Revista **U.D.C.A** Actualidad & Divulgación Científica
July-December 2024-Volumen 27 No. 2:e2656
ISSN: 2619-2551 **online version**
ISSN: 0123-4226 **printed version**

Scientific Article
http://doi.org/10.31910/rudca.v27.n2.2024.2656

Development of functional panela cheese enriched with prebiotics and probiotics

Desarrollo de queso panela funcional enriquecido con prebióticos y probióticos

Karina Tiscareño-Ortega[1]; Norma Angélica Chávez-Vela[2]*; Julieta Domínguez-Soberanes[3]; Gabriela Mariana Rodríguez-Serrano[4]

[1]Universidad Panamericana. Escuela de Dirección de Negocios Alimentarios. Aguascalientes - México; e-mail: 0241321@up.edu.mx.
[2]Universidad Autónoma de Aguascalientes, Departamento de Ingeniería Bioquímica. Aguascalientes - México; e-mail: angelica.chavez@edu.uaa.mx
[3]Universidad Panamericana. Facultad de Ingeniería. Aguascalientes - México; e-mail: jdominguez@up.edu.mx
[4]Universidad Autónoma Metropolitana, Iztapalapa, Departamento de Biotecnología, Ciudad de México - México; e-mail: gmrs@xanum.uam.mx
*corresponding author: angelica.chavez@edu.uaa.mx

How to cite: Tiscareño-Ortega, K.; Chávez-Vela, N.A.; Domínguez-Soberanes, J.; Rodríguez-Serrano, G.M. 2024. Development of functional panela cheese enriched with prebiotics and probiotics. Rev. U.D.C.A Act. & Div. Cient. 27(2):e2656. http://doi.org/10.31910/rudca.v27.n2.2024.2656

Official publication of the Universidad de Ciencias Aplicadas y Ambientales U.D.C.A, a Higher Education Institution Accredited of High Quality by the Ministry of National Education.

Received: May 9, 2024 **Accepted:** November 27, 2024 **Edited by:** Helber Adrián Arévalo Maldonado

ABSTRACT

Food trends show interest in functional foods, which provide health and nutrition. This research is focused on developing a functional Mexican-style panela cheese that incorporates the probiotics *Bifidobacterium animalis* subsp. *lactis*, *Lactobacillus delbrueckii* subsp. *bulgaricus* NCFB 2772, *Lacticaseibacillus rhamnosus* GG and 4% inulin as a prebiotic. Yield, and content of lactic acid bacteria (LAB) and CFU of total coliforms were evaluated. Scanning Electron Microscopy was performed to evaluate the incorporation of the bacteria in the matrix. Furthermore, shelf life was established. The cheese yielded 26.6% (w/v), and its microbial content reached 7.12×10^8 CFU/g, exceeding the Mexican Norm NOM 181. Inulin enhanced probiotic growth, and electron microscopy confirmed the successful integration of bacteria within the cheese matrix. Shelf-life studies demonstrated sustained LAB growth above 10^8 CFU/g for 22 days, though the shelf life was determined to be 13 days under refrigeration at 4 °C due to coliform bacteria detection. Sensory evaluation revealed through just about right within the 60% range where dairy flavor, mouthfeel moisture, creaminess, and adhesiveness; showing that no reformulation should take place. Furthermore, CATA analysis showed that attributes that better described the product were dairy flavor, mouth moisture, fresh milk smell, white color, and smooth texture. The cheese scored 5.03 ± 1.23 on a 7-point acceptance scale and achieved a 69% purchase intention rate. Therefore, a functional cheese product was obtained with LAB and inulin with adequate sensory characteristics.

Keywords: *Bifidobacterium*; Dairy product; Functional fermented product; Inulin; *Lactobacillus*.

