Study of the Shelf Life of a Low-Calorie Jam

Added with Microencapsulated Probiotics

Rafael E. González-Cuello, Kelvin Pájaro, Wendy Acevedo and Rodrigo Ortega-Toro

Programa de Ingeniería de Alimentos, Facultad de ingeniería
Universidad de Cartagena, Carrera 6 # 36-100
Cartagena de Indias D.T. y C., Colombia

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Abstract

The development of functional foods is essential to meet food needs and prevent some health problems of the population. The objective of this work was to standardise the process of making a tree tomato jam (Solanum betaceum) with functional properties. The jam was added with Aloe vera (Aloe Barbadensis Miller), stevia (Stevia rebaudiana) as a sweetener and Lactobacillus bulgaricus as a probiotic. Experimental design of 15 treatments was generated, according to the Box-Behnken design for three factors. These treatments were subjected to sensory analysis establishing the general acceptability of a panel of trained tasters. The computer program "Minitab Statistical Software" version 15 for Windows was used, obtaining the treatment with maximum acceptability. The best treatment had 50% pulp, 0.21% stevia, 0.18% pectin, 0.05% benzoate, 1.06% citric acid and 21.6% sucrose with a pH of 4.27 and 41.43 °Brix. This treatment was characterised physicochemically and microbiologically.

Keywords: Functional Foods, Jam, Shelf Life, Lactobacillus bulgaricus

Introduction

The preparation of jams has gone from being a homemade process, to become an essential activity of the food industry, especially in the area of fruit processing [1]. Also, this type of product allows the use of various components in its formulation, such as antioxidants, vitamins, minerals, probiotics and prebiotics, among others, conferring functional properties to the food. Aloe vera is one of the
most used ingredients in the design of functional foods. This plant can relieve stomach ailments such as heartburn, gastritis, ulcers and indigestion. In Colombia, there is a great diversity of fruits that can be used for making jams. The tree tomato is the fruit of a small tree native to the Andes mountain range; it belongs to the Solanaceae family [2]. It has some beneficial properties for health such as cholesterol reduction, high fibre content and low-calorie level [3]. Regarding the content of sugar in food, it is known that excess sugar can bring various adverse effects such as diabetes, which is why the use of sweeteners such as stevia can help prevent this type of disease [4].

Currently, the use of bacteria that contribute to the development of the sensory, physicochemical and rheological characteristics of food, and also generates specific benefits such as preventing constipation [5] has been explored. In this context, the food industry has focused on various investigations such as the incorporation of some probiotic microorganisms [3]. Taking advantage of these benefits, we use microencapsulation techniques, which consist of coating a component with a natural polymer [6]. By means of this technique, cellular losses can be reduced during the processing and storage of food, as well as reinforcing the viability of the bacteria of interest [7]. In the present work the formulation of a tomato jam was optimised using the response surface methodology (RSM), and in turn, the viability of Lactobacillus bulgaricus was evaluated.

Materials and Methods

Microencapsulation of L. bulgaricus

The probiotic microorganisms L. bulgaricus were microencapsulated and then incorporated into the jam by internal ionic gelation, following the methodology described previously [7]. Lyophilized inoculum of L. bulgaricus (0.02 g) was added into 5 mL of distilled water (0.4% p/v) at 25°C. This fact was done to create a suspension in a passive state [3].

The dispersion was carried out using a combination of 0.175 g of low acyl gellan gum (LAG) and 0.06 g of CaCO₃ in 20 mL of distilled water at a concentration of 1.1% wt/v, (with prior sterilisation at 121 °C for 15 min). This dispersion was then placed on a heating plate with constant magnetic stirring of 500 r.p.m. at a temperature of 80 °C for 10 min. Subsequently, it was cooled to 38 °C, in which the inoculum of L. bulgaricus was added. Then, 0.2 g of glucono-alpha-lactone was added to the system until a final pH of 4.0 was reached to obtain the microcapsules by gelling the biopolymer. The microcapsules were formed by dispersing the aqueous mixture (LAG, CaCO₃, L. bulgaricus and glucono-alpha-lactone) into 50 mL of vegetable oil under constant agitation for 15 minutes.
Jam formulations

