eISSN: 1984-3909
Coffee Science, e192178, 2024

Doi: https://doi.org/10.25186/.v19i.2178

Evaluation of *Lactobacillus Coagulans* in the design of coffee-based probiotic beverages by using different preparation methods

Angela María Ormaza-Zapata[1], Sneyder Rodriguez- Barona[2], Félix Octavio Díaz-Arango[1]

[1]Universidad de Caldas, Departamento de Engenharia, Faculdade de Engenharia, Manizales, Caldas, Colombia
[2]Universidad Nacional de Colombia, Sede Manizales, Departamento de Engenharia Química, Faculdade de Engenharia e Arquitetura, Manizales, Caldas, Colombia
[3]Universidad de Caldas, Departamento de Engenharia, Faculdade de Engenharia, Manizales, Caldas, Colombia
Contact authors: angela.ormaza@ucaldas.edu.co, snrodriguezba@unal.edu.co, felix.diaz@ucaldas.edu.co
Received in October 7, 2023 and approved in January 29, 2024

ABSTRACT

The purpose of the presented work was to assess the viability of microcapsules containing *Lactobacillus coagulans,* incorporated into coffee-based beverages; using hot and cold water preparation methods. These microcapsules were produced through spray drying and employed two different combinations of wall materials, based on coffee extract (EXT), maltodextrin (MD), and inulin (INU). The evaluation was carried out within the context of creating coffee-based beverages using nine distinct preparation methods. Various characteristics of the microcapsules were measured, including solubility, wettability, hygroscopicity, moisture content, and water activity. Sensory analysis was used to determine the proportion of probiotic incorporation in the roasted and ground coffee. Moreover, the viability and count of probiotics were quantified in the nine preparations, both in cold and hot water. Among the preparation methods, the Chemex approach demonstrated enhanced viability when utilized for hot water preparation, while the Cold Tower method excelled in cold water scenarios. The outcomes of the study underscore the considerable potential of *Lactobacillus coagulans,* with microcapsules consisting of MD-INU:EXT (50:50); in developing coffee-based probiotic beverages in hot and cold water, with remarkable chemical, sensory and functional stability.

Key words: Coffee extract; Cold coffee; Functionality; Microencapsulation.

1 INTRODUCTION

Coffee holds a venerable status as a traditional, economically pivotal, and socially significant crop within the Colombian agricultural landscape. On a global scale, it commands a market share of 10.75% (International Coffee Organization - ICO, 2018). In this sense, the inclusion of differentiating features that align with emerging market trends for traditional products like coffee becomes paramount (Federação Nacional das Cooperativas de Crédito - FNCC, 2014; International Coffee Organization - OIC, 2015). In the year 2021, Colombia recorded a production of 12.6 million 60-kilogram bags of green coffee, marking a 9% decline in comparison to the preceding year's harvest of 13.9 million bags. The final month of 2021 observed a further decrease, with production dwindling to 1.4 million bags—a 21% drop from the 1.7 million bags documented in the corresponding period of 2020. Similarly, production in 2021 experienced a substantial decline of 19%, totaling 3.5 million bags as opposed to the 4.3 million bags harvested during the corresponding period in the previous year. This decline in production was further accompanied by a marginal 1% decrease in exports throughout 2021 when compared to the export figures of the preceding year (Coffee Production in The Country IN 2021 WAS 12.6 Million Bags, 2021). In light of these circumstances, a strategy for value-added coffee-based products must be formulated. This strategy should not only boost exports and enhance marketing prospects but also bolster income generation for producers.

A notable shift towards the consumption of health-conscious foods, aimed at preserving both physical and mental well-being, has emerged. This shift has precipitated the proliferation of probiotic food products within the market (Zendeboodi et al., 2020). A transformation in product development paradigms is evident, emphasizing reduced allergens and additives, coupled with a heightened resemblance to their natural sources (Aschemann-Witzel; Varela; Peschel, 2019). While a significant proportion of available probiotic foods are presently confined to chilled dairy items, a discernible surge in non-dairy probiotic product offerings has emerged. These encompass an array of options such as meat, fruit, legume products, baked goods, mayonnaise, and chocolate-based products. This diversification serves as an alternative, addressing concerns surrounding dairy protein allergies, saturated fat intake, lactose intolerance, cholesterol consumption, and other related considerations (De Prisco; Mauriello, 2016; Cunha Júnior et al., 2023).

For a food product to qualify as probiotic, it requires containing live microorganisms in a specific concentration, substantial enough to modify the host's microflora through colonization or implantation. Furthermore, these microorganisms should have the potential to bestow beneficial health effects upon the host, irrespective of the host's initial state (Zielińska; Kołożyn-Krajewska, 2018). A noteworthy aspect to consider is that the food matrices must support the survival of probiotics as they traverse the gastrointestinal tract and facilitate their successful colonization (De Prisco; Mauriello, 2016). Hence, the choice of appropriate food systems for

administering probiotics stands as a pivotal determinant in crafting functional probiotic foods (Tripathy; Giri, 2014; De Prisco; Mauriello, 2016). *L. Coagulans*, notably, has gained traction for its application in functional products centered around fruits, vegetables, grains, and even within the domain of infant nutrition (Cardona-Arengas; López-Marín, 2019).

The focal point of the current study revolved around the formulation and design of probiotic beverages based on coffee. The methodology encompassed both cold and hot preparation methods. The design of each probiotic prototype was orchestrated to ensure harmlessness, a consumer-friendly sensory profile, and a harmonious balance of physicochemical variables with the microcapsules. These elements collectively contributed to maintaining the viability of the probiotic strain in line with prevailing Colombian regulations (Ministry of Social Protection - MPs, 2021) for it to qualify as a marketable probiotic food. Recent endeavors have undertaken the development of probiotic beverages across diverse food matrices. These endeavors have culminated in functional, ready-to-consume products that feature an array of probiotic strains, with or without lactic fermentation (Álvarez; Lamas, 2021; Souza et al., 2022; Cunha Júnior et al., 2023). Technologically speaking, it's noteworthy to emphasize that one of the most extensively employed techniques for probiotic microencapsulation is spray drying. Its efficacy in preserving microorganism viability and its cost-effectiveness render it a preferred choice (Tripathy; Giri, 2014).

Similarly, within the scope of this study, the creation of Cold coffee-based probiotic beverages has been considered. This approach aligns with the global trend in coffee consumption, aiming to encompass diverse sensory profiles by exploring various preparation methods for this beverage (Angeloni et al., 2019). Simultaneously, the intention was to optimize probiotic stability and the viability of encapsulated probiotic microorganisms. This pursuit of developing cold coffee-based beverages is geared towards enhancing the resilience and steadiness of the incorporated probiotic strain within the coffee formulations. For this reason, Lactobacillus coagulans was chosen for its well-known properties of thermoresistance, sporulation and stability at acidic pH (Palop et al., 1996), as in coffee. In addition, L. coagulans has the ability to produce lactic acid and spores, with a high ability to resist stomach acids with a good colonization rate in the intestine (Chaudhari et al., 2022).

