Minimally processed fruit salad enriched with *Lactobacillus acidophilus*: Viability of anti-browning compounds in the preservation of color

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Minimal processing promotes browning of some vegetal tissues due to cell membrane disruption, which results in the release of oxidative enzymes. This study evaluated the efficiency of citric acid, ascorbic acid, sodium metabisulfite and L-cysteine hydrochloride to retard enzymatic browning of minimally processed fruit salad and enriched this product with *Lactobacillus acidophilus* LA-5. Control treatment was fruit salad immersed in water. Polyphenol oxidase (PPO) and color (L*, a*, b*, index color - CI, browning index - BI, c*, and h°) were analyzed. The viability of *L. acidophilus* was also evaluated using Rogosa agar in fruit salads containing anti-browning compounds in higher concentrations. PPO presented a significant difference among control and fruit salad treated with ascorbic acid and L-cysteine hydrochloride, indicating the highest anti-browning activity of these compounds. The fruit color was affected by processing and storage time, with a reduction in the values of L* over time. Values of a*, c*, h° angle and CI indicated a predominance of red color in the fruit salad. Salads containing anti-browning compounds in higher concentrations presented viability of *L. acidophilus* above 7.43 log CFU/g up to the fifth day of storage, indicating that the product can be promised as probiotic. Thus, the fruit salad treated with anti-browning compounds has potential use as a probiotic carrier.

**Key words:** Fresh-cut fruits, color, ascorbic acid, vegetable matrix, probiotic culture.

**INTRODUCTION**

In recent years, concerns about health and wellness have lead consumers to seek a healthy diet by eating more fruits. Then, a rapid market growth for fresh cut fruits and vegetables has been observed once consumers increased...
demand for high nutritive value, convenience and fresh-like quality (Rico et al., 2007; James and Ngarmasak, 2010). According to De Ancos et al. (2011), minimally processed fruits and vegetables are plant products that have been subjected to physical changes to maintain their freshness. Fruit mixes, in which two or more fruits are combined to obtain a product with special characteristics, are often used to meet consumer needs regarding flavor, texture and the nutritional value of fruit (Rojas-Grãu et al., 2011). Some studies focus on the use of probiotic microorganisms in minimally processed fruit (Rôblie et al., 2010a, b; Alegre et al., 2011) and in juices from fruits and vegetables (Yoon et al., 2004; Yoon et al., 2005; Yoon et al., 2006; Sheehan et al., 2007). These products have presented promising results and as such are considered promoters of health and wellness. Probiotic microorganisms benefit the host organism by balancing intestinal microbiota. Microorganisms of the genus Lactobacillus are among the most commonly used as probiotics in food applications (Soccol et al., 2010). Fermented milk products are generally good carrier matrixes for these microorganisms. However, other food matrixes have been studied as potential carriers for probiotic microorganisms. According to Soccol et al. (2010) and Vitali et al. (2012), raw and processed fruits may provide an ideal substrate for probiotic cultures since they contain essential nutrients for microorganism multiplication, such as minerals, vitamins, antioxidants and fibers. Furthermore, the use of fruit matrixes as probiotic carriers allows the consumer to choose probiotic foods free of cholesterol and lactose constituents present in dairy products.

However, a major problem with minimally processed fruits is their short shelf life, caused by enzymatic or microbial deterioration that causes undesirable visual effects on food (Rôblie et al., 2009; Rojas-Grãu et al., 2009). The process of peeling and cutting promotes the release of enzymes, causing the fruit surface to brown and negatively affecting product appearance (Ragaert et al., 2011). Thus, researchers are constantly looking for new ways to extend the shelf life of minimally processed fruits. Among these technologies, the use of anti-browning compounds, such as cysteine, ascorbic acid, citric acid and sulfite, which act to inhibit enzymatic browning, have emerged as potential alternatives (Baldwin and Bai, 2011).

