

## Stability of *Bacillus coagulans* LBSC and Effect on Sensory Attributes in Tortillas, Tortilla Chips, and Chocolate

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### Abstract

*Bacillus coagulans* LBSC (DSM 17654) is a heat-stable spore-forming probiotic which has been found to provide several health benefits, such as alleviating gastrointestinal conditions. This study evaluated the stability of *Bacillus coagulans* LBSC in food products, including tortillas, tortilla chips, and chocolate with ganache, when added using conventional processes, without the need for additional equipment. *Bacillus coagulans* LBSC had good stability in the food products tested, which ranged from a survival rate of 42.0% to 98.4%. Each product was also compared to a control (no probiotic added) to determine if the added probiotic affected product quality. Foods with the added probiotic were rated similar in sensory attributes, when compared to their corresponding control. This research suggests that *Bacillus coagulans* LBSC is a promising probiotic for economically developing tortillas, tortilla chips, chocolate with ganache, and similarly processed products for gut health.

**Keywords:** probiotics, survival, viability, gut health, immunity, baked products.

**Study Area:** Food Microbiology.

## 1. INTRODUCTION

There is growing interest in foods with probiotics [1]. Research suggests that probiotics can help strengthen the immune system, reduce cancer risks, decrease cholesterol, prevent digestive disorders, and reduce gastrointestinal infections [1-8]. An imbalance in the gut microbiome can lead to several disease conditions. Probiotics offer a promising approach to restoring the normal intestinal environment and can be used to prevent antibiotic associated diarrhea [4,9]. Probiotics are defined by the World Health Organization as “live microorganisms which when administered in adequate amounts confer a health benefit on the host” [8]. *Lactobacilli* and *Bifidobacteria* are commonly used non-spore-forming probiotics, but they are sensitive to pH and cannot survive heat treatment. Spore-forming probiotics are more stable to heat and pH, and have better survival rates [1, 10].

*Bacillus coagulans* is a spore-forming probiotic which has been widely studied for human consumption. This probiotic can survive at high temperatures and acidic environments, and its probiotic activity and health benefits is dependent on the strain, dosage, final formulation, and medical conditions [1]. *B. coagulans* strain LBSC (DSM 17654) is a safe probiotic strain [2] that has been shown to be stable in acidic pH and bile [3], alleviate gastrointestinal conditions, such as acute diarrhea [4] and irritable bowel syndrome [5], and to reduce calories of foods under *in vitro* conditions suggesting that it could potentially help with management of obesity [6]. This probiotic strain can also help with healthy gut microbiome modulation [3]. The stability of *B. coagulans* LBSC was studied in beverages, frosting, frozen dairy desserts, condiments, cereal, and cough syrups [6], but research on the stability and effect of this probiotic on the organoleptic properties of corn tortillas, tortilla chips, and chocolate with ganache has not yet been published.

The aim of this study was to evaluate the stability of *B. coagulans* LBSC in tortillas, tortilla chips, and chocolate with ganache. Food products with and without *B. coagulans* LBSC were evaluated for taste and other sensory attributes.

## 2. MATERIALS AND METHODS

### 2.1 *B. coagulans* LBSC and viable count enumeration

*B. coagulans* LBSC ( $9.1 \times 10^9$  CFU/g) was supplied by Enzyme Innovation, Chino, CA. Viable spores of *B. coagulans* LBSC were enumerated based on a method by Bagkar et al. [6]. Viable spores of *B. coagulans* LBSC were enumerated based on the standard pour plate method. Briefly, 1 g of withdrawn samples was suspended in 0.1% peptone and serially diluted. Samples were heat shocked at 75°C for 30 minutes (mixed every 10 minutes), followed by immediate cooling down to below 45°C in a room temperature water bath. 1 ml of the heat-treated spore suspension was dispensed in Petri plates and mixed with molten PCA agar (52°C). The media were set to solidify and plates were incubated at 37°C for 48-72 h. *B. coagulans* LBSC was expressed in logarithm of colony forming units per gram (log CFU/g), using the mean of three independent analyses.

## 2.2 Stability of *B. coagulans* LBSC

*B. coagulans* LBSC was tested in tortillas, tortilla chips, and chocolate with ganache. Stability was evaluated by the survival rate of *B. coagulans* LBSC after processing and storage. The survival rate was calculated as % Survival Rate = ([CFU after processing] / g) / ([initial CFU] / g).

