Development of a Shelf-Stable Banana Purée by Combined Factors: Microbial Stability

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ABSTRACT

A preservation process based on the "hurdle" effect for obtaining a shelf-stable banana purée was developed. The microbial stability of the purée was challenged with inoculation of osmophilic and non-osmophilic yeasts, various molds, Bacillus coagulans, Clostridium pasteurianum and Clostridium butyricum. It was shown that growth of both native and inoculated flora may be prevented for at least 120 days storage in banana purée by adjusting water activity ($a_w$) to 0.97, pH to 3.4, adding 250 ppm of ascorbic acid (AA), 100 ppm of potassium sorbate (KS) and 400 ppm of sodium bisulphite (NaHSO₃), and applying a mild heat treatment.

Key Words: Banana, microbial stability.

Banana (Musa paradisiaca) is abundantly grown in many developing countries and holds an important position among the commodities exported by developing nations. An average of 15% of the bananas produced for export is rejected due to size, shape, skin stains and other factors (5). As processed banana products intended to substitute fresh bananas have not been widely accepted, a considerable amount of rejected bananas is wasted.

Surplus banana utilization has traditionally been confined to canned slices and purée, dehydrated products, osmotic dried slices and recently, to intermediate moisture food products (IMF). Many of these traditional preservation methods involve high processing costs (e.g., overcooked texture and flavor of canned products, shrinkage and toughness of dried fruit). Newly developed IMF banana is not sufficiently palatable since addition of large amounts of humectants like sugars and glycerol to developed IMF banana is not sufficiently palatable since addition of large amounts of humectants like sugars and glycerol to reduce $a_w$ to 0.75 to 0.85 changes appreciably the natural flavor of the fruit.

On the other hand, other simple procedures designed to obtain banana products with quality characteristics as close as possible to those of the fresh material only preserve the fruit for approximately 4 weeks at room temperature (5) or for 7 to 10 weeks in cool storage (12).

The purpose of the present work was to develop a simple and minimal preservation process for obtaining "shelf-stable" banana purée, based on the "hurdle" effect (10). The addition of NaHSO₃, KS and phosphoric acid; slight reduction of $a_w$ and a mild heat treatment were the inherent hurdles combined to accomplish the desired microbiological stability of the fruit for near 4 months storage at room temperature.

MATERIALS AND METHODS

Materials.

Ripe banana (M. paradisiaca) of Giant Cavendish variety from Brazil were used in this study. The average moisture content of the bananas was 77% (wt/wt), $a_w$ was approximately 0.98, and the pH ranged between 4.8 to 5.0. Additives used included: Cerelose (glucose monohydrate, food grade), food grade KS, NaHSO₃ (analytical grade, Mallinckrodt Chemical Works, St. Louis, MO), orthophosphoric acid and AA (analytical grade, Merck, Quimica, Argentina).

Determination of moisture and pH.

Banana purée moisture was determined gravimetrically by drying in a forced convention oven over magnesium perchlorate (MgClO₄) at 70°C for 16 h. The pH of the homogenized sample was determined with a glass electrode attached to a Metrohm pH Meter E 632 (Metrohm Herisam, Switzerland).

Determination of $a_w$.

The $a_w$ of fresh and preserved banana purée was measured using a Novasina Humidat TH2 Thermoconstanter hygrometer (Novasina AG, CH-8050, Zurich, Switzerland). The hygrometer was operated following the procedure described in detail by Kitic et al. (9) and was calibrated using unsaturated solutions of sodium chloride (NaCl) (4). The $a_w$ determinations were made in duplicate and the average reported.

Proposed preservation method for banana purée.

Ripe bananas were hand peeled and cut longitudinally into equal halves. Banana halves were blanched in saturated water vapor for 1 min and immediately cooled to about 15°C in a water bath. Halves were then processed with a Sorvall Omni-Mixer to obtain the purée. Glucose was added to reduce $a_w$ to 0.97. The amount of glucose needed was calculated using Ross’ equation (16):

$$ (a_w)_{\text{preserved banana}} = (a_w)_{\text{banana}} (a_w)_{\text{glucose}} $$

where $(a_w)_{\text{banana}}$ is the $a_w$ of the fresh fruit (approximately 0.984) and $(a_w)_{\text{glucose}}$ is the $a_w$ of the solution of glucose, plus the water...
of banana. Values of \( a_{w} \) were obtained from the following equation:

\[
(a_{w})_{\text{glucose}} = x_{1} \exp (-2.25 x_{2}^2)
\]  

where \( x_{1} \) and \( x_{2} \) are molar fractions of water and glucose (3). Predicted \( a_{w} \) value of preserved banana was in very good agreement with measurement. After the addition of NaHSO\(_{3}\) (400 ppm), KS (100 ppm) and AA (250 ppm), phosphoric acid was used to adjust the pH to 3.4. The puree was then packed under vacuum conditions in moisture-proof pouches (Cryovac CN530 R film). Finally, the pouches (approximately 1 cm thick) were placed in a boiling water bath for 1 min and rapidly cooled.

