

Shelf Life of Ice Cream: Effect of Microencapsulated *Lactobacillus bulgaricus*

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Abstract

In the present research, the effect of the microencapsulation of *Lactobacillus bulgaricus* on the microbiological stability of ice cream of milk and aloe vera was studied. In the first assay, the ice cream was made by adding microencapsulated probiotics before maturation at 4°C for 24 h. In the second assay, the ice cream was prepared by adding microencapsulated probiotics after ripening and in last one, ice cream without capsules as a control sample. Sensorial acceptance, microbiological shelf life and viability of *Lactobacillus bulgaricus* after storage time were evaluated. Product with the higher acceptance was those with microencapsulated probiotics added before ripening. This ice cream obtained 26 days of shelf life. Finally, microencapsulated probiotics have a final count of 10^3 .

Keywords: Ice cream, Shelf Life, Microencapsulation, *Lactobacillus bulgaricus*

Introduction

Ice cream is a sweet dairy product, consisting of milk, sweeteners, stabilizers and emulsifiers, is prepared by the mixture of its ingredients, followed by pasteurization, homogenization, ripening and freezing [1]. Fruits are also an important part because they transfer colour and flavour expressing the cultural diversity of a region. In the market there are many products that have been considered as functional foods. These products contain the specific components, which improve the health of consumers.

Aloe vera is a plant belonging to the *Liliaceae* family, the most used part is its gel, due to its functional, antioxidant and therapeutic properties. In the food industry, aloe vera has been used in various functional products [2]. Similarly, food products with the addition of lactic acid bacteria with counts of at least 10^8 cell/mL can be considered functional foods [3]. The aim of the present work was to determine the effect of the microencapsulated *Lactobacillus Bulgaricus* on the stability of the microbiological and sensory properties of ice cream with aloe vera gel.

Materials and Methods

Microencapsulation of L. bulgaricus

The microcapsules were prepared through the internal ionic gelation process. This technique is based on the formation of a water/oil emulsion. The aqueous phase was prepared by mixing 1 ml of the cell suspension (10^9 cell/mL of *Lactobacillus bulgaricus*) with 99 ml of the low acyl gellan dispersion (AGD) (with previous sterilisation at 121 ° C for 15 min). Calcium carbonate was used as a Ca^{2+} donor at a ratio of 30 mM Ca^{2+} . Subsequently, vegetable oil containing 2% v/v of surfactant (Span 80) was added at a ratio of 1:2 under constant agitation (800 rpm). After 15 min, glucono-alpha-lactone was incorporated into the system until reaching a pH of 4.2 to obtain the microcapsules by gelling the biopolymers. Finally, the oil was removed by adsorption.

Ice cream formulations

Three ice cream formulations were studied. In the first case, the probiotic microcapsules were added before the ice cream ripening at 4 ° C for 24 hours. The second test was performed by adding the microcapsules after the ripening of the ice cream. And the third assay did not contain the microcapsules.

Viability of L. bulgaricus into ice cream

For the microorganism viability study, 11 g of the ice cream was added to 99 ml of peptone water (0.1%) for 3 min. Subsequently, serial dilutions were made. For the release of the microorganisms in the microcapsules, maceration of the microcapsules was carried out. 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.

Shelf life study of ice cream

The microbial development in the matrices was studied for 20 days. For this, 11 g of each sample were weighed and homogenised in 99 mL of 0.1% peptone water for 3 min, making dilutions from 10^{-1} to 10^{-3} . Sabouraud agar was used at 25 ° C for 3 days. After the time, microbial counts of obtained colonies were made, expressing the results as CFU / g.

The collected data are modelled using the Baranyi and Roberts equation (equation 1) using DMFit 2.0 software for Windows to obtain the kinetic growth parameters.

$$y(t) = y_0 + \mu_{max} + \frac{1}{\mu_{max}} \ln(e^{-vt} + e^{-h_0} - e^{-vt-h_0}) - \frac{1}{m} \ln\left(1 + \frac{e^{m\mu_{max} + \frac{1}{\mu_{max}} \ln(e^{-vt} + e^{-h_0} - e^{-vt-h_0})}}{e^{m(y_{max}-y_0)}}\right) \quad (1)$$

Where: $y(t)$, cell concentration or diameter of the colonies; y_0 , concentration or initial diameter; μ_{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).

$$ts = \frac{\log N_s - \log N_0}{\log 2} \times Td \quad (2)$$

Where: ts , 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 in the product; and Td , time of duplication of the specific alteration population.

Sensorial analysis of formulations

A hedonic scale of 10 points was carried out to evaluate the acceptability of all the studied formulations. The score range was set between 1 (very annoying) and 10 (I like it a lot). A panel of 20 trained tasters was used. The tests were carried out in triplicate. Samples were evaluated after 1 week of storage.

Chemical composition

The chemical composition of ice cream was analysed by bromatological analysis. The determination of fat [4], dry extract [5], protein content [6] and titratable acidity [7] was carried out according standard methods.

Results and Discussion

*Viability of *L. bulgaricus* into ice creams*

The microencapsulation technique used in the ice creams had a significant effect on the viability of the microorganism within the dairy product, evidencing a slight growth of the *L. bulgaricus* for ice cream inoculated with probiotic microencapsulated before ripening (pH of 7.1 and 0.2% of lactic acid). This fact shows that these were the ideal conditions after a week of storage. Additionally,

their growth kinetics were checked every 5 days for 20 days, reaching a maximum rate of 10^3 UFC/g or 2.48 log.