RESUMEN

Las tendencias alimentarias muestran un creciente interés por alimentos funcionales que ofrecen beneficios para la salud y nutrición. Esta investigación desarrolló un queso panela funcional de estilo mexicano que incorpora los probióticos *Bifidobacterium animalis* subsp. *lactis*, *Lactobacillus delbrueckii* subsp. *bulgaricus* NCFB 2772, *Lacticaseibacillus rhamnosus* GG y un 4% de inulina como prebiótico. Se evaluó el rendimiento, y el contenido de bacterias ácido lácticas (BAL) y UFC de coliformes totales. La microscopía electrónica de barrido confirmó la incorporación de bacterias en la matriz del queso. El rendimiento fue del 26,6 % (p/v), y el contenido microbiano alcanzó $7{,}12 \times 10^8$ UFC/g, superando el requisito de la Norma Mexicana NOM 181. La inulina promovió el crecimiento probiótico, logrando BAL superiores a 10^8 UFC/g durante 22 días. Sin embargo, la vida útil del queso se estableció en 13 días bajo refrigeración a 4 °C, debido a la detección de bacterias coliformes. La evaluación sensorial con la escala just about right mostró valores dentro del rango del 60 % para sabor lácteo, humedad en boca, cremosidad y adhesividad, indicando que no se requiere reformulación. El análisis CATA destacó atributos como sabor lácteo, humedad en boca, olor a leche fresca, color blanco y textura suave. El queso obtuvo una puntuación de aceptación de 5,03 ± 1,23 en una escala de 7 puntos y una intención de compra del 69 %, demostrando su funcionalidad y adecuadas características sensoriales. Por lo tanto, se obtuvo un producto de queso funcional con BAL e inulina con características sensoriales adecuadas.

Palabras clave: *Bifidobacterium*; Inulina; *Lactobacillus*; Producto funcional fermentado; Producto lácteo.

INTRODUCTION

Food market trends for 2030 aim to introduce functional foods, low-fat foods with greater health benefits, and better nutritional profiles. Cheese is one of the best options among the various food matrices for incorporating probiotics due to its status as one of the most widely consumed dairy products globally, attributed to its nutritional contribution, sensory appeal, and potential inherent health benefits (Hammam & Ahmed, 2019; Machado *et al*. 2023).

Probiotics are defined as selected and viable microbial dietary supplements that, when introduced in sufficient quantities, beneficially affect the human organism through their effects on the intestinal tract. These beneficially affect the human body through their effects on the intestinal tract when introduced in sufficient quantities. Probiotic-rich foods constitute 60 to 70% of the total functional foods market (Fuentes-Berrio *et al.* 2015). These bacteria are crucial in repairing intestinal microbiota and restoring their functions. They primarily benefit human health through three general mechanisms of action: exclusion or inhibition of pathogens, improvement of the epithelial barrier function, and modulation of host immune responses (Pandey *et al.* 2015). The mechanism of action of probiotics includes the normalization of disturbed microbiota, regulation of intestinal transit, increased renewal of enterocytes, reinforcement of the intestinal barrier, resistance to colonization, production of acids and short-chain fatty acids, synthesis of vitamins, and metabolism of bile salts. The main sources of probiotics are kefir, yogurt, and some pickles (Pandey *et al.* 2015).

The main bacterial groups among probiotics are *Bifidobacterium* and *Lactobacillus*, which contribute to the immune system's development and are considered beneficial for enhancing overall health (Thompson *et al.* 2022; Ağagündüz *et al.* 2023). Recent attention to probiotics, termed immunobiotics by Villena *et al.* (2016), emphasizes their beneficial interactions and immune system regulations at mucosal levels.

Some of the most used probiotic strains in dairy food products are *Bifidobacterium animalis* subsp. *lactis,* BB-12® that promotes gastrointestinal health and improves immunity, and furthermore, they are widely used because they don't affect the taste, appearance, or mouthfeel of products (Jungersen *et al.* 2014), *Lactobacillus delbrueckii* subsp. *bulgaricus* NCFB 2772*,* which produces extracellular polysaccharides (EPS), enhancing viscosity, preventing syneresis, and improving sensory characteristics in fermented dairy products. Furthermore, it regulates immune response (Stachelska & Foligini, 2018; Bancalari *et al.* 2022), and *Lacticaseibacillus rhamnosus* (formerly *Lactobacillus rhamnosus* GG), which is a potential probiotic with resistance to acid and bile, inhibits harmful bacteria, acting as a natural preservative in yogurt-type products, secretes inhibitory substances against various bacterial species and significantly, balances the intestinal micro ecological system, providing immunomodulation. Studies show that these bacteria promote immune response and exhibit substantial antioxidant activity under physical stress, which benefits athletes (Rani & Yadav, 2018). With probiotics, the microbiota can be recolonized with beneficial bacteria, however, with prebiotics, the growth of these bacteria is stimulated.