The significance of this research's progression becomes evident, as it amalgamates insights into bacteria stabilization with probiotic potential and the application of microencapsulation technologies. This amalgamation allows for the design and formulation of probiotic products centered around coffee. The journey entails meticulous oversight over technological, functional, and sensory attributes of the probiotic prototype. This attention to detail aims at enabling mass production and creating a vantage point founded on scientific and technological understanding. This vantage point, in turn, generates value for both traditional and widely consumed items like coffee beverages.

In the course of this research's development, spray drying was leveraged as the microencapsulation technique for *L. coagulans*. Moreover, two distinct combinations of wall materials were assessed to safeguard microorganisms against the impact of water temperature during the coffee beverage preparation process. This entailed evaluating beverage preparation through diverse cold and hot methods. The aim was to scrutinize the microbiological viability and chemical stability of encapsulated B. coagulans and to evaluate the sensory profiles of the formulated probiotic coffee beverages.

2 MATERIAL AND METHODS

The study utilized parchment coffee (*Coffea arabica*) of the Castillo variety, sourced from the municipality of Palestina in the Caldas department. The coffee was subjected to threshing and classification, specifically retaining grains that passed through the number 14 mesh sieve (designating excelso quality/ U.G.Q.). The coffee extract (EXT) was produced at the Freeze-Dried Coffee Factory (Chinchiná, Caldas), from medium roasting, 30-35 °Brix and coarsely ground Prebiotic substances, namely chicory inulin (INU) and maltodextrin (MD), were procured from the commercial company Tecnas S.A. The biomass of the probiotic strain *Lactobacillus coagulans* was obtained through the commercial company Annar S.A. The strain was cultivated using microaerophilic fermentation on MRS agar medium (Scharlab, Italy) within a 2L bioreactor of the Ez-control brand (Applikon Biotechnology, Netherlands). The recovered biomass was obtained through centrifugation (8000 g) and subsequently washed in a saline solution (1%) (Fritzen-Freire et al., 2012).

2.1 Formulation of mixtures for the microencapsulation of *L. coagulans* from soluble coffee

Two distinct wall material formulations were employed, both incorporating coffee extract (EXT), maltodextrin (MD), and inulin (INU). The first formulation, denoted as MD-INU:EXT, featured a ratio of 50:50 between maltodextrin and inulin, combined with the coffee extract. The second formulation, labeled as EXT-Water, combined the coffee extract with water in a 60:40 ratio. These formulations were determined based on preliminary growth and expected viability tests of B. coagulans, the colour of the mixture obtained and the effect of the flavour given by the microcapsules (wall material and L. coagulans) to the beverage.

Both of these formulations were infused with a concentration of *Lactobacillus coagulans* at 1 x 10^8 CFU/mL.

2.2 Encapsulation of probiotic microorganisms by spray drying

A Mini Spray Dryer model (B-191, Büchi Labortechnik AG, Switzerland) was employed for the encapsulation process. The air inlet was maintained at temperatures of 80 ± 3 °C. The process incorporated a suction percentage of 75%, a pump yield of 0.05%, a feed flow rate of 6 mL/min, and an air flow rate of 600 nNL/h (equivalent to 90 mL/h). The resultant encapsulated probiotic powders were securely stored in medium-barrier metallized bags, specifically utilizing metallized BOPP/PA/PE packaging material. These bags were subjected to vacuum packaging and then stored under refrigeration conditions, maintaining a temperature of 5°C ± 2, until they were utilized. The evaluation of encapsulation performance was carried out subsequent to the encapsulation process, as outlined in previous research (Fritzen-Freire et al., 2012).

2.3 Probiotic Coffee Drink Formulation from Roasted and Ground Coffee

The roasting process was performed at 180°C and 100% power, in a laboratory-scale roaster (Quantik, TC-150 A/R, Armenia, Colombia). The milling of the roasted samples was carried out in a mill (Grindmaster 810, Mexico); The degree of grind was defined according to the coffee brewing method. .The roasted and ground coffee (U.G.Q.) were combined in a dry state and arranged in bulk within a powder mixer (PerMix, China). This amalgamation was performed alongside the previously microencapsulated material. The amount of encapsulated material introduced was adjusted to correlate with the viability percentage ascertained post-encapsulation. This adjustment was made while consistently ensuring that the initial count subsequent to the addition exceeded 1 x10^8 CFU/mL. This correlation was established in congruence with the inherent sensory attributes characteristic of a coffee-based beverage, established from sensory ratings, closer to a U.G.Q. coffee; which correspond to ratings by attribute, as follows: Fragrance of 8, aroma of 8, acidity of 5, bitter of 5, body of 7, residual flavor of 1 and overall impression of 8 (Instituto Colombiano de Normas Técnicas y Certificación - ICONTEC, 2000).

2.4 Assessment of the viability of the probiotic strain

To assess the impact of the prebiotic agents (INU, MD, and EXT) within the formulated beverages, the quantification of viable cells was conducted. This quantification transpired the day after microencapsulation, as well as following the cooling (to 21°C) of beverages prepared using hot water temperatures of 55, 70, and 90°C. Furthermore, this analysis was performed after the cold beverages (initially within the range of 4-10°C) attained room temperature (21°C).

The assessment of viable microorganism counts was executed in triplicate, and the results were expressed as Log CFU/mL in dry basis (d.b.). This assessment involved performing counts on 1 g of encapsulated material, which was suspended in 9 mL of 0.1% buffered peptone water. This mixture was vortexed and allowed to rest for 30 minutes, facilitating the release of the microorganisms (Fritzen-Freire et al., 2012). Subsequently, a 100 µL volume of the diluted solution was surface-seeded onto MRS solid medium with aniline blue (0.1%). The seeded medium was then incubated under microaerophilic conditions at 37°C for a duration of 48-72 h (Semyonov et al., 2010). Viability stability criteria were employed, ensuring that the count of viable cells did not fall below$_{106}$ CFU/mL, and factors such as CH (color intensity) and a_w (water activity) were taken into consideration.

2.5 Microbiological Viability of microencapsulated probiotic microorganism by spray drying

The percentage of Microbiological viability was assessed at regular intervals, including post-encapsulation and once the probiotic beverages (both hot and cold) reached room temperature (21 °C). This assessment involved quantifying the CFU/mL within the *L. coagulans* microcapsules produced through the spray drying process. The calculation of percentage viability was conducted according to equation 1, as described in previous studies (Fritzen-Freire et al., 2012; Rodríguez-Barona; Montes; Ramírez, 2012).

$$\%Viability = \left(\frac{N}{N_0}\right) x100 \quad (1)$$

Log N and Log N_0, correspond to the number of viable cells encapsulated (or live cells within the microcapsules) and inoculated cells (live cells prior to the completion of the microencapsulation process), respectively. This follow-up was carried out to counts not less than 10^6 CFU/mL, in alignment with prevailing regulations.

2.6 Determination of moisture content and water activity of the material microencapsulated with *L. coagulans*

Moisture content (MC) was assessed using 1 g of the sample through an MOC-120H infrared moisture analyzer balance (Shimadzu, Japan) at a temperature of 100 °C. Meanwhile, water activity (a_w) measurements were conducted

using a Thermoconstanter TH200 dew point hygrometer (Novasina, Switzerland). These determinations were executed in triplicate to ensure accuracy and reliability.