In this context, some of the anti-browning agent, such as ascorbic acid acts as an oxygen scavenger and it is benefic to the probiotic food. Oxygen content and redox potential have been show to be important factors that contribute for the viability of probiotic bacteria during the shelf life of the food. According to Barbagallo et al. (2012), ascorbic acid acts as scavenger by removing the molecular oxygen from the reactions catalyzed by PPO and moreover it has a chelating effect on Cu present on prosthetic group of the polyhenol oxidase enzyme. So its action anti-browning promotes a favorable environment to the probiotic bacteria in fruits. Dave and Shah (1997) evaluated the viability of probiotic bacteria in yogurt supplemented with four levels of ascorbic acid and they verified that the addition of ascorbic acid favored the viability of Lactobacillus delbrueckii ssp. bulgaricus.

Therefore, considering the sensitivity of fruits to enzymatic browning and the positive effect that the anti-browning agents could promote on the viability of probiotic culture, this study aimed to evaluate the efficiency of citric acid, L-cysteine hydrochloride, ascorbic acid and sodium metabisulfitel in minimally processed fruit salad enriched with Lactobacillus acidophilus, as well as to determine the viability of L. acidophilus in this minimally processed food product.

**MATERIALS AND METHODS**

**Minimal processing of fruits, fruit salad preparation and inoculation with L. acidophilus**

Pineapple, banana, guava, apple, papaya and mango were selected in the Rio Pomba, Minas Gerais, Brazil, market for fruit salad preparation. These fruits were obtained at ripening stage and sent to the Fruit and Vegetable Processing Unit at the Food Science and Technology Department at the Federal Institute of Education, Science and Technology of Southeast of Minas Gerais. Fruits were washed in tap water to eliminate dirt and impurities. Following this, fruits were immersed in water at 5°C with sodium dichloroisocyanurate (Sumaq®, Diversey Lever) with an active residual chlorine concentration of 200 mg/L for 20 min. Afterward, fruits were peeled and manually cut using stainless steel knives. After cutting, fruit salads were prepared using the same proportion of fruits in each salad. Prepared fruit salads were immersed in a citric acid and sodium citrate buffer solution containing approximately $10^{10}$ CFU/mL of L. acidophilus LA-5 (Christian Hansen®). This strain is recognized as probiotic being widely used in the food industry. The probiotic culture was prepared and added to fruit salads according to Rôblie et al. (2010a). Initially, the probiotic culture was activated twice in Man Rogosa Sharpe broth (MRS) and kept at 37°C for 18 h. The probiotic culture was then activated in MRS broth for 16 h, and centrifuged at 5°C for 15 min at 7000 g in a centrifuge model Sorvall Biofuge Stratos (Thermo Scientific). The broth supernatant was discarded and the obtained probiotic cell pellet was aseptically resuspended in a buffer consisting of a 1:1 ratio of citric acid and sodium citrate, pH 3.8 in order to wash cells. Then, it was centrifuged again in the same conditions. Afterwards, for each gram of probiotic cells it was added 10 mL of buffer solution of citric acid: sodium citrate (1:1, pH 3.8) to obtain at least $10^{10}$ cells/mL.

Finally, 1 mL of probiotic cell suspension was added to each gram of fruit salad to obtain this food containing L. acidophilus. The salads were in contact with the probiotic cell suspension for 15 min at 5°C. The control treatment was a minimally processed fruit salad without the addition of L. acidophilus.

**Treatment of fruit salad with anti-browning compounds**

To evaluate the effectiveness of the anti-browning compounds, salads containing L. acidophilus were immersed in 1 and 2% citric acid, 1 and 2% ascorbic acid, 0.01 and 0.03% sodium metabisulphite and 0.5 and 1% L-cysteine hydrochloride. Fruit salads were immersed for 3 min at 5°C. The control treatment was a fruit salad immersed in water. Fruit salads (1,000 g) were drained to remove excess solution, packed in polypropylene containers of 0 g and 2
stored at 8°C for further analysis at 0 hour and after 24, 72 and 120 h of processing. All experiments were done in three replicates.