Prebiotics are substrates selectively used by organisms to provide health benefits (Gibson *et al.* 2017). Some examples of these are fructooligosaccharides, inulin, pectins, mycelium or resistant starch. They influence lipid and mineral metabolism and cause changes in the intestine. Prebiotics are functional only in the colon, where beneficial microbiota like *Bifidobacteria* spp. and *Lactobacillus* spp. consume them. They provide energy for large intestine bacteria, fermenting into short-chain fatty acids (Rawi *et al.* 2020; Thompson *et al.* 2022). Non-digestible oligosaccharides, such as inulin-based fructo-oligosaccharides and galacto-oligosaccharides, are found in foods like garlic, Jerusalem artichokes, jicama, dandelion greens, and onions (Thompson *et al.* 2022). These prebiotics can alter intestinal microbiota, reduce harmful bacteria, and increase beneficial bifidobacteria (Fuentes-Berrio *et al.* 2015). Benefits include aiding mineral absorption, improving immune function, lowering blood cholesterol, potentially preventing cancer, and relieving constipation and irritable bowel syndrome. They also enhance the immune system, control appetite and weight, promote regularity, and increase bone density (Shoaib *et al.* 2016).

Inulin, a water-soluble, non-digestible fructan is recognized for health benefits and is generally considered safe in the US. It is abundant in chicory roots and has reduced caloric value, dietary fiber benefits, and prebiotic effects. It is used in dairy and non-dairy products as a fat replacement and texture enhancer (Karimi *et al.* 2015). Inulin promotes intestinal barrier repair, detoxifies carcinogens, supports beneficial microbes, and increases butyrate production (Hutchinson *et al.* 2023). It lowers blood glucose, improves liver and kidney functions, and helps counteract hyperglycemia and hypercholesterolemia as a low-calorie, plant-based fat supplement (Tresina *et al.* 2022). The addition of inulin to different types of cheese can be beneficial in the manufacture of a reduced or low-fat textured symbiotic product.

The objective of this research is to develop a functional Mexican-style cheese that is a healthy choice for consumers, presenting a high-protein and low-fat product. This product should be functional, which means that prebiotics and/or probiotics should be included. Moreover, it should have adequate sensory characteristics.

MATERIALS AND METHODS

Standardization of the Panela Cheese Production Procedure. The production process of panela cheese is typically low in fat (González-Córdova *et al.* 2016). Raw milk from the private company Lácteos del Camino, located in Aguascalientes, México, was used. The 4 L milk was skimmed using an 80-100 L Electric Milk Cream Separator from Motor Sich (Ukraine) and pasteurized at 74°C for 1 minute. Milk with 0.02% fat was obtained, according to the technical sheet of the equipment. To make the cheese, rennet (Cuarmix, Chr Hansen México S.A. de C.V., Mexico) was added according to the manufacturer's instructions, and calcium chloride (MABI, Mexico) was added at 0.02%. The milk was allowed to coagulate for 1 h. After the milk starts to coagulate, the probiotic culture is added, like the procedure performed by other researchers (Escobar *et al.* 2012). The solids were recovered, and the characteristic shape of panela cheese, resembling a basket, was given.

Preparation of bacterial Inoculants. Assays were conducted with three lactic acid-producing microbial strains: 1) *Bifidobacterium animalis subsp. lactis,* BB-12® (BB-12), 2) *Lactobacillus delbrueckii subsp. bulgaricus* NCBF 2772 (Lb. 2772), and 3) *Lacticaseibacillus rhamnosus GG* (Lb. GG).