2.7 Solubility

To determine the solubility of the microcapsules, 1 g of sample was dissolved in 100 mL of distilled water, as described by Cano-Chauca et al. (2005). The solution was placed on a C-MAG H57 heating plate (IKA Labortechnik, Staufen, Germany) at a temperature of 30 ± 2 °C, with continuous stirring at ١٢٠ rpm for a duration of ٥ minutes. The suspension was then subjected to centrifugation at ٣٠٠٠ rpm for ٥ minutes. A ٢٥ mL portion of the liquid located above the sediment (supernatant) was carefully extracted and transferred to a pre-weighed Petri dish. The Petri dish with the supernatant was placed in a forced convection oven (Binder, Fisher Scientific, Germany) at a temperature of ١٠٥ °C for a period of ٥ hours, until a constant weight was achieved. The Petri dish with the supernatant was placed in a forced convection oven (Binder, Fisher Scientific, Germany) at a temperature of ١٠٥ °C for a period of ٥ hours, until a constant weight was achieved. The solubility was calculated using equation 2.

m_i is 0.25 g. This determination was made in triplicate.

2.8 Hygroscopicity

One gram of *L. coagulans* microencapsulation powder was carefully positioned on a mesh with fine pores. This setup was established to prevent any unintended release or leakage of the material. This assembly was placed on an airtight glass container with a saturated NaCl solution (75.3% RH) at 25°C. Following a week-long period, the samples were removed from the container and weighed. Hygroscopicity was quantified as g moisture adsorbed per 100 g dry solids (g H_2O/ 100 g d.s.) (Fritzen-Freire et al., 2012). This determination was made in triplicate.

2.9 Wettability

The procedure was conducted in a cuboid-shaped static wetting device, modified by Rodríguez-Barona et al. (2012). The wetting time was determined in s and corresponds to the time taken for the complete immersion of 1 g of powder, gently deposited onto the surface of 100 mL of water at 20 °C. This determination was made in triplicate.

2.10 Determination of the minimum percentage of incorporation of the probiotic strain

Three levels of concentrations of microcapsules containing *L.coagulans*, with 10, 20 y to 30% of incorporation, were examined within U.G.Q. Coffee (With medium roasting, and grinding according to the preparation method), respectively. The evaluation centered around establishing a sensory profile that garnered high acceptance, drawing parallels with the attributes of U.G.Q. coffee (ICONTEC, 2000). These evaluations were carried out in triplicate.

2.11 Sensory analysis

The attributes encompassing fragrance, aroma, acidity, bitterness, body, residual flavor, and overall impression of the coffee cup (roasted and ground coffee infused with the incorporated probiotic microorganism) were evaluated through the methodology outlined in NTC-4883 (Q.D.A) (ICONTEC, 2000). These assessments were carried out in triplicate, aiming to examine various concentrations of the wall material formulations containing the probiotic. These formulations were blended with roasted and ground coffee, with the intention of achieving a sensory profile akin to that of an excelso coffee (Fragrance: 8, Aroma: 8, Acidity: 5, Bitter: 5, Body: 7, Residual Taste: 1 and Overall Impression: 8).

2.12 Preparation of coffee-based beverages

Upon establishing the optimal percentage of microcapsule incorporation into the roasted and ground coffee, the subsequent step involved the preparation of coffee beverages using nine distinct methods. These methods were divided into five that employed hot water and four that utilized cold water. The roasted and ground coffee was mixed with the microcapsules in a dry state. This ensured the viability of the intended strain of microorganisms. The mixture obtained from the dry mixing was dosed as per the specific methodology associated with each coffee preparation method. This involved considerations such as the ratio of coffee to microcapsules, the amount of water used, and the grinding level. The hot water preparation methods were assessed across three different temperature points: 55, 70, and 90°C. On the other hand, the methods utilizing cold water were executed at temperatures as specified by the manufacturer of each method.

The preparation procedures for the hot coffee beverage methods are described below:

2.13 French press

Coarse ground coffee (≥ 700 µm) with *L. coagulans* microcapsules was utilized and combined with hot water at 90 °C for 4 min. The ratio used was 14 g of coffee-microcapsule mixture with *L. coagulans* solubilized in 150 mL of water. Every 2 minutes, the mixture was stirred with a contact time of 4 minutes (Ormaza-Zapata; Díaz-Arango; Rojano, 2019). The same preparation was repeated with water temperatures of 55 and 70 °C, respectively.

2.14 Japanese siphon

This beverage was prepared with 21 g of coffee with microcapsules with *L. coagulans,* with a medium grind (501–700 µm) and 225 mL of hot water (90 °C). The water was deposited in

the round-bottomed glass containerwith an alcohol burner beneath it. The *L. coagulans*coffee-microcapsule mixture was placed in a glass funnel, with a cloth filter inside and over the round-bottomed in a glass funnel with a cloth filter. The burner was on. The water boiled in the round-bottomed container and traveled up the funnel, and came into contact with the roasted, microcapsule-ground coffee with *L. coagulans*. It was mixed manually, with a coffee spoon, and the water went up the funnel for 1 min. At that time, the microcapsule coffee with *L. coagulans* was fully brewed, but still with a quantity of ground coffee. The round-bottomed glass jar was removed from the burner. It was allowed to cool and the brewed coffee was collected through gravity filtration using the fabric filter of the funnel, into the round-bottomed glass container. Extraction time was 1 min (Ormaza-Zapata; Díaz-Arango; Rojano, 2022a). This preparation was also carried out with the water at a temperature of 55 and 70 °C, respectively.

2.15 Chemex

A medium grind coffee (501–700 µm) was used. Exactly 14 g of microcapsule coffee with *L. coagulans* was mixed with 150 mL of water. The contact time with water at 90°C is 4 min. The Chemex paper filter (FS-100) was placed inside the glass cone, hot water was poured over the coffee, and the filtration process was allowed to conclude (Ormaza-Zapata; Díaz-Arango; Rojano, 2022a). The same preparation was repeated with water temperatures of 55 and 70 °C, respectively.

2.16 Expresso

A total of 14 g of coffee with microcapsules containing *L. coagulans* (400 µm), solubilized in 75 mL of hot water at 90 °C with a fine grind, was used. The extraction time took only seconds. An Expresso machine was used to control the machine pressure, temperature and extraction time (Ormaza-Zapata; Díaz-Arango; Rojano, 2019). This preparation was repeated with water temperatures of 55 and 70 °C, respectively.

2.17 American

With this coffee maker, a medium grind roast coffee (501–700 µm) was utilized. For the extraction, a paper filter was employed, adhering to the specifications of the drip coffee maker. Exactly 21 g of coffee roasted and ground with *L. coagulans* microcapsules in 225 mL of hot water (90 °C) were used. The contact time between roast and ground coffee and water was 3 minutes (Illy, 2016; Ormaza-Zapata; Díaz-Arango; Rojano, 2022a). This procedure was also repeated with water temperatures of 55 and 70 °C, respectively.

The preparation procedures for cold coffee beverage methods are described below:

2.18 Cold tower

Coarse ground roasted coffee (701-900 µm) was utilized. The contact time with water at 4°C (± 0.2), was 12 h. Exactly 24 g of coffee was mixed with microcapsules of *L. coagulans* with 240 mL of water (Ormaza-Zapata; Díaz-Arango; Rojano, 2022b).