Populations of native flora and inoculated flora were determined in purees with diverse treatments in order to evaluate the effectiveness of different "hurdles" combination. In the case of native flora the purees used were:

A - Puree preserved with proposed method.
B - Puree preserved with proposed method but without \( a_{w} \) adjustment.
C - Puree preserved with proposed method without thermal treatment of pouches.
D - Fresh banana puree ("natural" puree) with thermal treatment of the pouches.

The following treatments were applied to test inoculated flora response to different hurdles:

E - Fresh banana puree without modification ("natural" purée).
F - Puree with \( pH \) adjusted to 3.4 with phosphoric acid.
G - Puree with \( pH \) adjusted to 3.4 and the addition of 400 ppm of NaHSO\(_{3}\) 250 ppm of AA; and 100 ppm of KS (without \( a_{w} \) adjustment).
H - Puree with \( pH \) 3.4 and \( a_{w} \) adjusted to 0.97 with glucose (without additives).
I - Puree preserved with proposed method with the exception of thermal treatment step.

Before inoculation, these purees were thermally treated with boiling water in 1 cm thick pouches at 100°C, for 2 min, to eliminate interfering microorganisms. The effect of a mild heat treatment after inoculation was not evaluated in the systems E to I.

Microorganisms.

The microbial stability of the preserved banana purees was challenged with the inoculation of \( B.\ coagulans \) Hammer ATCC 8038, \( C.\ pasteurianum \) ATCC 6013, Zygosaccharomyces rouxii ATCC 8393 and Zygosaccharomyces bailii NRRL Y-1448, as well as \( C.\ butyricum \), Aspergillus niger, Penicillium roquefortii, Eurotium amstelodami, Paecilomyces variotii and Saccharomyces cerevisiae, provided by the Laboratory of Industrial Microbiology of the Facultad de Ingeniería, University of Buenos Aires. These test microorganisms were selected on the basis of their ability to grow in fruits (some of them have usually been found in banana) (1,15,18), in the presence of sorbate (13) at low pH values or in a variety of substrates.

Inocula preparation and incubation of samples.

\( Clostridium\ pasteurianum \) and \( C.\ butyricum \) inocula were prepared by growing cells in nutrient broth (E. Merck AG, Darmstadt, Germany) for 24 h. Cultures of \( C.\ butyricum \) and of \( C.\ pasteurianum \) were diluted 100-fold. A 2-ml portion of each suspension was inoculated with a sterile syringe in vacuum-sealed pouches containing 18 g of sterile purée. The initial concentration of microorganisms was \( 1 \times 10^5 \) to \( 10^4 \) colony forming units (CFU)/g (No). The inoculated pouches were stored at 33°C.

Tubes containing nutrient broth were heated 10 min to deaerate the medium and were rapidly cooled to the incubation temperature. A 2-ml portion of clostridia suspension was inoculated into the tubes. Then were paraffin plugged and periodically checked as a growth control.

\( B.\ coagulans \) inoculum was prepared by growing cells in a nutrient broth recommended by American Type Culture Collection (ATCC) (beef peptone 7.8 g; casein peptone 7.8 g; yeast extract 2.8 g; glucose 1.0 g; NaCl 5.6 g; distilled water 1.0 L) for 24 h. \( B.\ coagulans \) suspension was diluted 10-fold. A 2-ml portion of this was inoculated into 250-ml sterile flasks, which contained 18 g of sterile purée. The initial count was \( 1 \times 10^3\) CFU/g (No). In order for the aerobic condition not to become a controlling growth factor for the microorganism, an adequate headspace was left in the flasks. After inoculation, they were hermetically closed and stored at 37°C. A 2-ml portion of \( B.\ coagulans \) suspension was inoculated into flasks containing 18 ml of the nutrient broth, and the flasks were periodically checked as a growth control.