Each strain was reactivated using 1 mL of it from previously inactivated cultures in glycerol. The strains were reactivated in 50 mL of a Man, Rogosa, and Sharp medium (MRS, Becton, Dickinson & Company, France) at 37 °C to obtain young and active cultures. Inoculants from the above bacterial cultures were prepared in milk for panela cheese preparation. From each reactivated culture, 1 ml was taken and added to the pasteurized milk and then incubated for 6 h at 37°C

Cheeses. Three cheeses were produced with the following formulations to find the optimal combination. 1. Control without any microorganism, 2. *Bifidobacterium animalis subsp. lactis* BB-12® at 5% and *Lactobacillus delbrueckii* subsp. *bulgaricus* NCBF 2772 at 5% was added for achieving a final probiotic concentration of 10%, and 3. *Bifidobacterium animalis subsp. lactis* BB-12® at 3.33%, *Lactobacillus delbrueckii subsp. bulgaricus* NCBF 2772 at 3.33% and *Lacticaseibacillus rhamnosus* GG at 3.33% were added for a final probiotic concentration of 10% (Figure 1).

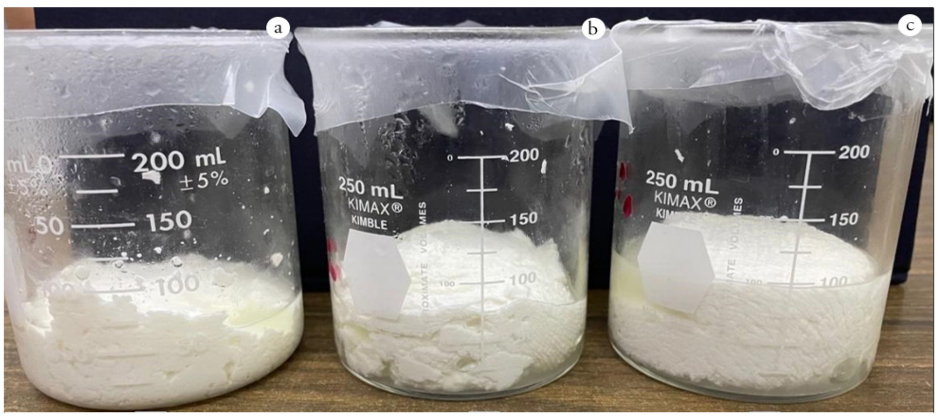

Figure 1. Appearance of cheeses obtained with different strains of lactic acid bacteria: a) cheese without lactic acid bacteria (prototype); b) Cheese supplemented with *Bifidobacterium animalis* subsp. *lactis* and *Lactobacillus delbrueckii* subsp. *bulgaricus* 2772; c) Cheese supplemented with *Bifidobacterium animalis* subsp. *lactis*, *Lactobacillus delbrueckii* subsp. *bulgaricus* 2772, and *Lacticaseibacillus rhamnosus* GG. The cheeses were incubated at 37°C for 1 hour and then refrigerated at 4°C.

Yield, microbial count, and sensory characteristics of cheeses were evaluated. Yield was measured by dividing the final weight of recovered solids by the initial milk weight. Furthermore, colony-forming unit (CFU) counts of lactic acid bacteria were performed on Man, Rogosa and Sharpe (MRS) agar plates. Additionally, coliform enumeration was evaluated by Violet Red Lactose Agar (VRLA) by seeding a sample from each cheese, and counts were obtained through serial dilutions.

Identification by electron microscopy of the presence of lactic acid bacterial cultures and inulin in functional cheese. Samples were dehydrated with alcohol 60 to 100%. Extent moisture was removed by liquid CO_2 in a critical point dryer (TOUSIMIS). Samples were then counted with gold using a DESK II chamber and photographed with a JEOL LV 5900 SEM). This was performed at the Universidad Autónoma de Aguascalientes, México using the protocol defined by that laboratory.

Determination of the shelf life of functional cheese. Viable counts of LAB were estimated after 1, 7, 15, 21, and 30 days of storage at 4 °C. Serial dilutions were made and seeded on MRS (Becton, Dickinson & Company, France) medium agar, which allows the development of lactic acid bacteria and VRLA, which is a selective medium for the detection and enumeration of coliform microorganisms (both manufactured and distributed by Becton Dickinson in Mexico). Incubation was carried out for 48 h at 37°C. Afterward, CFUs/g were counted. When coliforms in the VRLA agar plates were observed, the shelf life was determined. In addition, the LAB was monitored to comply with Mexican NOM 181, which must have a range between 10^6 CFU/g and 10^8 CFU/g to be considered a functional product.