**Determination of polyphenol oxidase (PPO) activity**

The PPO activity of the control (without probiotic and anti-browning compounds) and the fruit salad containing *L. acidophilus* that was previously immersed in the anti-browning compounds was determined at 0 h and after 24, 72 and 120 h of processing, according to Teisson (1979). The extract was obtained by macerating 10.02 g of fruits (1.67 g of each one) with 0.5 g of polyvinylpolypyrrolidone (PVPP), three drops of Triton X-100 and 20 mL of 0.05 M phosphate buffer (pH 7), kept refrigerated. The homogenate was immediately filtered through a cheese cloth and centrifuged for 10 min at 5000 g at 0°C in a centrifuge model Sorvall Biofuge Stratos (Thermo Scientific). The resulting supernatant was used to determine enzymatic activity. An aliquot of 1 mL of the obtained supernatant was added to 3.6 mL of 0.1 M phosphate buffer (pH 7) and 0.1 mL of 10 mM catechol that was left for 30 min in a water bath at 30°C. Afterward, the reaction was interrupted by adding 1.6 mL of 2 N perchloric acid. The enzymatic activity of polyphenol oxidase was expressed as units per gram of fresh weight per minute (U/g/min). One unit corresponded to the enzymatic activity capable of altering 0.001 absorbance at 395 nm.

**Color determination**

The surface color of the fruit salads was evaluated after inoculation with *L. acidophilus* and treatment with anti-browning compounds. The fruit surface color was measured using the colorimeter MiniScan EZ, HunterLab (Reston, Va., USA) at 0 h and after 24, 72 and 120 h of processing. Color determination was performed in reflectance mode by direct reading of the coordinates L*, a* and b*, using the CIELAB L* scale, where L* measures lightness and varies from 0 (black) to 100 (white), a* measures the tonality, from +a (red) to -a (green), and b* measures the saturation, from +b (yellow) to -b (blue). The color index (CI), which measures the color of the product, was determined according to Mazzuz (1996): CI = 1000 x a* / L* x b*. The browning index (BI) was determined according to Palou et al. (1999): BI = [100 (X-0.31)]/0.172, where X = (a* + 1.75L*) / (5.645L* + a* - 3.02b*). The color intensity or chroma (C*) and hue angle (h°) were calculated according to McGuire (1992): c* = [(a*)² + (b*)²]¹/² and h° = arctan (b*/a*). To measure color, control and treated fruit salads were crushed and placed on a glass plate, and values of L*, a* and b* for each sample were read directly from the product. It was used at the same quantity of each fruit to measure the color.

**Viability of L. acidophilus in minimally processed fruit salads**

Samples of fruit salads (25 g) were homogenized in 225 mL of peptone saline solution (0.85% NaCl and 0.1% peptone). Serial dilutions were performed and cultured by the pour-plate method, in which 1 mL of the respective dilutions was added to a small amount of Rogosa SL agar (Himedia) in Petri dishes. Following this, the Petri dishes were kept in anaerobic jars at 37°C for 72 h. The viability of *L. acidophilus* was determined in the control treatment as well as in the fruit salads treated with anti-browning compounds at the highest concentrations (2% citric acid, 2% ascorbic acid, 0.03% sodium metabisulphite and 1% L-cysteine hydrochloride) at 0 h and after 120 h of fruit salad preparation in order to demonstrate that they do not inhibit the growth of probiotic bacteria. All fruit salads were stored at 8°C.

