DEVELOPMENT OF PROBIOTIC GRAPE JUICE AND LACTOBACILLUS PARACASEI VIABILITY UNDER COLD STORAGE

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ABSTRACT – The aim of this study is to develop a probiotic grape juice with Lactobacillus paracasei and to evaluate microorganism viability and the effect of adding inulin to the formulation. Two formulations were developed, being one with 10 % (w/v) inulin added. The beverage was kept at 4°C for 28 days and cell viability, pH and Brix were monitored. In this period, the pH remained around 3.3 not statistically differing from the initial product. The soluble solids did not change with the juice containing inulin remaining at a higher level. Cell viability reduced about 3 logarithmic cycles in 14 days and remained about 6 log CFU/mL until the end of storage. It is concluded that grape juice is a promising matrix for the production of a beverage with probiotic L. paracasei but inulin did not improve cell viability in used concentrations.

KEYWORDS: probiotic, grape juice, Lactobacillus paracasei, inulin, cell viability.

1. INTRODUCTION

The demand for healthy foods have been one of the most important trends of food consumption in recent years (Bigliardi & Galati, 2013). People are increasingly aware on their own health, as well as the social and environmental impacts of their food consumption (Falguera, Aliguer, & Falguera, 2012). Functional products with addition of probiotics and prebiotics substances are in the center of this health style trend (Granato et al., 2010; Martins et al., 2013). Previously, probiotic cultures were employed only in dairy products but today, research is carried out for introduction in vegetable foods (Kandylis et al., 2016) especially beverages, which facilitate its consumption by lactose intolerant people and vegans.
Except for the low pH, fruit juices are adequate vehicles for the incorporation of probiotics since they are rich in sugars, antioxidants and vitamins (Granato et al., 2010; N. P. Shah et al., 2010). Agte et al. (2010) also demonstrated prebiotic activity in grape varieties and hybrids that showed from 21.2 to 72.5% of the activity of fructooligosaccharide (FOS) used as a standard prebiotic. In this sense, grape juice could be a potential matrix for a synbiotic beverage. The term synbiotic is used when a product contains both probiotics and prebiotics (Tripathi & Giri, 2014).

Grape juice is widely produced in the Americas and Europe, being United States, Spain, Argentina and Brazil the world’s top producers (FAOSTAT, 2013). *Vitis vinifera* L. grape varieties such as Merlot and Cabernet Sauvignon are typically used to fine wine production in the world and they are also the varieties used to juice production in Europe (Granato et al., 2016). Otherwise, American grapes (*Vitis labrusca* L.) such as Concord, Niagara and Bordo, and their hybrids with *Vitis vinifera* are the main cultivars for grape juice processing in American countries, including United States and Brazil (Granato et al., 2016; Yamamoto et al., 2015). Most grapes juice production in Brazil is located in Rio Grande do Sul State, with greater emphasis in the Vale dos Vinhedos region which is the first region of the country to be officially recognized as a geographical indication (Almeida et al., 2015).

Studies of food and beverages with probiotics are found widely in literature (Kandylis et al., 2016; Tripathi & Giri, 2014). Probiotics are “live microorganisms which when administered in adequate amounts confer a health benefit on the host” (FAO/WHO, 2001; Hill et al., 2014; Reid, 2016). The most common probiotics microorganisms used commercially in food are bacteria from the genera *Lactobacillus* and *Bifidobacterium* (Tripathi & Giri, 2014). Probiotics are widely used in commercial functional products of animal origin, mainly fermented milk, such as yogurt, and cheeses. Nevertheless, non-dairy probiotic products are found to a lesser extent in market, and are usually restricted to traditional products based on cereals or soy (Kandylis et al., 2016). Fruit and vegetable juices can be considered as a new category of food matrices for probiotic bacteria (Prado et al., 2008) with some developments reported in literature (Nagpal, Kumar, & Kumar, 2012; Nematollahi et al., 2016) (Nualkaekul, Salmeron, & Charalampopoulos, 2011; R. H. Shah, 2016). Nevertheless, to the best of our knowledge, previous works of grape juice as a matrix to *Lactobacillus paracasei* was not reported.