2.19 Mizudashi

For this method, 100 g of microcapsule roasted coffee containing *L. coagulans*, with medium grinding (500-700 µm) in 500 g of ice inside a plastic mesh and 500 mL of cold water at 10 °C were used. This method is provided with a filtration system, below this an ice container placed inside a jar, the coffee mixture underwent cold filtration for a span of 24 h, while stored in refrigeration at 4 °C (Ormaza-Zapata; Díaz-Arango; Rojano, 2022b).

2.20 Toddy

For this method, 100 g of coffee with microcapsules with *L. coagulans*, with medium grinding (500 -700 µm) mixed with 500 mL of cold water and 500 g of ice were used. The three ingredients were mixed in a plastic container. The contact time was 24 h of storage in the refrigerator at 4 °C. This content was passed through a porous filter using gravity to a glass jar, which was attached to a plastic container, to obtain the final drink (Ormaza-Zapata; Díaz-Arango; Rojano, 2022b).

2.21 Fretta

This method featured a reservoir that housed ice. The coffee interacted with cold water and fell dropwise onto the ice through a Melitta #4 paper filter (Melitta, Germany). Specifically, 14 g of medium grind coffee (701-900 µm) were mixed with microcapsules with *L. coagulans* and 150 mL of water, at 4 °C (± 0.2). The entire coffee-water contact extended to 4 h maintaining refrigerated conditions (Ormaza-Zapata; Díaz-Arango; Rojano 2022b).

2.22 Statistical analysis

An experimental design based on a one-factor approach was employed in this study, with each measurement being replicated three times. The experimental units were assessed longitudinally, capturing data over time. The outcomes were then presented as the mean value along with the standard deviation of the determined replicates. For data analysis, an ANOVA (Analysis of Variance) was conducted. Statistical significance was considered when the p-value was less than 0.05 ($p<0.05$).

3 RESULTS

3.1 Viability and physicochemical parameters of the probiotic strain within the wall material mixtures evaluated

The percentage viability of the probiotic strain, along with MC, a_w, hygroscopicity, solubility and wettability of the

microcapsules containing *L. coagulans* inside, and produced through spray drying, were assessed. The results are presented in Table 1. The spray-drying process yielded microcapsules with a viability exceeding 99%. The wall material formulation demonstrating the highest viability (%) was EXT-Water (60:40). This emphasizes the potential of EXT as a suitable wall material for microencapsulation of *L. coagulans*. ANOVA (Analysis of Variance) revealed no significant difference ($p > 0.05$) in viability between the *L. coagulans* microcapsules and the wall material mixture used. Concerning MC of the wall material formulations, values consistently remained below 4% humidity, with the EXT-Water formulation exhibiting the highest MC. Generally, the MC values achieved by the evaluated wall material formulations indicated that the *L. coagulans* microcapsules possessed a low humidity content, placing them within the realm of high safety products. This characteristic reduces the risk of chemical or biological degradation (Esquivel-Gonzalez; Ochoa-Martinez; Rutiaga-Quiñones, 2015). ANOVA showed no significant difference ($p > 0.05$) between the MC and the two evaluated wall formulations. As for the a_w, the microcapsules consistently demonstrated values below 0.5, with the EXT-Water formulation also displaying the highest a_w value. ANOVA revealed a significant difference ($p < 0.05$) in a_w among the wall formulations due to their composition. Hygroscopicity varied considerably among the wall formulations, with the EXT-Water (60:40) formulation boasting the highest hygroscopicity value. ANOVA indicated a significant difference ($p < 0.05$) in hygroscopicity due to the varying compositions of the wall formulations. In terms of capsule solubility, both formulations generally exhibited favorable values for this functional property. The EXT-Water (60:40) formulation displayed the highest solubility value, with the MD-INU:EXT (50:50) formulation slightly lower in this aspect. ANOVA revealed no significant difference ($p > 0.05$) in the solubility of the evaluated wall formulations. Regarding wettability, all values were under 12 seconds, and the EXT-Water (60:40) formulation exhibited the shortest time for complete wetting in water. ANOVA indicated a significant difference ($p < 0.05$) in wettability among the evaluated wall formulations.

Table 1: Viability and physicochemical parameters of the probiotic strain within the wall material mixtures evaluated.

Parameters	MD-INU: EXT (50:50)	EXT-Water (60:40)
Viability (%)	99.3 [a]	99.6 [a]
MC	2.98 [a]	3.77 [a]
a_w	0.31 [a]	0.45 [b]
Solubility	95.1 [a]	99.7 [a]
Hygroscopicity (g H_2O/100 g d. s.)	19.3 [a]	33.2 [b]
Wettability (s)	11.4 [a]	7.6 [b]

Average values (n=3) with different letters (a – b) in the same row indicate statistical differences at the 5% significance level ($p < 0.05$).

3.2 Determination of the Optimal Probiotic Strain Incorporation Percentage in Formulated Coffee-Based Beverages

Figures 1a, 1b, and 1c illustrate the sensory profile of coffee-based beverages, formulated with microcapsule incorporation percentages of 10, 20, and 30% relative to the MD-INU:EXT (50:50) wall material mixture and roasted ground coffee, respectively. The sensory analysis conducted indicates that elevating the microcapsule concentration leads to enhanced aroma, body, acidity, and overall impression of the beverage, culminating in a sensory profile akin to the U.G.Q. pattern. The resultant probiotic drink exhibits a robust aroma and a pronounced equilibrium between bitter and acidic notes, particularly when utilizing a 30% microcapsule proportion alongside the probiotic strain, in conjunction with roasted and ground coffee. This concentration is identified as the optimal blend, producing a harmonious sensory experience, In relation to the sensory qualification of the pattern U.G.Q.

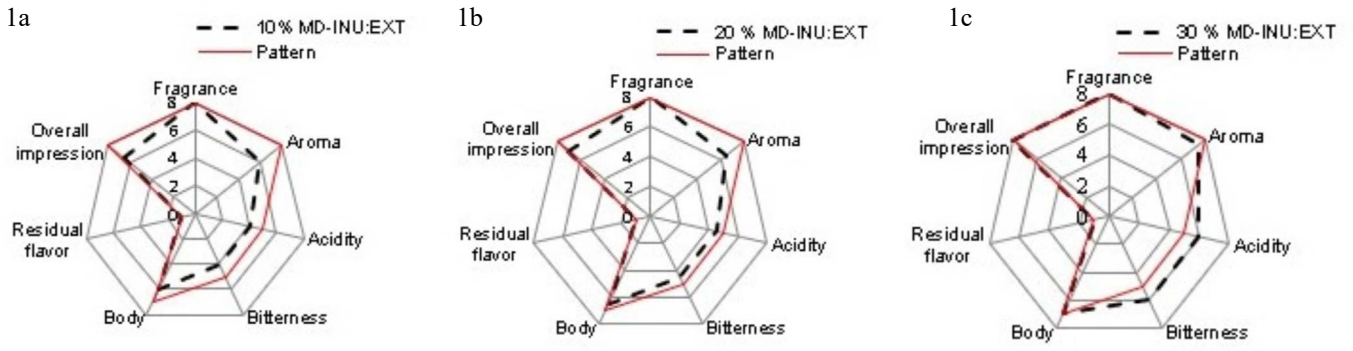

Figure 1: Percentage of incorporation of microcapsules formed with MD-INU:EXT (50:50) with roasted and ground coffee at a) 10% b) 20% and c) 30% of wall materials

Figures 2a, 2b, and 2c depict the sensory profile of coffee-based beverages created using microcapsule proportions of 10%, 20%, and 30% in relation to the EXT-Water (60:40) wall material mixture and roasted ground coffee. Through the conducted sensory analysis, it becomes evident that the optimal blending percentage for EXT-Water (60:40) stands at 20%. At a concentration of 30% within the wall mixture, the overall impression diminishes, and distinct bitter notes become prominent. Statistical analysis underscores a noteworthy distinction ($p < 0.05$) between the microencapsulated material's incorporation percentage and roasted ground coffee, with respect to the sensory profile of the samples.