Sensory evaluation of functional cheese. One hundred sensory questionnaires were administered to consumers aged 18-70 in Aguascalientes, Mexico. All participants agreed to participate in the sensory analysis of this research and signed the informed consent form. This work is part of a project approved by Universidad Panamericana by the Ethics Committee 2002-2024.

A sensory questionnaire was generated with six sections. Information on gender, age, and product consumption was collected, asking consumers if the product was just right or needed more or less of a specific attribute. The just-about-right (JAR) test evaluated the following attributes (lactic flavor, acidic flavor, moisture in mouth, creaminess, gumminess, adhesiveness, and aftertaste).

The Check All That Apply (CATA) method assessed 20 cheese-related attributes (Hernández-Cervantes *et al.* 2010; CEA, 2018). A 7-point hedonic scale was used to evaluate the acceptability of the product. Finally, consumers were asked if they would buy this product.

RESULTS AND DISCUSSION

Selection of bacterial cultures for making functional cheese. The cheese with the highest CFU count for lactic acid bacteria was panela cheese supplemented with *Bifidobacterium animalis* subsp. *lactis, Lactobacillus delbrueckii* subsp. *bulgaricus NCFB 2772,* and *Lacticaseibacillus rhamnosus* GG (Table 1) reached a 7.12 x 108 CFU, and the cheese yield relative to the volume of milk reached 26.66%.

This response can be attributed to a synergistic effect between the probiotic bacteria (Pranckutė *et al.* 2016), and the increased water retention generated by the presence of exopolysaccharides. Using EPS-producing bacteria increases moisture content, resulting in improved sensory attributes for low-fat products due to the water-absorbing capacity of EPS, increasing product viscosity. Certain lactobacilli can produce exopolysaccharides (EPS), used in the food industry to enhance the texture of products such as yogurt, cheese, and other dairy products. Additionally, EPS has health benefits, including immune system stimulation, anti-mutagenic properties, and anti-tumor activity (Harutoshi, 2013). In this case, it is an EPS producer (Fox *et al.* 2017) notably, EPS also possesses prebiotic abilities since their composition allows for slower metabolism by the intestinal microbiota, reaching parts of the distal colon (Harutoshi, 2013). Therefore, *Lactobacillus delbrueckii* subsp. *bulgaricus* NCFB 2772 was selected, aiding in cheese yield, contributing to the prebiotic effect (in addition to inulin), and achieving the desired symbiotic effect.

Ideally, probiotic strains for food applications should originate from humans, be acid and bile-tolerant, adhere to the gastrointestinal tract linings, compete with pathogenic bacteria, and have a safe dosage for human consumption. Selection criteria for probiotics include benefiting the host, surviving transit through the intestines, adhering to the intestinal epithelial cell membrane, creating antibiotic substances against infections, and stabilizing the intestinal microflora (Tegegne & Kebede, 2022). The chosen cultures BB12, Lb. 2772, and Lb. GG have been successfully used in dairy matrices before (Castro *et al.* 2015). These microorganisms, especially lactobacilli and bifidobacteria are associated with health benefits such as immune system activation, inhibition of pathogenic bacteria, and vitamin production (Hugenholtz & Smid, 2002).

After selecting the appropriate probiotic combination, the influence of inulin on bacterial growth was evaluated. A growth of 1.27 $x10^8$ UFC/g was observed, compared to 4.53 $x10^7$ UFC/g without inulin, indicating 2.8 times more growth. Logarithmically the increase in microbial growth aligns with the literature, which states that inulin favors the growth of lactic acid bacteria by being selectively metabolized (Table 2). In cheese, inulin can reduce fat by 63% with 3.4% added inulin, but more than 8% affects sensory appeal (Talbot-Walsh *et al.* 2018). Additionally, the effect of inulin is not limited to cheese; it also promotes the growth of bifidobacteria in the intestine, known as the bifidogenic effect of prebiotic compounds (Hughes *et al.* 2022).