The aim of this study is to develop a probiotic grape juice by adding *Lactobacillus paracasei* and to evaluate the microorganism viability in this matrix under cold storage. Furthermore, we intend to evaluate the effect of inulin as a prebiotic ingredient.

### 2. MATERIAL E MÉTODOS

#### 2.1 Microorganism

This study used microorganism *Lactobacillus paracasei* (FD-DVS nu-trish® L. casei-01, Chr. Hansen, Denmark) freeze-dried and stored at -18 °C.

#### 2.2 Raw Material and Ingredients

Grapes (*Vitis labrusca* L. cv Bordo) was harvested at the optimum point of ripeness. They were grown in the Vale dos Vinhedos region, Bento Gonçalves (Brazil), in the following coordinates -29° 10' 18.88" . -51° 32' 34.68.

Pectolytic enzyme (Rohapect 10L, AB Enzymes, Amazon Group, Brazil) was added during juice processing to lower pectin content and for extraction of pigments and flavor compounds. Inulin (Orafti Beneo® GR, Clariant, Chile) was used as a standard prebiotic. Preliminary tests were performed by adding 5, 10, 15, 20 and 25% (m/v) inulin to the juice in order to set the best concentration.
2.3 Grape Juice Production

The processing of the grape juice was held on the same place where they were grown. 60 kg of grapes were processed and the clusters were selected so that there were no rotten berries or contaminants. The grains were extracted in crushe and subsequently pressed to obtain the wort. This wort was subjected to heating at 75 °C and pectolytic enzyme was added. Then, filtration was carried out by separating the skin grape from the liquid (juice). The juice was subjected to pasteurization at 90 °C and filled into 500 mL glass bottles previously sterilized and cooled.

2.4 Inoculum Preparation

The freeze-dried *L. paracasei* strain was inoculated in MRS broth and incubated for 18 h at 30 °C in shaker (Tecnal, Brazil). The culture broth was centrifuged at 4 °C and 5000xg for 15 min. (Hanil Science Industrial Co., Ltd., Korea). The pellet was suspended in sterile saline to wash the cells and centrifuged again. After centrifugation the cells were suspended in pasteurized grape juice for subsequent homogenization and addition to the beverage.

2.5 Formulation of Probiotic Juices

Two formulations from pasteurized grape juice were developed for beverage production: (1) *L. paracasei* inoculum 7.5% (v/v); and (2) *L. paracasei* inoculum 7.5% (v/v) and inulin 10% (w/v).

2.6 Survival Assays

After formulation, glass bottles of juice were stored at 4 °C. Every week, for a period of 28 days, the viability of the *L. paracasei* in grape juice was evaluated by lactic acid bacteria counts in agar MRS (Dowens & Ito, 2001). At the same time, pH and soluble solids of juice were analyzed.

2.7 Chemical and Microbiological Characterization

pH, soluble solids (°Brix), total solids content, total acidity, and water activity were measured in grape juice with and without probiotic microorganism and inulin. Moreover, lactic acid bacteria, yeast and molds counts, coliforms NMP and *Salmonella* sp. were also determined (Dowens & Ito, 2001). Analyzes were performed considering two replicates.

2.8 Statistical analysis

Results were subjected to ANOVA, and the difference between the means was assessed by the Tukey test (p ≤ 0.05), using the Assistat 7.7. software (Brazil).

3. RESULTADOS E DISCUSSÃO

To set the amount of inulin, which was sensorially suitable for producing the beverage, preliminary tests were performed by adding different amounts of inulin in grape juice. 10% inulin was the maximum concentration to be added without damaging the sensorial characteristics of product. With this content, the juice remained liquid, limpid and homogeneous, with typical odor and color, but with a small amount of precipitated material after a few days of storage.