3.3 Viability and Viable Cell Count in Hot Water Coffee Brewing Methods

Table 2 shows the viability and viable cell count when using the MD-INU:EXT wall material formulation (50:50) in coffee-based beverages prepared by 5 methods with hot water at 55, 70 and 90 °C, respectively. Hot methods indicated a Microbiological viability between 53.0 to 66.3%; 31.7 to 49.6% and 21.3 to 33.2% at 55, 70 and 90°C, respectively. The methods that used hot water with greater viability were the Chemex and the Americano preparations, at the 3 water temperatures evaluated. The drink prepared with the wall material and the coffee roasted and ground with water at 55 °C, had a maximum greater viability of 66.3% with the Chemex method. This outcome suggests that the composite wall material, in conjunction with roasted ground coffee, did not provide sufficient thermal isolation for the microcapsules containing the microorganism, resulting in a notable decrease in probiotic viability. Similarly, in terms of Colony-Forming Units per milliliter (CFU/mL) of *L. coagulans*, the hot beverages fell short of attaining the viable cell count (> $1x10^6$ CFU/mL) required to meet the stipulations of resolution 810 (MPs, 2021). Within the Chemex and Americano hot beverages, a range of 7.4 to 7.1 x 10^5 CFU/mL of viable *L. coagulans* cells was achieved. ANOVA analysis revealed significant differences ($p < 0.05$) in both the percentage of viability and the *L. coagulans* count of the MD-INU:EXT (50:50) formulation concerning coffee preparations prepared with hot water.

Table 3 presents the outcomes of viability and viable cell count analysis using the EXT-Water (60:40) wall material formulation within coffee-based beverages prepared through five distinct methods involving hot water at 55, 70, and 90°C.

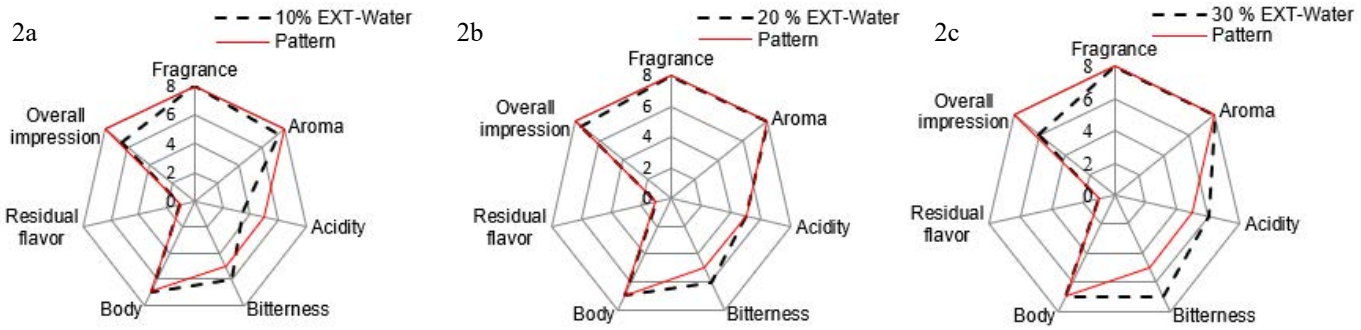

Figure 2: Percentage of incorporation of microcapsules formed with EXT-Water (60:40) with roasted and ground coffee at a) 10% b) 20% and c) 30% of wall materials

Table 2: Viability of microcapsules composed of MD-INU:EXT (50:50) with *L. coagulans* in the preparation of hot beverages.

Preparation method	Hot water temperature (°C)					
	55°C		70 °C		90°C	
	Viability (%)	Viable cell count (CFU/mL)	Viability (%)	Viable cell count (CFU/mL)	Viability (%)	Viable cell count (CFU/mL)
French press	63.9±0.3 [a]	6.6 x 10^5 [a]	45.4±0.2 [a]	5.3 x 10^4 [a]	30.7±0.8 [a]	3.3 x 10^3 [a]
Japanese siphon	53.2±0.9 [b]	6.1 x 10^4 [b]	31.7±0.9 [b]	4.6 x 10^3 [b]	21.3±0.2 [b]	3.6 x 10^2 [b]
Chemex	66.3±0.4 [a]	8.7 x 10^{5a}	48.8±0.5 [a]	7.4 x 10^4 [a]	33.2±0.7 [a]	4.8 x 10^3 [a]
Expresso	53.0±0.5 [b]	6.0 x 10^4 [b]	37.2±0.2 [c]	4.2 x 10^3 [b]	25.8±0.1 [b]	6.3 x 10^2 [b]
American	66.1±0.2 [a]	8.3 x 10^{5a}	49.6±0.3 [a]	7.1 x 10^4 [a]	32.6±0.6 [a]	7.3 x 10^3 [a]

Average values (n=3) with different letters (a – c) in the same column indicate statistical differences at the 5% significance level ($p < 0.05$).

In this context, the hot brewing techniques yielded viability rates spanning from 34.7% to 48.6% at 55°C, 24.6% to 36.9% at 70°C, and 19.3% to 25.9% at 90°C. Among these methods, the Americano and Chemex preparations demonstrated superior viability rates when utilizing hot water, albeit still falling below the viability achieved with the MD-INU:EXT (50:50) wall material mixture (as indicated in Table 2). ANOVA analysis highlighted significant differences ($p < 0.05$) in both the percentage of viability and *L. coagulans* count linked to the EXT-Water (60:40) formulation in relation to the distinct hot water coffee preparation methods at varying temperatures. Notably, this group of experiments exhibited reduced viability and viable cell counts in comparison to the MD-INU:EXT (50:50) formulation at corresponding temperatures.

3.4 Viability and Viable Cell Count in Cold Water Coffee Brewing Methods

Table 4 provides insights into the viability and viable cell count of coffee-based beverages containing microcapsules formed by MD-INU:EXT (50:50) and *L. coagulans*, prepared through five distinct cold brewing methods. Among the cold methods employed, Cold tower, Midzudashi, and Toddy yielded notably higher viability rates of 89.8%, 89.7%, and 87.6%, respectively. This outcome aligns with expectations, as the viability of *L. coagulans* is indeed influenced by the water's temperature during the preparation process. Moreover, all cold methods surpassed the regulatory minimum for probiotic counts ($1x10^6$ CFU/mL as specified by MPs, 2021), exhibiting counts ranging from 3.7×10^6 to 8.6×10^7 CFU/mL. ANOVA analysis highlighted significant differences ($p < 0.05$) in both the percentage of viability and *L. coagulans* count pertaining to the MD-INU:EXT (50:50) formulation in relation to coffee-based beverages obtained through the applied cold water preparation methods.