Three formulations were prepared at different concentrations. In the first treatment, 0.28% of stevia, 0.72% of pectin and 0.10% of sodium benzoate were mixed. In the second treatment, 0.21% stevia, 0.18% pectin and 0.07% sodium benzoate were added to the jam. For the third treatment, 0.14% of stevia, 0.36% of pectin and 0.05% of sodium benzoate were added. Fruit and Aloe vera content was set at 50% and 5% respect to total weight. Response Surface Designs (RSD) was used in this study to determine the optimal levels of the ingredients for tree tomato jam with functional characteristics. The effect of three independent variables (Levels: pectin ($X_1$), stevia ($X_2$) and benzoate ($X_3$)) in one response variable ($Y$, general acceptability reported by sensorial analysis) [8]. It was evaluated using the Box-Behnken design with 3 factors and two levels per factor. Each test was performed 3 times. The response variables were analysed by analysis of variance (ANOVA) using SPSS software version 17 for Windows, with a confidence level of 95%. Additionally, the DMS test was performed to evaluate the relationship between the average data of each response variable. The optimal formulation was chosen to evaluate its physicochemical and microbiological properties.

Sensorial analysis of formulations

A hedonic scale of 7 points was carried out to evaluate the acceptability of all the studied formulations. The score range was set between 1 (very annoying) and 7 (I like it a lot). A panel of 20 trained tasters was used. The tests were carried out in triplicate. Samples were evaluated at the initial time and after 1 week of storage.

Shelf life study of jam

The jams with the highest percentage of acceptance were selected and inoculated with microcapsules of L. bulgaricus. Jellies were studied with and without microcapsules. The follow-up was carried out for 20 days. The growth kinetics of moulds and yeasts were determined since they are directly related to the shelf life of the products. A procedure similar to that described in the viability section of L. bulgaricus was followed, being used as Sabouraud agar at 25 °C for 3 days. Microbial counts were performed expressing the results as CFU/g. The data obtained are modelled using the Baranyi and Roberts equation (equation 1) using the DMFit 2.0 software to get the kinetic growth parameters.

\[
y(t) = y_0 + \mu \text{max} + \frac{1}{\mu \text{max}} \ln \left( e^{-\nu t} + e^{-h_0} - e^{-\nu t-h_0} \right) \\
- \frac{1}{m} \ln \left( 1 + \frac{e^{m\mu \text{max} + \frac{1}{u \text{max}} \ln \left( e^{-\nu t + h_0} - e^{-\nu t-h_0} \right)}}{e^{m(y_{\text{max}} - y_0)}} \right) 
\]  
(1)
Where: \( y(t) \), cell concentration or diameter of the colonies; \( y_0 \), concentration or initial diameter; \( \mu_{\text{max}} \), specific growth rate; \( m \), curvature parameter to characterize the transition of the exponential phase; and \( h_0 \), a dimensionless parameter that quantifies the initial physiological state of the cells.

Once the kinetic parameters of microbial growth are obtained, the microbiological shelf life is estimated through the Monod-Hinshelwood equation (equation 2).

\[
ts_t = \frac{\log N_s - \log N_0}{\log 2} \times T_d
\]

Where: \( t_s \), estimated time for the microorganisms to develop causing the alteration in the food; \( N_s \) (CFU / g), value corresponding to the security population; \( N_0 \) (CFU / g), amount of the initial population present in the product; and \( T_d \), time of duplication of the specific alteration population.

**Bromatological Analysis**

The protein content was determined using the Kjeldahl procedure described by AOAC 920.52 [9]. Fat content was determined by Soxhlet extraction method according AOAC 920.39 [10]. Moisture content was determined by AOAC method 925.45 [11] and total carbohydrates were calculated as the difference between jam total weight and the sum of protein, fat, water and ash.

**Viability of L. bulgaricus into jam**

For the microorganism viability study, 1 g of the jam was added to 99 ml of peptone water (0.1%) for 3 min. Subsequently, serial dilutions of \( 10^{-1} \) were made. Aliquots of 1 mL of each dilution were extracted and transferred to MRS agar (Man Rogosa and Sharpe), and it was incubated at 37 °C for 24 h. After the storage time, the microbial count of colonies obtained was performed, expressing the results in CFU / g.