Yeast inocula were prepared by growing cells in Sabouraud broth at 25°C for 24 h. A 2-ml portion of each suspension (approximately \( 1 \times 10^7\) CFU/ml) was inoculated in sterile flasks containing 28 g of banana purée. The inoculated flasks were stored at 25°C. Growth controls of each yeast were tested in Sabouraud broth flasks.

Mold inocula were prepared by growing cells in slant tubes with Sabouraud agar at 25°C for 1 week. The growth was scraped off and suspended in a 5 ml of Tween 80 solution (0.1% w/v). Inocula suspension of spores contained 5 x 107-108 spores per ml. 10 µl of suspension of spores was inoculated at the center of petri dishes containing 20 g of sterile purée. The inoculated samples were incubated at 25°C. A petri dish with potato dextrose agar and 40 ppm tetracycline (analytical grade, Merck) was used as a growth control for each mold.

For every microorganism studied, each experiment was done in duplicate and the average was reported. The microbial population of the banana purees before the inoculation with \( B.\ coagulans \), \( C.\ pasteurianum \), \( C.\ butyricum \), yeasts and molds was determined. The aerobic mesophilic plate count averaged \( 1 \times 10^6\) CFU/g mold, yeast and anaerobic bacterial counts resulted in no detectable organisms. These results indicated that thermal treatment to obtain "sterile" purée was enough to destroy interfering microorganisms for the subsequent counts.

Native flora.

Pouches containing 40 g of banana purée were stored at 25°C for the analysis of aerobic and anaerobic mesophilic yeasts and molds and at 37°C for the analysis of aerobic and anaerobic thermophilic. Analysis was performed in duplicate and the average was reported.

Enumeration of microorganisms.

The microbial population of the different kinds of purees previously described was determined during storage. \( B.\ coagulans \) plate counts were performed on thermoacidurans agar (pH:5) incubated at 37°C for 48 h. Anaerobic mesophilic, \( C.\ pasteurianum \) and \( C.\ butyricum \) concentrations were estimated using the Most Probable Number (MPN) technique in tubes with reinforced clostridial + casein red phenol agar incubated at 33°C for 7 to 10 days. Anaerobic thermophilic concentrations were estimated in a similar way except by the incubation of the tubes at 55°C for 1 week. Aerobic mesophilic and thermophilic plates counts were performed on plate count agar incubated at 33°C for 48 h or at 55°C for 1 week. Molds and yeasts were plated on potato dextrose agar with 40 ppm tetracycline and incubated at 33°C.
25°C for 1 week. Microbial counts were performed in quadruplicate for each sample and the average reported as CFU/g (N). Occurrence of absence of spoilage was reported for inoculated molds.

All media were obtained from Merck.

RESULTS AND DISCUSSION

Microorganisms assayed as "native" flora were aerobic and anaerobic mesophilic and thermophilic yeasts and molds. Neither counts of anaerobic thermophilic nor of aerobic thermophilic were detected during 100-day storage at 37°C in any type of puree. Figure 1 shows the changes in the aerobic mesophilic plate count during incubation of banana purees at 25°C. The number of counts in the "natural" puree with thermal treatment (D) increased rapidly during incubation and odor, and visual and textural signs of microbial spoilage were evident. In the preserved puree without thermal treatment (Fig. 1, line C), plate count declined during storage but microbial population reached $7 \times 10^5$ CFU/g during the first days, looking like the puree fully deteriorated. In the preserved puree without $a_w$ adjustment (Fig. 1, line B), the aerobic plate count grew by about two logarithmic cycles and then declined during incubation reaching values similar to initial ones. The combination of all "hurdles" proposed (e.g., thermal treatment, addition of 100 ppm KS, 250 ppm AA and 400 ppm NaHSO$_3$ and adjustment of pH to 3.4 and $a_w$ to 0.97) (Fig. 1, line A) resulted in growth inhibition. It is interesting to note that aerobic mesophilic microorganisms seemed to be more sensitive to heat in banana purees with additives and pH 3.4 (Fig. 1, lines A and B) than in "natural" puree (Fig. 1, line D) since initial counts for A and B systems were at least two orders lower than for D system. Table 1 and Table 2 show the estimation of anaerobic mesophilic concentration and yeast and mold counts, respectively, in the banana purees during 102-day storage at 25°C. It can be seen that the thermal process is not enough treatment to prevent the growth of yeasts, molds and anaerobic mesophilic bacteria. In all other preserved purees (A, B, C), the number of cells declined during incubation. In particular, the viable population was almost lost in the puree preserved by using all "hurdles" (A).