Table 5 elucidates the viability and viable cell count of coffee-based beverages augmented with microcapsules crafted from EXT-Water (60:40) and *L. coagulans*, produced through five diverse cold brewing methods. Among these methods, Midzudashi, Cold Tower, and Toddy emerged as top contenders, boasting viability rates of 85.5%, 83.0%, and 71.1%, respectively. This outcome aligns with expectations, as the viability of *L. coagulans* is indeed influenced by both the water temperature during preparation and the composition of the wall material utilized within this experimental category. Notably, the viable cell counts spanned from 3.3×10^6 to 6.3×10^7 CFU/mL, surpassing the stipulated minimum. ANOVA analysis accentuated significant differences ($p < 0.05$) in both the percentage of viability and *L. coagulans* count with respect to the EXT-Water (60:40) formulation in relation to coffee-based beverages obtained through the applied cold water preparation methods. This group of experiments exhibited diminished viability and viable cell count compared to the MD-INU:EXT (50:50) formulation under analogous conditions of coffee-based cold beverage preparation.

In summary, for both wall material formulations, namely the MD-INU:EXT (50:50) and EXT-Water (60:40), certain brewing methods like Chemex and Americano share common principles of gravity filtration and employ filter paper

Table 3: Viability of microcapsules composed of EXT:Water (60:40) with *L. coagulans* in the preparation of hot beverages.

Preparation method	Hot water temperature (°C)					
	55°C		70 °C		90°C	
	Viability (%)	Viable cell count (CFU/mL)	Viability (%)	Viable cell count (CFU/mL)	Viability (%)	Viable cell count (CFU/mL)
French press	34.7 ± 0.7^{a}	$5.3 \times 10^{5\ a}$	29.4 ± 0.7^{a}	$3.8 \times 10^{3\ a}$	21.6 ± 0.7^{a}	$5.8 \times 10^{2\ a}$
Japanese siphon	36.4 ± 0.4^{a}	$5.4 \times 10^{4\ a}$	27.3 ± 0.5^{a}	$3.9 \times 10^{3\ a}$	19.4 ± 0.3^{a}	$5.5 \times 10^{2\ a}$
Chemex	48.6 ± 0.8^{b}	$7.8 \times 10^{5\ b}$	36.9 ± 0.8^{b}	5.7×10^{4}	22.8 ± 0.4^{a}	$6.2 \times 10^{2\ a}$
Expresso	35.8 ± 0.4^{a}	$5.8 \times 10^{4\ a}$	24.6 ± 0.4^{c}	$3.4 \times 10^{3\ a}$	19.3 ± 0.6^{a}	$5.7 \times 10^{2\ a}$
American	46.3 ± 0.4^{b}	$7.5 \times 10^{5\ b}$	36.3 ± 0.4^{b}	$5.6 \times 10^{4\ b}$	25.9 ± 0.9^{b}	$6.9 \times 10^{2\ a}$

Average values (n=3) with different letters (a – c) in the same column indicate statistical differences at the 5% significance level ($p < 0.05$).

Table 4: Viability of microcapsules composed of MD-INU:EXT (50:50) with *L. coagulans* in the preparation of cold beverages.

Preparation method	Viability (%)	Viable cell count (CFU/mL)
Cold tower	89.8 ± 0.7^{a}	8.6×10^{7a}
Midzudashi	89.7 ± 0.3^{a}	8.1×10^{7a}
Toddy	87.6 ± 0.8^{a}	6.9×10^{7b}
Fretta	77.8 ± 0.4^{b}	$3.7 \times 10^{6\ c}$

Average values (n=3) with different letters (a – c) in the same column indicate statistical differences at the 5% significance level ($p < 0.05$).

during coffee beverage preparation. The results of *L. coagulans* viability and cell count analysis in these methods suggest that the presence of a paper filter might create an insulating effect during the brief 4-minute extraction process. This likely mitigates the impact of temperature on the probiotic's viability. Conversely, French Press and Express methods utilize metallic components for compacting and separating insoluble coffee solids from the brewed infusion. This choice of materials enhances thermal conductivity, preserving higher water temperatures. The Japanese siphon method, while mostly incorporating glass components, exhibits lower viability due to two reasons. Firstly, the use of a burner within the coffee maker raises the temperature of the brew. Additionally, the vacuum-based operation fails to provide adequate cooling to achieve optimal viability and cell count for *L. coagulans* in this infusion. In contrast, the cold methods that demonstrated superior viability and *L. coagulans* count, namely Midzudashi and Cold Tower, achieve this due to the notably lower water temperature employed during coffee beverage preparation. In conclusion, this study underscores that temperatures exceeding 55°C exert a drastic negative impact on the viability and cell count of *L. coagulans* when utilized in the preparation of coffee-based infusions. The choice of brewing method, its components, and the temperature it entails collectively contribute to the observed variations in probiotic viability across different scenarios.

Table 5: Viability of microcapsules composed ofEXT:Water (60:40) with *L. coagulans* in the preparation of cold beverages.

Preparation method	Viability (%)	Viable cell count (CFU/mL)
Cold tower	83.0 ± 0.8 [a]	5.6 x 10^{7a}
Midzudashi	85.5 ± 0.3 [a]	6.3 x 10^{7a}
Toddy	71.1 ± 0.4 [b]	3.3 x 10^{6b}
Fretta	63.1 ± 0.2 [c]	8.7 x 10^{6c}

Average values (n=3) with different letters (a – c) in the same column indicate statistical differences at the 5% significance level ($p < 0.05$).

4 DISCUSSION

This discrepancy arises from the higher sugar content in the EXT formulation (Brix between 29.15 to 35.65) (Castaño; Quintero, 2004), in contrast to the MD-INU:EXT (50:50) formulation, which comprises higher molecular weight polysaccharides (MD) and oligosaccharides (INU). The sugar composition in EXT enhances the hygroscopicity phenomenon of the mixture (Castaño; Quintero, 2004, Fritzen-Freire et al., 2012).

High hygroscopicity, solubility, and wettability necessitate the incorporation of microcapsules into matrices with low water content, as microcapsules might dissolve in aqueous media, jeopardizing the survival of the probiotic microorganism (Rodríguez-Barona; Giraldo; Montes, 2016).

This choice of the optimal blending percentage for EXT-Water (60:40) at 20 %; is supported by ratings that closely approximate an excellent pattern while incorporating subtle bitter notes, attributed to the inclusion of EXT in the wall mixture (Castaño; Quintero, 2004).

Prior studies focusing on probiotic drink formulation have embraced a comprehensive product design approach that encompasses sensory considerations. This is essential due to the integration of various food-grade extracts that can impact the final probiotic product's taste, as well as the chemical and biological stability of the incorporated probiotic (Rodríguez-Barona; Giraldo; Montes, 2016; Souza et al., 2022).

The resolution 810 (MPS, 2021) dictates a minimum number of probiotic microorganism colonies for the coffee-based drink to be classified as a "probiotic product."