### 2.2.1 Tortillas

Tortillas were made using a method adapted from Bueso-Ucles [11] with modifications, using 0.2179g *B. coagulans* LBSC (SEB LBSC) per serving (2 tortillas). *B. coagulans* LBSC was pre-mixed into the water prior to adding to the dry ingredients to form the dough for more uniform distribution. Control tortillas were also made, where no probiotic was added. The dough was divided into 31g balls. Each dough ball was pressed with a tortilla press for 1 second. The tortillas were cooked at 135°C for 45 seconds each side. Tortillas were cooled to 23-24°C and stored in polyethylene bags at room temperature.

### 2.2.2 Tortilla chips

Tortilla chips were coated with oil (2.9% of the tortilla chips weight) and a dry blend containing maltodextrin and *B. coagulans* LBSC in a coating drum. 0.0792g of *B. coagulans* LBSC was added per serving (28.35g of tortilla chips). Samples were stored in polyethylene bags at room temperature.

### 2.2.3 Chocolate with ganache

Chocolate with ganache was made by forming the chocolate shell using chocolate with 54% cocoa content (90.7%), cocoa butter (9.1%), and soy lecithin (0.2%) which was melted to 48-57°C and tempered to 31-32°C, and making the ganache filling. The ganache filling was made by mixing the *B. coagulans* LBSC into heavy cream, and then bringing this heavy cream mixture to a simmer (90-93°C) and pouring over the chocolate to melt the chocolate. 0.0934g *B. coagulans* LBSC (SEB LBSC) was added per serving (one 43g chocolate bar which contained 14.4g ganache), and was compared to control chocolate with ganache (no probiotic added). The ganache filling was added to the chocolate shell and the filling was sealed with tempered chocolate.

## 2.3 Sensory Analysis

Each of the products (with and without *B. coagulans* LBSC) were evaluated for sensory by four sensory panelists using a hedonic rating scale of 1-9, where detailed written descriptions and instructions were provided for rating each sensory attribute. Samples were presented in random order to panelists and were labeled with randomized 3-digit codes.

### 2.3.1 Tortillas

Tortillas were re-heated in a 700 watt microwave for 45 seconds. Heated tortillas were stored in individual tortilla warmers for sensory analysis. Tortillas were rated on a scale of 1 to 9 for aroma (1=strong off-aroma, 5=neutral, 9=pleasant), softness (1=very rough, 5=neither rough nor soft, 9=very soft), rollability (1=unrollable/breaks

significantly, 9=stays completely intact when rolled without any cracks or breaks on the surface), tear strength (1=very rubbery, 9=very easy to tear), tenderness (1=very brittle and dry, 9=very tender), and taste (1=strong off-flavor, 5=neutral, 9=very pleasant).

### 2.3.2 Tortilla Chips

Tortilla chips were rated on a scale of 1 to 9 for appearance (1=dislike extremely, 5=neither like nor dislike, 9=like extremely), aroma (1=strong off-aroma, 5=neutral, 9=very pleasant), taste (1=strong off-flavor, 5=neutral, 9=very pleasant flavor), texture (1=not crispy/rubbery, 9=very crispy), and overall acceptability (1=dislike extremely, 5=neither like nor dislike, 9=like extremely).

### 2.3.3 Chocolate with Ganache

Chocolate with ganache was rated on a scale of 1 to 9 for appearance (1=pale/ chalky/ bloomed appearance, 9=shiny, dark brown color), aroma (1=strong off-aroma, 5=neutral, 9=sweet cocoa, fruity, slight bitter aroma), hardness (1=very soft, 9=very hard/crisp crack), mouthfeel (1=very grainy, 9=creamy/smooth/melts nicely), sweetness (1=very unsweet, 9=very sweet), flavor (1=strong off-flavor, 5=neutral, 9=bittersweet cocoa/slight fruity flavor), and overall likability (1=strongly dislike, 5=neither dislike nor like, 9=strongly like).

## 2.4 Data Analysis

A one-way ANOVA was used to analyze the data, and a Tukey's t-test was used for comparing the values using a program from Assaad et al. [12]. Differences were considered statistically significant at a p value < 0.05.