Figure 2A and 2B show the behavior of C. pasteurianum and C. butyricum, respectively, during incubation at 33°C of banana purees with the different treatments mentioned in Materials and Methods section. The bacteria in the control (Fig. 2A and 2B, dashed lines) and in the "natural puree" (Fig. 2A and 2B, lines E) grew well being almost $1 \times 10^6$ CFU/g after 10 days of incubation. Clostridium butyricum showed better growth in the "natural puree" (Fig. 2B, line E) than in the control broth (Fig. 2B, dashed line). All treatments tested were lethal to C. pasteurianum and C. butyricum. Moreover, in pH 3.4 puree (Fig. 2A and 2B, lines F), no counts were detected beyond 40-day storage. As can be seen in Fig. 2A and 2B, control of pH alone led to growth inhibition, and when other combinations of hurdles were studied ($a_w$ - pH or additives - pH or $a_w$ - additives - pH), no additional effect was observed.

Figure 3 shows the changes in the B. coagulans plate count during incubation at 37°C of banana purees with different treatments. The number of counts in the "natural"

**TABLE 1. Estimation of anaerobic mesophilic (CFU/g) in uninoculated banana purée during storage.**

<table>
<thead>
<tr>
<th>Banana purée</th>
<th>Storage time (days) at 25°C</th>
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<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>A</td>
<td>$4 \times 10^3$ &lt;20 &lt;20 &lt;20 &lt;20</td>
</tr>
<tr>
<td>B</td>
<td>$2 \times 10^3$ 7 $2 \times 10^2$ &lt;20 &lt;20 &lt;20</td>
</tr>
<tr>
<td>C</td>
<td>&lt;20 3 $3 \times 10^2$ &lt;20 &lt;20 &lt;20</td>
</tr>
<tr>
<td>D</td>
<td>&lt;20 5 $5 \times 10^2$ &lt;20 5 $5 \times 10^1$</td>
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<table>
<thead>
<tr>
<th>Banana purée</th>
<th>Storage time (days) at 25°C</th>
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<tbody>
<tr>
<td></td>
<td>0</td>
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<tr>
<td>A</td>
<td>&lt;20 ND ND ND ND</td>
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<tr>
<td>B</td>
<td>$2 \times 10^2$ 7 $2 \times 10^2$ &lt;20 &lt;20 &lt;20</td>
</tr>
<tr>
<td>C</td>
<td>ND ND ND &lt;20 &lt;20 &lt;20</td>
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<tr>
<td>D</td>
<td>$2 \times 10^1$ 2 $4 \times 10^2$ &lt;20 &lt;20 2 x $10^1$</td>
</tr>
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</table>

**Table 1:** Estimation of anaerobic mesophilic (CFU/g) in uninoculated banana purée during storage.

**Table 2:** Yeast and mold counts (CFU/g) in uninoculated banana purée during storage.

**Figure 1. Changes of aerobic mesophilic plate count of banana purees adjusted to selected combinations of "hurdles" during incubation at 25°C ($N = $ microbial concentration).

- (A) Purée preserved by thermal treatment, $KS = 100$ ppm, $NaHSO_3 = 400$ ppm, AA = 250 ppm, pH 3.4, $a_w$ 0.97.
- (B) Purée with thermal treatment, $KS = 100$ ppm, $NaHSO_3 = 400$ ppm, AA = 250 ppm, pH 3.4.
- (C) Purée with $KS = 100$ ppm, $NaHSO_3 = 400$ ppm, AA = 250 ppm, pH 3.4, $a_w$ 0.97.
- (D) "Natural" purée with thermal treatment.
Figure 2. Changes of C. pasteurianum ATCC 6073 (A) and C. butyricum (B) concentration of banana purées adjusted to selected combinations of $a_w$, pH and addition of KS, NaHSO$_3$, and AA ($N =$ microbial concentration; $No =$ initial microbial concentration).

(E) Banana purée "natural".
(F) Purée with pH 3.4.
(G) Purée with pH 3.4, KS = 100 ppm, NaHSO$_3$ = 400 ppm and AA = 250 ppm.
(H) Purée with pH 3.4 and $a_w$ = 0.97.
(I) Purée with pH 3.4, KS = 100 ppm, NaHSO$_3$ = 400 ppm, AA = 250 and $a_w$ = 0.97.

"Control" (nutrient broth).