**Statistical analyses**

For the anti-browning compound experiments, we used a completely randomized statistical design using subdivided plots. This consisted of eight treatments and one control in the plots, with 3 replicates. Subplots were differentiated by assessments over storage time (0, 24, 72 and 120 h). The viability of *L. acidophilus* was evaluated using a completely randomized design with three replicates and a 4x2 factorial design. In this factorial design, four anti-browning compounds (1% L-cysteine hydrochloride, 2% ascorbic acid, 2% citric acid, 0.03% sodium metabisulphite) and two storage times (0 and 120 h) were evaluated. Analysis of variance (ANOVA) and the Dunnett’s test for multiple comparisons among means were used to analyze the results, considering a 5% level of significance, using the R software (R Core Team, 2012).

**RESULTS AND DISCUSSION**

The color of the fruits present in the salad was affected by processing (Table 1) and storage time. The polyphenol oxidase activity presented significant differences (*p<0.05*) among the control treatment and fruit salads treated with 1 and 2% ascorbic acid and those treated with 0.5 and 1% L-cysteine hydrochloride. These results indicate that ascorbic acid and L-cysteine hydrochloride were the best browning inhibitors, since these treatments presented lower enzyme activity than the fruit salads treated with 0.01 and 0.03% sodium metabisulphite and those treated with 1 and 2% citric acid. Sodium metabisulphite and citric acid did not differ significantly from control treatment, presenting an average PPO activity value of 1.67 U/g/min (Table 1). However, we verified with the naked eye that apple and banana present in fruit salads treated with 0.5% L-cysteine hydrochloride showed undesirable pinkish surface coloration over storage time. Melo and Vilas Boas (2006) found that the lower the concentration of cysteine, the higher the pinkish intensity in minimally processed 'apple' banana. According to Richard-Forget et al. (1992), this is probably due to the regeneration of phenols when the applied amount of cysteine is low, which probably explains the pink coloration in apple and banana treated with only 0.5% of this anti-browning compound. The browning of vegetal tissues is due to disruption of the cell membrane during the processing, which promotes the release of enzymes that come in contact with the phenolic substrates, thus having an uncontrolled oxidation, using molecular oxygen (De Ancos et al., 2011), as we observed in this work. The lightness (L*) of fruit salads was not significantly different among treatments (*p>0.05*). However, storage time did have a significant effect (*p<0.05*) on this colorimetric parameter, as a result of the fruits salad browning over time (Figure 1A). Browning is an undesirable characteristic of minimally processed fruit. González-Aguilar et al. (2008) observed less browning in fresh cut mango treated with ascorbic acid, as we verified. Colorimetric parameters a* and b* did not present a significant difference (*p>0.05*) among treatments (Table 1).