Figure 1 shows that the growth of the probiotics in both samples have variations during the 20 days of storage. The first treatment had a slight growth from day 10 to day 20. In the case of the second one, after day 15 a state of latency is shown.

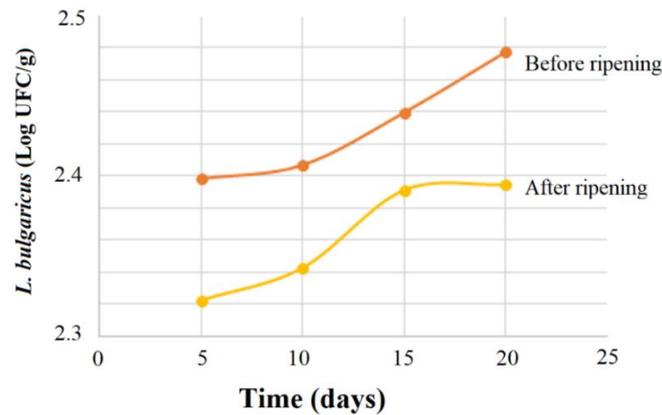


Figure 1. Growth of microencapsulated *L. bulgaricus* before and after the ripening of ice cream

Shelf life analysis

One of the most important factors of the perishability of ice cream is the development of moulds and yeasts that alter both their organoleptic and physicochemical properties. The microbiological analysis of moulds and yeasts was carried out on the ice creams during storage and those to which the microcapsules were added before ripening had less proliferation of moulds and yeasts was found (μ_{\max} : 0.413). As was expected, the reduction of the process temperature promotes the destruction and inactivation of cells and spores.

The microbiological shelf life of a food product can be defined by obtaining the kinetic parameters of microbial growth in the logarithmic phase, where the bacterial cells are healthy and stable from a physiological point of view [8]. The shelf life of the ice cream was determined by the Monod-Hinshelwood equation, taking into account that the safe population of moulds and yeasts in this type of products is 10^6 CFU / g.

The shelf life determined for ice creams without probiotics was 18 days, and this one for ice creams with probiotics (added before ripening) was 26 days. The addition of probiotics promoted an increase in the shelf life of ice cream by 44%. The results found can bring both economic and public health benefits to the ice cream industries.

Sensorial analysis of formulations

Figure 2 shows the scale of assessment obtained from the sensory panel for each ice cream with and without the microencapsulated probiotic concerning the sensory evaluation.

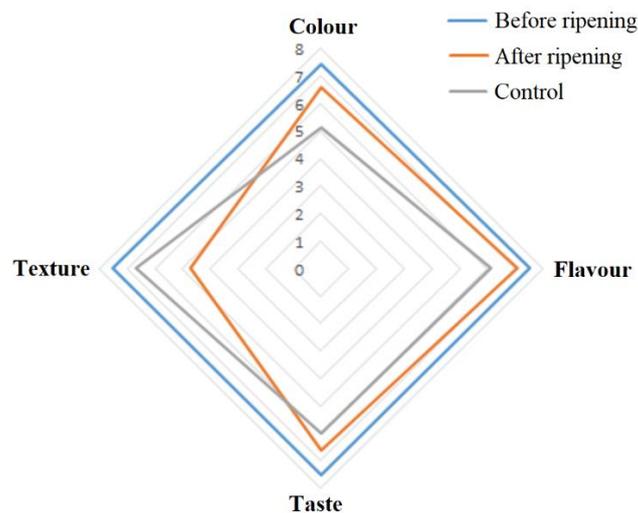


Figure 2. Mean values of sensory analysis of ice creams with and without microencapsulated *L. bulgaricus*

The analysis shows that the ice cream with higher acceptability was those to which the microcapsules were added before ripening, presenting higher values in colour, taste, flavour and texture than the other formulations.

Chemical composition

The chemical composition of the ice cream with higher acceptability was determined. Table 1 shows results of this analysis.

Table 1. Mean values and standard deviation of the chemical composition of ice cream with microcapsules added before ripening

Component	Content (%)
Water content	80 ± 1
Fat	10.3 ± 0.2
Protein	1.0 ± 0.2
Titrateable acidity (lactic acid)	0.22 ± 0.04
pH	7.1 ± 0.2

As can be seen, the pH is neutral, and this promotes the proliferation of probiotic bacteria. As was expected, the acidity of ice cream was around of 0.2 expressed as lactic acid percentage, according to with the activity of *L. bulgaricus* and exogenous lactic acid bacteria [9]. Regarding other components (water content, fat and protein), their values were consistent with ice cream formulation.

Conclusion

Incorporation of *Lactobacillus bulgaricus* microcapsules into ice cream before and after ripening was studied. In the first case, the ice cream had the higher acceptability compare to those added after ripening. After 20 days of storage, the probiotic bacteria showed the viability of 10^3 UFC/g; this value is not enough higher for been considered a functional food. On the other hand, the probiotic added before ripening had high survivor capacity because of they had better adaptation to food matrix.

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