This highlights the potential of hot coffee-based beverages when extracted at a lower temperature due to the inherent heat-resistant nature of *L. coagulans* (Konuray; Erginkaya, 2018). Furthermore, the combined protective effects of maltodextrin, inulin, and coffee extract contribute to the microorganism's preservation by supplying solids and structural support, as demonstrated in previous research (Rodríguez-Barona; Giraldo; Montes, 2016).

Maltodextrin finds widespread utility as a principal wall-forming agent due to its multifaceted attributes. Notably, its low viscosity aids in maintaining favorable processing conditions. Furthermore, its role encompasses diminishing adhesion and hygroscopic tendencies within mixtures. Additionally, it contributes a significant volume of solids, bolstering the polymerization of protective walls for bioactive compounds. Crucially, maltodextrin's inclusion neither imparts any discernible odor nor alters the intrinsic taste of the encompassing product (García et al., 2004). The synergy of inulin and maltodextrin, combined with other polysaccharides like alginate and xanthan gum, has garnered attention across diverse applications. This amalgamation has been particularly instrumental in microencapsulation endeavors, notably in encapsulating bioactive and probiotic constituents like *Lactobacillus Acidophilus*. The encapsulation process, often via spray drying, adheres to temperature thresholds around 160°C to safeguard product integrity (Parra, 2010).

Notably, these results (Table 2) underscore the capacity of the composite wall mixture's varied composition to effectively insulate the probiotic microorganism thermally, thereby mitigating declines in viability. In terms of Colony-Forming Units per milliliter (CFU/mL) of *L. coagulans*, this collection of hot coffee-based beverages failed to attain the necessary viable cell count to meet the requirements stipulated by resolution 810 (M.P.S., 2021). Consequently, these

beverages do not meet the criteria for designation as "probiotic products."

Regarding the preparation of hot coffee-based beverages, the utilization of water at a minimum temperature of 55°C emerges as a feasible approach to attain elevated *L. coagulans* microorganism viability. This outcome can be attributed to the microorganism's robust thermostability, facilitated by its spore-forming capability, as substantiated by recent studies. These findings hold even when the microorganism is integrated with coffee extract (Konuray; Erginkaya, 2018).

Regarding viable cell counts, the coffee-based beverages crafted via the five cold preparation methods successfully attained a level of viable cells sufficient to meet the criteria for designating these products as probiotics, in line with regulatory guidelines (M.P.S., 2021).

The protective qualities of EXT contributed to the safeguarding of the probiotic microorganism. Nevertheless, the achieved viability and viable cell count were comparatively lower than those observed with the MD-INU:EXT (50:50) mixture (as indicated in Table 4). This divergence can be attributed to the solubilizing tendency exhibited by EXT, which is more pronounced compared to INU and MD due to its elevated soluble solids content (°Brix) (Castaño; Quintero, 2004). It's noteworthy that the coffee-based beverages within this experimental cluster reached a viable cell count exceeding the threshold required for designating them as probiotics, in accordance with regulatory guidelines (M.P.S., 2021).

According to the findings of a study conducted by Ormaza-Zapata, Díaz-Arango and Rojano (2022b), cold coffee-based beverages exhibited a delicate equilibrium between acidity and bitterness. Interestingly, these cold preparations often showcased reduced acidity and body, yet managed to garner a positive overall impression, aligning with the UGQ pattern. This was in contrast to beverages prepared with hot water at 90 °C. Moreover, in terms of percentage viability and viable cell count, the use of cold water methods resulted in higher viability compared to those employing hot water methods. A comparative analysis of the two wall material formulations indicated that the MD-INU:EXT (50:50) blend outperformed in terms of viability and *L. coagulans* count, emerging as the superior choice for both hot and cold coffee-based beverages. In a study by Rodríguez-Barona, Giraldo and Montes (2016), an assessment of *L. casei* and *L. rhamnosus* viability was conducted, both with and without the incorporation of microencapsulated prebiotics via spray drying. The results exhibited cell viability percentages of approximately 96.67 % and 84.04 %, respectively. These outcomes resonate with the viability achieved using cold water methods for coffee-based beverage preparation in the present study, with both evaluated wall material mixtures. Another study by Rodríguez-Barona et al. (2012) documented the viability of L. casei within a mixture of maltodextrin and inulin, reaching approximately 82 % when microencapsulated through spray drying at a temperature of 80 °C. A study carried out by Konuray and Erginkaya (2020), reported with the incorporation of L. coagulans in wheat flour pastes, achieving a viability of above 1x108 CFU/g of L. coagulans, after performing the extrusion and drying process at 50 °C for 30 min, achieving the design of a probiotic paste, the feasibility data of this study are slightly higher than those of the present study. A study conducted by Polo et al. (2022), managed to encapsulate L. coagulans in an herbal extract of tea with inulin, at different brewing temperatures, where at 75 °C they achieved a viability of 1x109CFU/g of the probiotic microorganism with a brewing time of 3 min; This corroborates the potential of L. coagulans in obtaining probiotic infusions. In contrast, the results of this study showed that, at making the coffee beverage at 70 °C, a viability of 5.7 x 104CFU/g of L. coagulans was obtained with a wall material composed of MD-INU:EXT (50:50), applying the Chemex method.

5 CONCLUSIONS

According to the objective of the study, the nine resulting probiotic beverages exhibited sensory attributes similar to those of hot and cold U.G.Q. quality coffee beverages, respectively, harboring L. coagulans with a microcapsule viability above 60%. In addition, the evaluation of different coffee brewing methods revealed that freshly brewed cold beverages showed superior viability compared to their hot counterparts. . In particular, beverages brewed with water at 90°C, a traditional method for infusion of hot coffee, produced markedly lower viability of L. coagulans. This result highlights the impact of temperature on the viability of the probiotic microorganism used. The study also demonstrated the remarkable potential of EXT as a wall material for the micro-encapsulation of L. coagulans by spray drying, as well as its good ability to combine with common wall materials, such as maltodextrin and inulin. The knowledge obtained from this study was used to design a functional product that stands out within the Colombian coffee industry, offering probiotic and biofunctional advantages, with the Chemex and Cold Tower methods. This research expanded the applications of beneficial microflora such as L. coagulans beyond dairy products to non-dairy foods. In addition, it introduced innovative consumption options for coffee-derived products, particularly cold beverages, enriched with a probiotic component.

6 AUTHORS' CONTRIBUTION

Conceptual idea: Ormaza-Zapata, A.; Rodríguez-Barona, S.; Methodology design: Ormaza-Zapata, A.; Rodríguez-Barona, S.; Data collection: Díaz-Arango, F.; Data analysis and interpretation: Rodríguez-Barona, S.; Díaz-Arango, F., and Writing and editing: Ormaza-Zapata, A.

7 REFERENCES

ÁLVAREZ, S. T.; LAMAS, D. Enrichment of a vegetable drink based on Macadamia Nut (Macadamia tetraphylla), with omega-3 and probiotics. Reportes científicos de la FACEN, 12(2):63-73, 2021.

ANGELONI, G. et al. Characterization and comparison of cold brew and cold drip coffee extraction methods. Journal of the Science of Food and Agriculture, 99(1):391-399, 2019.