However, storage time had a significant effect on these parameters (Figures 1B and 1C), occurring a increasing
Table 1. Mean values of polyphenol oxidase (PPO) activity (U/g/min) and color parameters 
(L*, a*, b*, CI, BI, c* and h°) of fruit salads treated with anti-browning compounds.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>PPO</th>
<th>L*</th>
<th>a*</th>
<th>b*</th>
<th>Cl</th>
<th>BI</th>
<th>c*</th>
<th>h°</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA1</td>
<td>1.26b</td>
<td>57.60a</td>
<td>23.89a</td>
<td>33.51a</td>
<td>12.56a</td>
<td>112.66a</td>
<td>41.28a</td>
<td>1.26a</td>
</tr>
<tr>
<td>AA2</td>
<td>1.23b</td>
<td>57.46a</td>
<td>23.70a</td>
<td>32.33a</td>
<td>12.85a</td>
<td>108.57a</td>
<td>40.13a</td>
<td>1.24a</td>
</tr>
<tr>
<td>CC05</td>
<td>1.30b</td>
<td>57.18a</td>
<td>23.99a</td>
<td>32.45a</td>
<td>12.98a</td>
<td>110.05a</td>
<td>40.30a</td>
<td>1.23a</td>
</tr>
<tr>
<td>CC1</td>
<td>1.18b</td>
<td>59.04a</td>
<td>22.57a</td>
<td>33.43a</td>
<td>11.57a</td>
<td>107.12a</td>
<td>40.39a</td>
<td>1.34a</td>
</tr>
<tr>
<td>MS001</td>
<td>1.67a</td>
<td>57.64a</td>
<td>22.40a</td>
<td>35.87a</td>
<td>11.11a</td>
<td>119.41a</td>
<td>42.40a</td>
<td>1.44a</td>
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<tr>
<td>MS003</td>
<td>1.53a</td>
<td>58.65a</td>
<td>21.74a</td>
<td>34.66a</td>
<td>10.81a</td>
<td>111.27a</td>
<td>40.96a</td>
<td>1.44a</td>
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<tr>
<td>AC1</td>
<td>1.65a</td>
<td>58.89a</td>
<td>21.83a</td>
<td>36.85a</td>
<td>10.09a</td>
<td>119.13a</td>
<td>42.86a</td>
<td>1.50a</td>
</tr>
<tr>
<td>AC2</td>
<td>1.44a</td>
<td>58.78a</td>
<td>22.75a</td>
<td>37.35a</td>
<td>10.48a</td>
<td>122.10a</td>
<td>43.80a</td>
<td>1.46a</td>
</tr>
<tr>
<td>Cont.</td>
<td>1.67a</td>
<td>56.69a</td>
<td>22.92a</td>
<td>34.12a</td>
<td>12.07a</td>
<td>116.74a</td>
<td>41.18a</td>
<td>1.34a</td>
</tr>
<tr>
<td>DMS-Dunnett</td>
<td>0.26</td>
<td>2.57</td>
<td>2.49</td>
<td>5.36</td>
<td>1.99</td>
<td>20.72</td>
<td>4.96</td>
<td>0.20</td>
</tr>
</tbody>
</table>

Cl, color index; BI, browning index; AA1, 1% ascorbic acid; AA2, 2% ascorbic acid; CC05, 0.5% L-cysteine hydrochloride; CC1, 1% L-cysteine hydrochloride; MS001, 0.01% sodium metabisulphite; MS003, 0.03% sodium metabisulphite; AC1, 1% citric acid; AC2, 2% citric acid; Cont, Control. Mean values followed by the same letter in lowercase in the column have no significant difference at 5% of probability by Dunnett’s test.

Figure 1. Regression equation and colorimetric coordinate determination coefficients L* 
(A), a* (B), and b* (C) of minimally processed fruit salads treated with anti-browning compounds for 3 min at 5°C and stored for 120 h.

Similar to our results, Pizato et al. (2013) observed increasing values of a* in minimally processed apple over
storage time for the treated samples. The color index (CI) ranges from -20 to +20, in which negative values indicate a greener fruit color and positive values indicate intense red coloration (Mazzuz, 1996). In the present study, fruit salads presented an increase in CI over storage time (p<0.05) (Figure 2). However, it was not observed significant differences (p>0.05) among treatments for this colorimetric parameter. The browning index (BI), which measures the purity of the brown color, increased over storage time in fruit salads at 8 °C (p<0.10). These results are in agreement with those of Javdani et al. (2013), who worked with minimally processed apple and observed increasing values of BI as a function of time. However, a significant difference among treatments was not observed (p>0.05) in this work (Table 1). Another colorimetric parameter assessed was the Chroma index (c*), which denotes the saturation or color intensity. Smaller values of c* correspond to weaker color patterns (matting color of an object), while higher values indicated stronger or brighter colors, with the latter being desirable in food products (Cardoso et al., 2007).