Table 1 presents the physicochemical and microbiological characteristics of the pasteurized grape juice with and without *L. paracasei* and inulin. The thermotolerant coliform, acid lactic bacteria, and yeast and molds counts demonstrate the microbiological quality of raw materials and the efficiency of heat treatment applied to grape juice. The number of lactic acid bacteria in juices added of *L. paracasei* was obviously higher, being in the range of 9 log CFU/mL. The addition of probiotic...
microorganism did not significantly change the physicochemical parameters analyzed in juices with and without *L. paracasei*, however the addition of inulin caused a significant increase in total and soluble solids of the juices added by this prebiotic ingredient.

Table 1 - Physicochemical and microbiological characteristics of the pasteurized grape juice with and without *L. paracasei* and inulin.

<table>
<thead>
<tr>
<th>Physicochemical and microbiological analysis</th>
<th>grape juice</th>
<th>grape juice with <em>L. paracasei</em></th>
<th>grape juice with <em>L. paracasei</em> and inulin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total acidity (tartaric acid g/mL)</td>
<td>0.193&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.194&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.209&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Soluble solids (ºBrix)</td>
<td>13.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>13.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>18.2&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>pH</td>
<td>3.31&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.30&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.28&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>Water activity</td>
<td>0.998&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.980&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.985&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Total solids (g/100 mL)</td>
<td>11.24&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.85&lt;sup&gt;a&lt;/sup&gt;</td>
<td>17.69&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Lactic acid bacteria (log CFU/mL)</td>
<td>&lt; 1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.98&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9.01&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Thermotolerant coliform (MNP/mL)</td>
<td>&lt; 0.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>&lt; 0.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>&lt; 0.3&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Yeast and molds (log CFU/mL)</td>
<td>&lt; 1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>&lt; 1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>&lt; 1&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>Salmonella</em> sp./25 mL</td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
</tr>
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</table>

Means followed by identical letters in same row are not significantly different (p ≤ 0.05) based on Tukey test. N.D., not detected.

During the cold storage, pH of both juices (with and without inulin) ranges between 3.33 and 3.22, and did not differ from grape juice freshly produced (p ≤ 0.05). Brix also remained constant over the 28 days for each juice, but grape juice containing inulin remained at statistically higher value, with 18.3 ºBrix in 28 days. During this period, the viability of *L. paracasei* was also evaluated. The results are shown in Figure 1.

A statistically significant decline in cells viability was observed for both juices from the inoculation to the first 14 days. There was a decreased from 9.0 log CFU/mL to 5.9 log CFU/mL and 6.1 log CFU/mL respectively for juices with and without inulin. This reduction in viability may be associated with typical low pH of the grape juice. Similar behavior was reported by Nematollahi et al. (2016) to the viability of *Lactobacillus casei* in cornelian cherry juice with pH adjusted at 3.5, during cold storage. According to Tripathi and Giri (2014), the concentration of undissociated organic acids increases at low pH, enhancing the bactericidal effect on probiotic microorganism.

![Figure 1: Viability of *L. paracasei* in grape juice with and without inulin during cold storage (4 °C)](image)
The supplementation of prebiotic ingredients as inulin could protect probiotic microorganisms from the acidic environment and enhance the viability of probiotic microorganisms (Tripathi & Giri, 2014), but it was not observed in this study. Viability of L. paracasei was not different between juices (p ≤ 0.05). On the other hand, grape juice has proved to be a promising plant-based matrix to production of a probiotic beverage since it was able to ensure the viability of L. paracasei above 6 log CFU/mL until the end of storage. Prebiotic potential of grape juice for other Lactobacillus and Bifidobacterium strains was also demonstrated by Agte et al. (2010).

According to Martins et al. (2013), there is no consensus about the minimum of probiotic microorganisms to be ingested in order to ensure their functionality, but values ranges from 10⁶ to 10⁹/g. Considering the inferior limit, beverage developed in this work has potential probiotic effect.

4. CONCLUSION

The results presented in this work demonstrated that Lactobacillus paracasei was able to survive in grape juice, even without addition of a prebiotic ingredient as inulin. Therefore, it could be concluding that grape juice could be employed as a matrix to delivery this probiotic microorganism. Additionally, inulin did not improve cell viability in tested concentrations.

5. REFERENCES