ASCHEMANN-WITZEL, J.; VARELA, P.; PESCHEL, A. O. Consumers' categorization of food ingredients: Do consumers perceive them as "clean label" producers expect? An exploration with projective mapping. Food Quality and Preference, 71:117-128, 2019.

CANO-CHAUCA, M. et al. Cal-vidal, Effect of the carriers on the microstructure of mango powder obtained by spray drying and its functional characterization. Innovative Food Science & Emerging Technologies, 6(4):420-428, 2005.

CARDONA-ARENGAS, M. A.; LÓPEZ-MARÍN, B. E. Probiotics: Functional foods for infants. Nutrición y Dietética, 32(2):31-39, 2019.

CASTAÑO, J. J. C.; QUINTERO, G. P. Quality of drilled coffee extracts obtained by cryoconcentration. Cenicafé, 55(3):183-201, 2004.

CHAUDHARI, K. et al. Invitro and in vivo evaluation of probioticpotential and safety assessment of Bacilluscoagulans SKB LAB-19 (MCC 0554) in humans and animal healthcare. Regulatory Toxicology and Pharmacology, 133:105218, 2022.

COFFEE PRODUCTION IN THE COUNTRY IN 2021 WAS 12.6 MILLION BAGS. **Portfolio,** 2021. Available in: https://www.portafolio.co/indicadores-economicos/produccion-de-cafe-en-colombia-durante-el-2021-560457**.** Access in: April, 20, 2023.

CUNHA JÚNIOR, P. C. et al. Elaboration, characterization, and probiotic viability of synbiotic non-dairy drink based on coconut. Ciência Rural, 53(3):1-15, 2023.

DE PRISCO, A.; MAURIELLO, G. Probiotication of foods: A focus on microencapsulation tool. Trends in Food Science & Technology, 48:27-39, 2016.

ESQUIVEL-GONZALEZ, B. E.; OCHOA-MARTINEZ, L. A.; RUTIAGA-QUIÑONES, O. M. Microencapsulation by spray drying of bioactive compounds. Revista Iberoamericana de Tecnología Postcosecha, 16(2):180-192, 2015.

FEDERAÇÃO NACIONAL DAS COOPERATIVAS DE CRÉDITO - FNCC. Comportamiento de la industria cafetera Colombiana 2014. 2014. Available in: https://www.federaciondecafeteros.org/static/files/Informe_Industrial_2014_Web.pdf. Access in: 22 February 2024.

FRITZEN-FREIRE, C. B. et al. Microencapsulation of bifidobacteria by spray drying in the presence of prebiotics, Food Research International, 45(1):306-312, 2012.

GARCÍA-GUTIÉRREZ, C. et al. Microencapsulation of green barley juice by spray drying. Ciencia y Tecnologia Alimentaria, 4(4):262-266, 2004.

INTERNATIONAL COFFEE ORGANIZATION - ICO. International Coffee Organization, Coffee Market Report, 2015. Available in: http://www.ico.org/documents/cy2014-15/cmr-0315-c.pdf. Access in: 22 February 2024.

INSTITUTO COLOMBIANO DE NORMAS TÉCNICAS Y CERTIFICACIÓN - ICONTEC. Colombian institute of technical standards and certification, sensory analysis. Coffee. Methodology for Descriptive Quantitative Sensory Analysis of Coffee, NTC 4883, 1-6, Bogotá, D.C., Republic of Colombia, 2000.

ILLYCAFFÈ S. P. A. Company - ILLY. The filter coffee, or American coffee: how it is prepared. 2016. Available in: https://www.illy.com/wps/wcm/connect/es/cafe/cafe-filtro. Access in: 1 March 2024.

INTERNATIONAL COFFEE ORGANIZATION - ICO. **Historical data on the global coffee trade**. 2018. Available in**:** https://www.ico.org/new_historical.asp?section=Statistics. Access in: 1 March 2024.

KONURAY, G.; ERGINKAYA, Z. Quality evaluation of probiotic pasta produced with Bacillus coagulans GBI30. Innovative Food Science and Emerging Technologies, 66:102489, 2020.

KONURAY, G.; ERGINKAYA, Z. Potential use of bacillus coagulans in the food industry. Foods, 7(6):92, 2018.

MINISTRY OF SOCIAL PROTECTION - MPS. Resolution 810 of 2021, 35-36, Bogotá, D.C., Republic of Colombia 2021. Available in: https://www.minsalud.gov.co/Normatividad_Nuevo/Resoluci%C3%B3n%20No.%20810de%202021.pdf. Access in: 1 March 2024.

ORMAZA-ZAPATA, A. M.; DÍAZ-ARANGO, F. O.; ROJANO, B. A. Analysis gravity-filtration preparation methods' antioxidant content, capacity, and coffee beverage acceptance. Ciencia Tecnología Agropecuaria, 23(2):1-22, 2022a.

ORMAZA-ZAPATA, A. M.; DÍAZ-ARANGO, F. O.; ROJANO, B. A. Effect of cold preparation of coffee (*Coffea arabica* L. var. Castillo) on antioxidant capacity and sensory quality. Información Tecnológica, 33(1):57-60, 2022b.

ORMAZA-ZAPATA, A. M.; DÍAZ-ARANGO, F. O.; ROJANO, B. A. The effect of pressure filtration coffee preparation methods (*Coffea arabica* L. var. Castillo) on antioxidant content and activity, and beverage acceptance. DYNA, 86(209):261-270, 2019.

PALOP, A. et al. Heat Resistance of Bacillus subtilis and *Bacillus coagulans*: Effect of sporulation temperature in foods with various acidulants. Journal of Food Protection, 59(5):487-492, 1996.

PARRA, R. A. H. Review: Microencapsulation of food. Revista Facultad de Ingeniería Universidad de Antioquia, 63(2):5669-5684, 2010.

POLO, A. et al. A novel functional herbal tea containing probiotic Bacillus coagulansGanedenBC30: An in vitro study using the Simulator of the Human IntestinalMicrobial Ecosystem (SHIME). **Journal of Functional Foods**, 88:10487, 2022.

RODRÍGUEZ-BARONA, S.; GIRALDO, G. I.; MONTES, L. M. Encapsulation of probiotic foods by lyophilization in the presence of prebiotics. Información Tecnológica, 27(6):135-144, 2016.

RODRÍGUEZ-BARONA, S.; MONTES, L. M.; RAMÍREZ, D. J. Microencapsulation of probiotics by spray drying in the presence of prebiotic. Applied Food Research, 19(1):186-188, 2012.

SEMYONOV, D. et al. Microencapsulation of Lactobacilllus paracasei by spray freeze drying. Food Research International, 43(1):193-202, 2010.

SOUZA, T. L. T. L. et al. Drink with probiotic potential based on water-soluble extract from cashew nuts. Ciência Rural, 52(11):1-9, 2022.

TRIPATHY, M. K.; GIRI, S. K., Probiotic functional foods: Survival of probiotics during processing and storage. Journal of Functional Foods, 9:225-241, 2014.

ZENDEBOODI, F. et al. Probiotic: Conceptualization from a new approach. **Current Opinion in Food Science**, 32:103-123, 2020.

ZIELIŃSKA, D.; KOLOŻYN-KRAJEWSKA, D. Food-origin lactic acid bacteria may exhibit probiotic properties. BioMed Research International, Article ID 5063185, 2018.