In this study, there was no significant difference among treatments (p>0.05) for this parameter, with an average c* value of 41.47 (Table 1). Values of c* close to zero indicate neutral colors (gray) and values around 60 indicate vivid colors. Thus, although the storage time negatively impacted the color intensity of fruit salads (p<0.01), these products retained brighter colors. Also, hue angle (h°) is a colorimetric parameter often used to express color variations in vegetable products (McGuire, 1992). Red is related to h° equal to zero, while yellow is related to h° equal to 90°, green to 180° and blue to 270°. Fruit salads presented h° values ranging from 1.23° to 1.50° (Table 1), located in the first quadrant. These results showed the predominance of red coloration in the salads. The analysis of L. acidophilus viability in minimally processed fruit salads showed that the concentration of this probiotic microorganism ranged from 8.38 Log CFU/g in the fruit salad treated with 0.03% sodium metabisulphite to 8.53 log CFU/g in the fruit salad treated with 1% L-cysteine hydrochloride immediately after processing (time 0). Moreover, the anti-browning compounds in the highest concentrations did not affect (p>0.05) the viability of L. acidophilus (Table 2) showing that they could promote a favorable environment to the probiotic bacteria in the salad. However, after 120 h of storage at 8 °C, we observed a significant reduction (p<0.05) in the viability of L. acidophilus. There was no growth of this bacterium in the control treatment. Rößle et al. (2010) used L. rhamnosus GG in minimally processed apples treated with the antioxidant Natureseal® AS1 and found that after 10 days of storage, the product contained 10^6 CFU/g of this microorganism.

Published works are not clear regarding the minimum concentration of probiotic microorganisms needed to promote beneficial effects on the host organism. Some researchers suggest concentrations greater than 10^5 CFU/g (Dave and Shah, 1997; Saad, 2006) while others suggest concentrations of at least 10^6-10^8 CFU/g (Lourens-Hattingh and Viljeon, 2001). Thus, based on reviewed literature and considering that the developed fruit salads treated with different anti-browning compounds contained over 10^7 CFU/g of L. acidophilus, the developed fruit salads have potential for use as probiotic carriers. This high count of L. acidophilus does not promote deterioration of the product because it is maintained on cooling. Thus, the population is only viable, as found, once the salad is not stored in the optimum temperature for growth of L. acidophilus.

**Table 2. Mean values of lactic acid bacteria count (Log CFU/g) in minimally processed fruit salad treated with anti-browning compounds, at 0 h and after 120 h of processing and storage at 8°C.**

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Treatment</th>
<th>CC1</th>
<th>AA2</th>
<th>AC2</th>
<th>MS 003</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>CC1</td>
<td>8.53</td>
<td>8.42</td>
<td>8.50</td>
<td>8.38</td>
<td>8.46^a</td>
</tr>
<tr>
<td>120</td>
<td>CC1</td>
<td>7.61</td>
<td>7.61</td>
<td>7.42</td>
<td>7.32</td>
<td>7.49^b</td>
</tr>
<tr>
<td>Mean</td>
<td>CC1</td>
<td>8.07^A</td>
<td>8.01^A</td>
<td>7.96^A</td>
<td>7.85^A</td>
<td></td>
</tr>
</tbody>
</table>

| CC1, 1% cysteine hydrochloride; AA2, 2% ascorbic acid; AC2, 2% citric acid; MS 003, 0.03% sodium metabisulphite. Mean values followed by the same uppercase letter on the line or lowercase letter in the column are not different according to the F test at 5% probability.

**Conclusion**

Ascorbic acid and L-cysteine hydrochloride were more effective in retarding the enzymatic browning of fruit salads.
salads. However, we suggest the use of ascorbic acid due to its low cost. Also, ascorbic acid is an innocuous and natural product that does not promote undesirable color changes in the product. Fruit salads presented brown color over storage time at 8°C, which was confirmed by the reduction of L* values. Moreover, values of a*, c*, h* and CI indicated that the fruit salad color tended toward red with brighter colors being predominant. Anti-browning compounds did not have a negative impact on the viability of L. acidophilus and this fruit salad can be used as a promising probiotic carrier. Therefore, minimally processed fruit salads treated with anti-browning compounds and enriched with L. acidophilus constitute a promising functional probiotic product that can be consumed by vegetarians, children, elderly people and those with cholesterol restricted diets.

Conflict of interests

The authors did not declare any conflict of interest.

REFERENCES