## 3. RESULTS AND DISCUSSION

### 3.1 *B. coagulans* LBSC Stability Results

As shown in Table 1, *B. coagulans* LBSC had good stability in the tortillas, tortilla chips, and chocolate. In tortillas, *B. coagulans* LBSC had 50.9% survival after processing and 2 weeks of storage at room temperature. In tortilla chips, *B. coagulans* LBSC had 98.4% survival when applied as a dry coating (mixed into maltodextrin) and after two weeks of room temperature storage. In chocolate with ganache where the probiotic was added to the ganache filling, *B. coagulans* LBSC had 43.6% survival after processing and two weeks of room temperature storage. The higher survival rate of *B. coagulans* LBSC in tortilla chips was expected since there was no heating step after applying the probiotic to the tortilla chips. A lower survival rate is expected for the tortillas and chocolate with ganache since these foods were heated. For the chocolate with ganache, *B. coagulans* LBSC could be added during tempering of the chocolate shell instead of in the ganache filling to minimize heat exposure to increase its survival rate. This study tested the probiotic in the ganache filling, which is cooked at a higher temperature, to evaluate the stability of *B. coagulans* LBSC in a more extreme cooking condition.

**Table 1.** Stability of *B. coagulans* LBSC in tortillas, tortilla chips, and chocolate ganache.

Product	<i>B. coagulans</i> LBSC initial count (log CFU/g)	<i>B. coagulans</i> LBSC after processing & storage (log CFU/g)	% Survival	Storage Conditions
Corn Tortillas	7.505 ± 0.020 <sup>a</sup>	7.212 ± 0.052 <sup>b</sup>	50.9%	2 weeks at room temperature
Tortilla Chips	7.405 ± 0.015 <sup>a</sup>	7.398 ± 0.043 <sup>a</sup>	98.4%	2 weeks at room temperature
Chocolate Ganache	7.771 ± 0.020 <sup>a</sup>	7.410 ± 0.037 <sup>b</sup>	43.6%	2 weeks at room temperature

Values are means ± SEM, n = 3 per treatment group.

Means in a row without a common superscript letter differ ( $p < 0.05$ ) as analyzed by one-way ANOVA and the TUKEY test.

These stability results are especially promising because *B. coagulans* LBSC was added using conventional processes for the food applications tested, and did not require additional non-standard equipment such as spraying equipment. Thus, this probiotic can be used at a lower cost while still providing good stability, so that food products can be developed economically to include sufficient quantities for providing a health benefit.

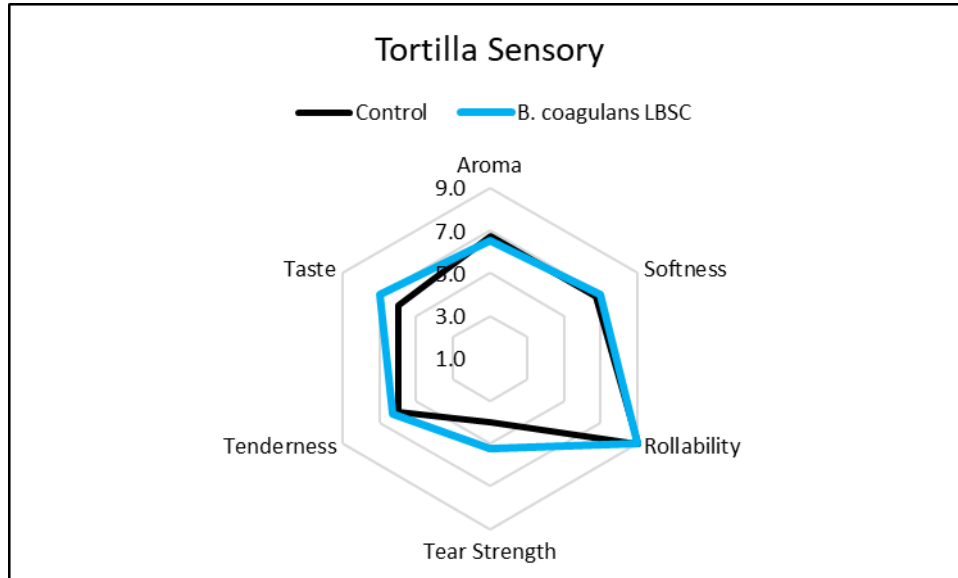
## 3.2 Sensory Analysis

### 3.2.1 Tortillas

Figure 1 shows tortillas made with and without *B. coagulans* LBSC. Table 2 and Figure 2 are a summary of the tortilla sensory data. Tortillas with *B. coagulans* LBSC were rated similar in appearance, aroma, softness, rollability, tear strength, tenderness, and taste to the control tortillas (no probiotics added).