**Results and Discussion**

**Sensorial analysis of formulations**

The selected panellists evaluated 3 samples from each of the 15 treatments according to the Box-Behnken design applied. Table 1 shows the composition of the samples and the acceptance degree values of the studied formulations.
Table 1. Composition of studied formulation and acceptance degree values from sensorial analysis

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Stevia (%)</th>
<th>Pectin (%)</th>
<th>Benzoate (%)</th>
<th>Y prom ± DE</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>0.14</td>
<td>0.18</td>
<td>0.07</td>
<td>4.0 ± 1.7</td>
</tr>
<tr>
<td>T2</td>
<td>0.28</td>
<td>0.18</td>
<td>0.07</td>
<td>6.3 ± 0.6</td>
</tr>
<tr>
<td>T3</td>
<td>0.14</td>
<td>0.72</td>
<td>0.07</td>
<td>6.3 ± 1.2</td>
</tr>
<tr>
<td>T4</td>
<td>0.28</td>
<td>0.72</td>
<td>0.07</td>
<td>5.7 ± 0.6</td>
</tr>
<tr>
<td>T5</td>
<td>0.14</td>
<td>0.45</td>
<td>0.05</td>
<td>6.0 ± 0.2</td>
</tr>
<tr>
<td>T6</td>
<td>0.28</td>
<td>0.45</td>
<td>0.05</td>
<td>6.3 ± 1.2</td>
</tr>
<tr>
<td>T7</td>
<td>0.14</td>
<td>0.45</td>
<td>0.10</td>
<td>6.7 ± 0.6</td>
</tr>
<tr>
<td>T8</td>
<td>0.28</td>
<td>0.45</td>
<td>0.10</td>
<td>6.3 ± 1.2</td>
</tr>
<tr>
<td>T9</td>
<td>0.21</td>
<td>0.18</td>
<td>0.05</td>
<td>7.0 ± 0.2</td>
</tr>
<tr>
<td>T10</td>
<td>0.21</td>
<td>0.72</td>
<td>0.05</td>
<td>5.3 ± 1.2</td>
</tr>
<tr>
<td>T11</td>
<td>0.21</td>
<td>0.18</td>
<td>0.10</td>
<td>6.0 ± 0.2</td>
</tr>
<tr>
<td>T12</td>
<td>0.21</td>
<td>0.72</td>
<td>0.10</td>
<td>6.0 ± 1.2</td>
</tr>
<tr>
<td>T13</td>
<td>0.21</td>
<td>0.45</td>
<td>0.07</td>
<td>5.7 ± 0.6</td>
</tr>
<tr>
<td>T14</td>
<td>0.21</td>
<td>0.45</td>
<td>0.07</td>
<td>4.3 ± 1.5</td>
</tr>
<tr>
<td>T15</td>
<td>0.21</td>
<td>0.45</td>
<td>0.07</td>
<td>5.0 ± 0.2</td>
</tr>
</tbody>
</table>

Different superscripts indicate significant differences between the formulations (p < 0.05).

Equation 3 was obtained to predict the acceptability (Y) of the studied jams. To do this, only the terms that had significant differences were taken into account (p ≤ 0.05).

\[
Y = 0.207(stevia) + 0.041(benzoate) + 0.041(stevia)^2 \\
+ 0.167(pectine)^2 + 0.917(benzoate)^2 \\
+ 0.417(pectine \times benzoate) 
\]  \hspace{1cm} (3)

Table 1 shows that T1 was the least satisfactory (acceptance degree of 4.0 ± 1.7). This formulation contained 0.14% stevia, 0.18% pectin, 0.07% benzoate, pH 4.23 and 48.06 °Brix. On the other hand, T9 obtained the highest acceptance value (7.0 ± 0.2). This formulation contained 0.21% stevia, 0.18% pectin, 0.05% benzoate, pH of 4.27 and 41.43 °Brix. Comparing T1 and T3, it can be observed that for constant values in stevia and benzoate (0.14% and 0.07%, respectively) a decrease in the pectin concentration produces a decrease in the values of acceptance. Furthermore, comparing treatments T2 and T4, whose concentrations of stevia and benzoate are constant (0.28% and 0.07%, respectively), but higher than treatments T1 and T3, a decrease in the concentration of pectin does not produce a significant change in the acceptance values of the final product.

Figure 1 shows the assessment scale obtained from the sensory panel for each of the treatments with and without probiotic microcapsules, added with Aloe vera and stevia, concerning the evaluated acceptability.
Figure 1. Mean values of the sensory analysis of tree tomato jam with probiotic microcapsules and control sample (without probiotic).