Figure 3. Changes of B. coagulans ATCC 8038 plate count of banana purées adjusted to selected combinations of $a_w$, pH and addition of KS, NaHSO$_3$, and AA ($N =$ microbial concentration; $No =$ initial microbial concentration).

(E) Banana purée "natural".
(F) Purée with pH 3.4.
(G) Purée with pH 3.4, KS = 100, NaHSO$_3$ = 400 ppm and AA = 250 ppm.
(H) Purée with pH 3.4 and $a_w$ = 0.97.
(I) Purée with pH 3.4, KS = 100, NaHSO$_3$ = 400 ppm and AA = 250 ppm and $a_w$ = 0.97.

"Control" (nutrient broth).

juice are in the range 3.8 to 5.1, depending on the type of acidulant, growth medium, strains examined and other factors (2). Our results do not support these literature values as can be seen from the fact that growth of B. coagulans was not inhibited in banana purée with pH adjusted to 3.4 (Fig. 3, line F). Examination of the combined effect pH-$a_w$ (Fig. 3, line H) indicates practically no synergism between $a_w$ and pH. Very little information is available on the spoilage (non-pathogenic) sporeforming bacteria and their $a_w$ limits for growth in foods or model food systems (7). Growth of Bacillus spp. can take place at significantly lower $a_w$ values than growth of clostridia. Jakobsen (7) found that the minimum $a_w$ for growth in optimal laboratory conditions in tryptone-glucose yeast extract broth (adjusted to various $a_w$ levels with NaCl or glucose) is 0.90 to 0.93 for about 350 strains isolated from spices, various dry products and environmental samples from canneries, bacon slaughter lines and poultry slaughter houses, but he did not report data about B. coagulans in particular. Sajur (17) reported that B. coagulans did not grow in preserved peaches with pH 3.5 and $a_w$ = 0.94 adjusted with glucose. These literature results might explain the little influence on growth response of an $a_w$ depression to a 0.97 value when the pH is 3.4, since this value is well above the minimum. When the combination of factors pH-$a_w$-additives is used (Fig. 3, line 1), the bacteriostatic-bactericidal effect (except a slight initial increase in counts) seems to be more pronounced and ensures a better banana preservation.

Clostridium pasteurianum and C. butyricum showed to be more sensitive to acidification (Fig. 2A and 2B) since a slight reduction of pH to 3.40 prevents spoilage in preserved banana. Even though the high concentration of carbohydrate present in banana, results obtained here seem
to agree with literature data in other media. Blocher and Busta (2) reported that the minimum pH for growth varies from 3.5 to 4.0 for C. pasteurianum in 10° Brix apricot juice, canned pineapple, pureed tomato and pear juices.

Jakobsen and Jensen (8) investigated the combinations of pH and $a_w$ for preventing growth of butyric anaerobes in canned pears and found that at pH = 3.8, just one strain was able to grow, and this occurred only at $a_w \geq 0.985$; but they only considered 30-day storage time.

The banana purées were inoculated with spores of A. niger, P. roquefortii, E. amstelodami and P. variotii. After 12 days, surface colony formation was observed in the "natural purée" (E), in the purée with pH 3.4 (F), in the purée with pH 3.4 and $a_w$ adjusted to 0.97 (H), and in the control. However, no growth was detected in any of the other purées (pH 3.4 and added sorbate, NaHSO$_3$, and AA with or without $a_w$ adjustment) (G,I) during 4 months of incubation at 25°C.

Figure 4A, 4B and 4C shows the behavior of S. cerevisiae, Z. bailii and Z. rouxii, respectively, inoculated in the banana purées and incubated at 25°C. None of them were able to grow in the banana purée with pH 3.4, additives and $a_w$ 0-97 (I). Moreover, this combination of "hurdles" was lethal to the three yeasts. In the absence of any of these factors (decreased $a_w$, decreased pH, additives), the yeasts grew very well, as in the "natural" purée and in the control.

Inoculated flora "hurdles" other than thermal treatment (additives - pH - $a_w$) resulted in inhibition of microorganisms. However, thermal treatment was essential because it enhanced these "hurdle" effects significantly, diminishing the native microbial population.

According to our results, a "shelf-stable" banana purée can be obtained with the combined application of various "hurdles" ($a_w$ 0.97, pH: 3.4; 100 ppm KS; 400 ppm NaHSO$_3$; 250 ppm AA and slight thermal treatment), being storable without refrigeration for at least 120 days.

It is important to remark that the product obtained is organoleptically acceptable as tested through a battery of sensorial evaluation tests that will be published somewhere.

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