**Figure 1.** Control tortillas (left) and tortillas with *B. coagulans* LBSC (right).



**Figure 2.** Tortilla sensory data.

**Table 2.** Sensory data for control tortillas and tortillas with *B. coagulans* LBSC.

<b>Attribute</b>	<b>Control</b>	<b><i>B. coagulans</i> LBSC</b>
Aroma	6.7 ± 0.8 <sup>a</sup>	6.5 ± 1.0 <sup>a</sup>
Softness	6.7 ± 0.3 <sup>a</sup>	7.0 ± 0.4 <sup>a</sup>
Rollability	6.7 ± 0.3 <sup>a</sup>	7.0 ± 0.4 <sup>a</sup>
Tear Strength	6.7 ± 0.3 <sup>a</sup>	7.0 ± 0.4 <sup>a</sup>
Tenderness	6.7 ± 0.3 <sup>a</sup>	7.0 ± 0.4 <sup>a</sup>
Taste	6.7 ± 0.3 <sup>a</sup>	7.0 ± 0.4 <sup>a</sup>

Values are means ± SEM,  $n = 4$  per treatment group.

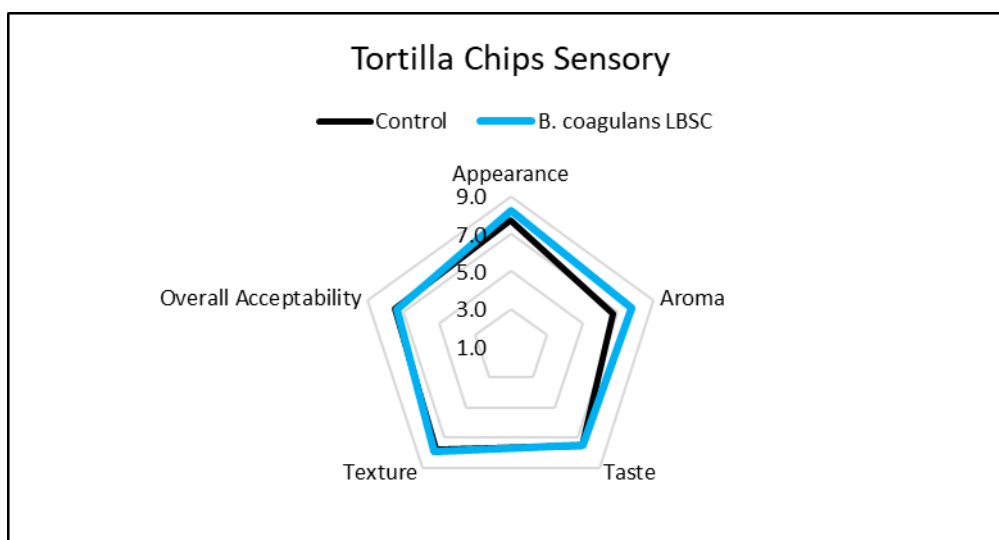
Means in a row without a common superscript letter differ ( $p < 0.05$ ) as analyzed by one-way ANOVA and the TUKEY test.

### 3.2.2 Tortilla Chips

Figure 3 shows tortilla chips made with and without *B. coagulans* LBSC. Table 3 and Figure 4 are a summary of the tortilla chips sensory data. Tortilla chips with *B. coagulans* LBSC were rated similar in appearance, aroma, taste, texture, and overall acceptability to control tortilla chips (no probiotics added).



**Figure 3.** Control tortilla chips (left) and tortilla chips with *B. coagulans* LBSC (right).



**Figure 4.** Tortilla chips sensory data.

**Table 3.** Sensory data for control tortilla chips and tortilla chips with *B. coagulans* LBSC.

Attribute	Control	<i>B. coagulans</i> LBSC
Appearance	7.7 ± 0.8 <sup>a</sup>	8.2 ± 0.5 <sup>a</sup>
Aroma	6.7 ± 1.0 <sup>a</sup>	7.7 ± 0.8 <sup>a</sup>
Taste	7.5 ± 1.0 <sup>a</sup>	7.5 ± 0.5 <sup>a</sup>
Texture	7.7 ± 0.8 <sup>a</sup>	7.8 ± 0.4 <sup>a</sup>
Overall Acceptability	7.5 ± 0.6 <sup>a</sup>	7.3 ± 0.2 <sup>a</sup>

Values are means ± SEM, n = 4 per treatment group.

