healthy and refreshing drinks, and are consumed regularly, quality that is essential to obtaining the benefits attributed to probiotics (Awaisheh, 2012; Pimentel, Prudencio, & Rodrigues, 2011). In addition, they contain no starter cultures to compete with the probiotic cultures for substrates; they can be supplemented with ingredients that promote anaerobic conditions, such as ascorbic acid; and they contain sugars that can be metabolised by probiotic cultures (Ding & Shah, 2008).

However, the addition of probiotic cultures to fruit juices presents numerous technological challenges, due to their acidity, the presence of oxygen, and inherent differences among fruits (Saeed, Zahid, & Sattar, 2013; Vasudha & Mishra, 2013). Furthermore, they have shown sensory barriers, resulting in fruit juices with flavours and aromas described as “dairy”, “medicinal”, “acidic”, “salty”, “bitter”, “astringent”, “artificial” or “earthy” (Granato et al., 2010; Luckow & Delahunty, 2004a, 2004b; Saeed et al., 2013). However, it is unclear whether all probiotic cultures give the product the same flavour at the same levels of intensity (Luckow, Sheehan, Dleahunty, & Fitzgerald, 2005).

Sucrose, derived from sugar cane or beet, has been part of the human diet for centuries, and the sweet taste that it provides to food products is naturally preferred by consumers (Al-Dabbas & Al-Qudsi 2010). Due to growing health concerns and nutritional recommendations to decrease sugar intake, many food companies are interested in reducing the sucrose contents of their products, including fruit juices and nectars (Rodbotten et al., 2009). A number of sugar substitutes have been used and can provide different characteristics to products, including a sweet taste, an oral tactile

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http://dx.doi.org/10.1016/j.lwt.2014.08.001
0023-6438/

sensation, stability and, in some cases, improved colour (O'Donnel, 2007).

Sucralose is a non-nutritive sweetener that is produced by replacing three hydroxyl groups with three chlorine atoms in the sucrose molecule. Sucralose is safe for use, has high sweetening power (600 times higher that of sucrose), a mild bitter aftertaste, and is stable at high temperatures and acidities (Al-Dabbas & Al-Qudsi 2010). Sucralose has been considered the best sweetener to replace sugar because it causes fewer sensory changes in the products compared with other sweeteners (Cadena et al., 2013).

Oligofructose has attracted special attention from researchers and industries due to its prebiotic, health, and technological properties (Renuka, Kulkarni, Vijayanand, & Prapulla, 2009). The consumption of oligofructose modulates the intestinal microbiota (prebiotic effect), inhibits the growth of pathogens in the intestine, increases calcium absorption from the diet, and relieves constipation (Saad, Delattre, Uudaci, Schmitter, & Bressolier, 2013). Technologically, oligofructose has properties comparable to those of sucrose and glucose syrups because it has free sugars. However, its sweetness in pure form is 30–60% of that of sucrose (Apolinário et al., 2014; Franck, 2002; Mussato & Mancilha, 2007).

In the development of functional products it is necessary to understand the sensory impacts of these components and to determine how their addition to products influences the acceptability and consumer preference in terms of appearance, aroma, flavour and texture in order to direct product development and formulation (Granato et al., 2010). No studies have evaluated the effect of the concomitant addition of probiotic cultures and sugar substitutes on the sensory properties of apple juices. Therefore, the objectives of this study were to assess the effect of the addition of oligofructose or sucralose as sugar substitutes and of *Lactobacillus paracasei* ssp. *paracasei* as a probiotic culture on the sensory profile and acceptance of clarified apple juice and to determine the influence of the sensory attributes on product acceptance.

## 2. Material and methods

### 2.1. Material

Royal Gala apples (*Malus domestica Borkh)* (Castel Frutas®), pectinolytic enzyme (Pectinex Ultra Clear, Novozymes®), natural sodium bentonite (Na-35; Schumacher®), colourless gelatin (Dr. Oetker®), sucrose (União®), oligofructose (P95, Orafti®), sucralose (Splenda®) and the *L. paracasei* ssp. *paracasei* (LC-01, Christian Hansen®) probiotic culture were used in the present study.