The sample with the highest acceptance value \(Y\) was T9 (0.21% stevia, 0.18% pectin and 0.05% benzoate) because when tasters evaluated the sample, the panellists did not perceive any undesirable residual taste or odour. Other researchers [12] reported that lactic acid bacteria (LAB) contribute to the bio-preservation of dairy foods and improve their sensory characteristics such as taste, smell, texture and increase their nutritional quality.

Considering the acceptance values for each sample, the optimised compositional values were calculated using Minitab 15 Software for Windows. Optimization of \(Y\) values close to 6 was obtained. As a result, the optimal composition should be 0.05% of benzoate, 0.18% of pectin and 0.14% of stevia.

Shelf life analysis

Figure 2 shows the growth of moulds and yeasts for 20 days for the best acceptance sample (T9) and control sample (T10). There are slight differences between the studied formulations. T9 starts with 18 CFU/g and ends with 25 CFU/g and T10 starts with 17 CFU/g and finished with 27 CFU/g.
Among the studied formulations, no marked differences were observed although the control showed a higher microbial count. Furthermore, there was no evidence of the high growth of these microorganisms, because sucrose is considered a hygroscopic agent, thus contributing to the reduction of moisture in the final product. The combination of stevia and sucrose in the jam formulation provided an increment in the soluble solids and thus increase the osmotic pressure.

Taking into account the kinetics of growth of moulds and yeasts and applying the model of Baranyi and Robert and the equation of Monod-Hinshelwood, the shelf life of the jams was obtained. The shelf life for T9 was 15 days and for the control formulation of 14 days. These results have not statistical differences because of the similarity in microbiological content.

**Microbiological results**

The microbiological results obtained for all the treatments were within the parameters established for canned fruits, according to the Resolution 3929 of the Ministry of Health and Social Protection [13] (Colombian legislation). The limits of the number of total coliforms are indicated, as well as faecal coliforms, mould and yeast count. The results are shown in Table 2 as CFU / g.

**Table 2. Microbiological results and legislation requirement for jam**

<table>
<thead>
<tr>
<th>Analysis</th>
<th>Method</th>
<th>Obtained results</th>
<th>Legislation requirement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mould and yeast (UFC/g)</td>
<td>Plate count</td>
<td>&lt; 10</td>
<td>20-50</td>
</tr>
<tr>
<td>Total coliforms (MPN)</td>
<td>Plate count</td>
<td>&lt; 3</td>
<td>&lt; 3</td>
</tr>
<tr>
<td>Faecal coliforms (MPN)</td>
<td>Plate count</td>
<td>&lt; 3</td>
<td>&lt; 3</td>
</tr>
</tbody>
</table>

MPN: most probable number

The pH of the jams was 4.6 ensuring the safety of the products. This pH inhibits the proliferation of microorganisms, and therefore, jams comply with legislation regarding microorganisms’ presence. Table 2 shows that the count of yeasts and moulds had values lower than 10 CFU/g for and coliforms values lower than 3 MPN.
Bromatological Analysis
The bromatological composition of the jam with greater acceptance (T9) was determined. The differences between the different treatments are expected to be minimal. T9 had 67.2 ± 0.5% of water, 0.4 ± 0.1% of protein, 0.020 ± 0.002% of fat and 34 ± 0.5% of total carbohydrates. The pH of the final product was 4.27 ± 0.11. A serving of tree tomato jam with aloe vera and stevia (15 g) provides less than 16 calories (calculated from protein, fat, and carbohydrate content). According to Resolution 333 of the Ministry of Social Protection of Colombia [14], this product could be considered low in calories.

Viability of L. bulgaricus into jam
The count of L. bulgaricus was studied after 20 days of storage to determine the viability of this probiotic. The treatment with the best result was T3 with 3.6 x 10^3. This content of microorganisms is lower than that of functional meals (1 x 10^8). It is possible that the method for encapsulating the microorganism is not appropriate or that the coating material has pores and allows the permeation of some components of the food matrix into the capsules causing the death of the probiotic bacteria.

Conclusion
It is possible to obtain a probiotic jam with low-calorie content from tree tomato, aloe vera, stevia and Lactobacillus bulgaricus encapsulated. The formulation with the highest sensory acceptance (T9) contained 50% tomato pulp, 21.6% sucrose, 1.06% citric acid, 0.21% stevia and 1% microencapsulated probiotics. The final viability of the microencapsulated L. bulgaricus during 20 days of storage was 3.6 x 10^3, far from the therapeutic level of 1 x 10^8. Therefore it is recommended to improve the procedure and the matrices used to encapsulate the microorganisms.

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