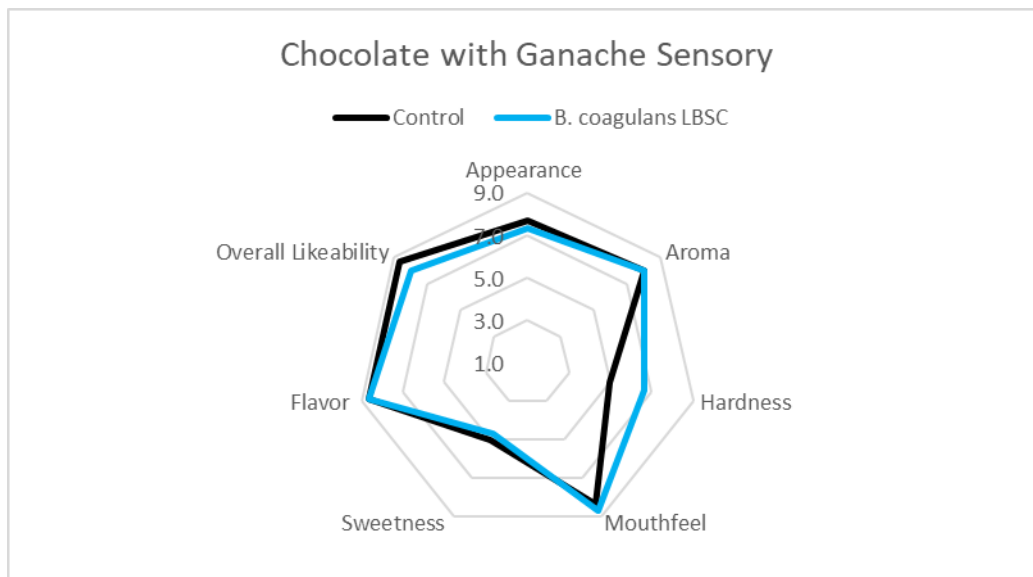
Means in a row without a common superscript letter differ ( $p < 0.05$ ) as analyzed by one-way ANOVA and the TUKEY test.

### 3.2.3 Chocolate with Ganache

Figure 5 shows chocolate with ganache made with and without *B. coagulans* LBSC. Table 4 and Figure 6 are a summary of the chocolate sensory data. Chocolate with ganache made with *B. coagulans* LBSC were rated similar in appearance, aroma, hardness, mouthfeel, sweetness, flavor, and overall likeability to control chocolate with ganache (no probiotics added).



**Figure 5.** Control chocolate (left) and chocolate with *B. coagulans* LBSC (right).



**Figure 6.** Chocolate sensory data.



**Table 4.** Sensory data for control chocolate and chocolate with *B. coagulans* LBSC.

Attribute	Control	<i>B. coagulans</i> LBSC
Appearance	7.7 ± 0.5 <sup>a</sup>	7.5 ± 0.6 <sup>a</sup>
Aroma	7.5 ± 0.9 <sup>a</sup>	7.5 ± 0.6 <sup>a</sup>
Hardness	5.7 ± 1.1 <sup>a</sup>	7.0 ± 1.4 <sup>a</sup>
Mouthfeel	8.5 ± 0.3 <sup>a</sup>	8.7 ± 0.3 <sup>a</sup>
Sweetness	5.3 ± 0.4 <sup>a</sup>	4.7 ± 0.3 <sup>a</sup>
Flavor	8.7 ± 0.3 <sup>a</sup>	8.7 ± 0.3 <sup>a</sup>
Overall Likeability	8.5 ± 0.3 <sup>a</sup>	8.2 ± 0.5 <sup>a</sup>

Values are means ± SEM, n = 4 per treatment group.

Means in a row without a common superscript letter differ ( $p < 0.05$ ) as analyzed by one-way ANOVA and the TUKEY test.

#### 4. CONCLUSIONS

*B. coagulans* LBSC had good stability in tortillas, tortilla chips, and chocolate with ganache without affecting their sensory attributes. This study suggests that *B. coagulans* LBSC is a promising probiotic for use in a variety of food products to provide health benefits without affecting taste.

#### 5. ACKNOWLEDGMENT

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