### 2.2. Methods

#### 2.2.1. Probiotic activated probiotic culture preparation

The method described by Ding and Shah (2008), with the modifications proposed by Pimentel et al. (2011) was used to activate the lyophilised probiotic cultures. A culture aliquot was inoculated into 5 mL of Man, Rogosa and Sharpe (MRS) media (Himedia®) and incubated at 37 °C for 15 h. After this time, 0.05 mL of the culture was again inoculated into 10 mL of MRS media and incubated at 37 °C for 15 h (pre-inoculum). To obtain the biomass, 0.1 mL of the pre-inoculum was transferred into 300 mL of MRS media and re-incubated under the same conditions. The biomass was separated by centrifugation in a refrigerated centrifuge (Eppendorf®, model 5804R) at 14,000 × *g* for 10 min at 4 °C and washed three times in 0.85 g/100 mL sterile saline solution (NaCl Dinamica®) to remove the residual MRS media. The biomass was then resuspended in 50 mL of 0.85 g/100 mL sterile saline solution to obtain the activated probiotic culture.

#### 2.2.2. Formulations

Seven formulations of clarified apple juice were prepared: PUR, SAC, PRE, PRO, SYNB, SUC and SUC-P, as shown in Table 1. These formulations were selected aiming to study the influence of the additions of sweeteners (oligofructose [also a prebiotic] and sucralose) and probiotic to clarified apple juices, as well as, a combination of prebiotic and probiotic (synbiotic). Pure and sucrose added juices were used as controls.

#### 2.2.3. Preparation of apple juices

Gala apples were washed in running water, sanitised (6 mL/L Pury Vitta® fruit and vegetable disinfectant with 0.96 g/100 g active chlorine) and crushed using a fruit processor (Walita®). Raw juices were subjected to enzymatic treatment (0.03 mL/L Pectinex Ultra Clear) in a water bath for 1 h at 50 °C and subsequently vacuum filtered through a Buchner funnel (Qualy® filter paper, 80 g/m$^2$; 205 μm; 25 cm diameter). Juice clarification was completed through treatment with 24 h advance activated bentonite (0.45 g/L) and gelatin (0.05 g/L) at 50 °C for 1 h followed by vacuum filtration through a Buchner funnel (Burdurlu & Karadeniz, 2003).

The clarified juices were then supplemented with sucrose (20 g/L; SAC), oligofructose (20 g/L; PRE and SYNB) or sucralose (0.03 g/L; SUC and SUC-P). The concentration of oligofructose added was based on Brazilian legislation defining the functional properties of oligofructose (prebiotic) (Anvisa, 2008a) and on the minimum daily intake suggested for fructans in order to obtain beneficial health effects (Keenan, Brunton, Butler, Wouters, & Gormley, 2011; Röβle, Brunton, Gormley, Ross, & Butler, 2010). Sucrose was added at the suggested levels for apple juice (Bleibaum et al., 2002; Ellendersen, Granato, Gguergoletto, & Wosiacki, 2012). The amount of sucralose was determined using the manufacturer's recommendation for the product (sweetness: 600 times that of sucrose), available studies (Cadena & Bolini, 2012; Marcellini, Chainho, & Bolini, 2005; Marchi, Montes-Villanueva, McDaniel, & Bolini, 2012, pp. 1–34) and preliminary sensory tests (Triangular Test) (results not shown), as well as in accordance with the maximum limits established by legislation (Anvisa, 2008b) and to establish a similar sweetness to that of the product sweetened with sucrose (SAC).

All formulations were placed in glass (Farma®) packages and pasteurized at 80 °C for 20 min in a water bath and cooled in an ice bath until reaching 37 °C. Formulations with probiotic cultures (PRO, SYNB and SUC-P) were supplemented with 20 mL/L activated probiotic culture, corresponding to $10^{11}$ viable cells per litre of juice. The apple juices were then stored at 4 °C for 2 days.

#### 2.2.4. Sensory profile

The sensory profile of clarified apple juice was established using Quantitative Descriptive Analysis (QDA). Twenty-six individuals were selected among students and staff members of the Federal Institute of Paraná - Campus Ivaiporã (Ivaiporã, Paraná, Brazil).

Initially, the consumers were subjected to tests to recognise basic odours and tastes. Individuals who failed to identify at least one solution for each basic taste or did not achieve the minimum of

**Table 1**
Experimental design of apple juice formulations.

| Formulations | Sucrose | Oligofructose | Sucralose | Probiotic[a] |
|---|---|---|---|---|
| PUR | – | – | – | – |
| SAC | 20 g/L | – | – | – |
| PRE | – | 20 g/L | – | – |
| PRO | – | – | – | 20 mL/L |
| SYNB | – | 20 g/L | – | 20 mL/L |
| SUC | – | – | 0.03 g/L | – |
| SUC-P | – | – | 0.03 g/L | 20 mL/L |

[a] Quantity of the activated probiotic culture.