Table 1. Comparison of attributes of panela cheese with lactobacilli strain combinations

Cheese	CFU/g	Volume	Yield (w/v)
Control Panela Cheese	5.26 x10^ 4	50 mL	15%
Panela Cheese with *Bifidobacterium animalis* subsp. *lactis* and *Lactobacillus delbrueckii* subsp. *bulgaricus* 2772	9.66 x10^ 6	75 mL	20.67%
Cheese supplemented with *Bifidobacterium animalis* subsp. *lactis*, *Lactobacillus delbrueckii* subsp. *bulgaricus* 2772, and *Lacticaseibacillus rhamnosus* GG	7.12 x10^8	100 mL	26.33%

Table 2. Influence of the presence of inulin on probiotic UFC count in panela cheese

Cheese	UFC/g
Control Panela Cheese	9.38 x10^ 04
Panela Cheese with *Bifidobacterium animalis* subsp. *lactis* and *Lactobacillus delbrueckii* subsp. *bulgaricus 2772*	4.53 x10^ 07
Cheese supplemented with *Bifidobacterium animalis* subsp. *lactis*, *Lactobacillus delbrueckii* subsp. *bulgaricus 2772,* and *Lacticaseibacillus rhamnosus* GG	1.27 x10^08

Identification by electron microscopy of the presence of lactic bacterial cultures and inulin in functional cheese. Images were taken through electron microscopy to confirm lactobacilli's presence in the protein matrix. In Figure 2, the presence of lactobacilli and bifidobacteria can be observed. Electron microscope photographs clearly show the presence of bifidobacteria and lactobacilli, notably, the cultures remain viable where a lactobacillus is dividing showing the possible interaction of bacteria with inulin and demonstrating the proliferation of lactobacilli within the protein and inulin matrix, according to the literature, demonstrating that lactobacilli can continue to grow within their environment as long as they have the means to survive and reproduce (Rolim *et al.* 2020).

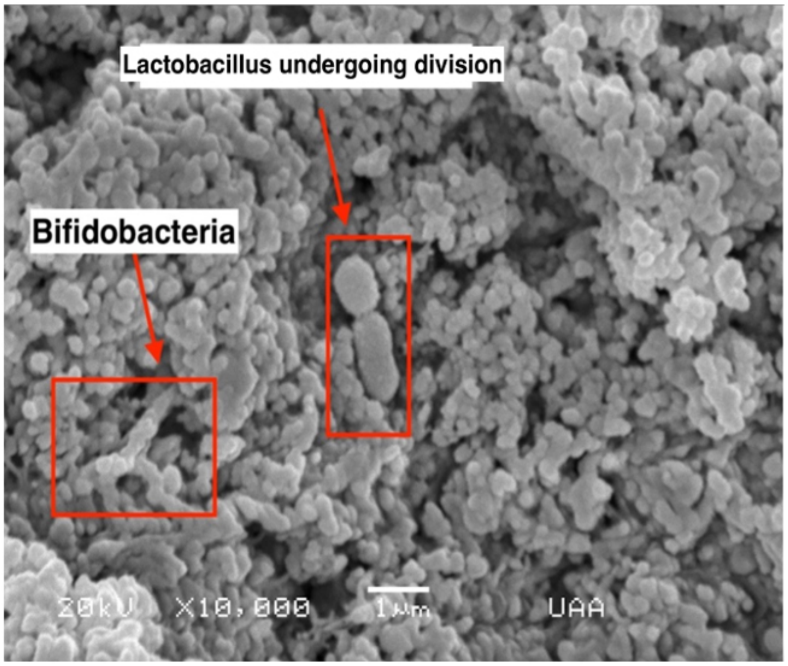

Figure 2. Electron microscope micrograph of *Bifidobacterium* and *Lactobacillus*, the latter can be observed in the process of dividing.

Shelf life of cheese. The shelf life of a food product, also known as its useful life, is the period counted from its production date, during which it maintains acceptable sensory and safety characteristics for consumers. The following steps were taken: Over 22 days, microbial growth was monitored in a standard counting medium, on bile red violet agar to determine coliforms, and on MRS agar to quantify the presence of lactobacilli. Microbial growth above 10^8 CFU/g was observed over 22 days, Figure 3a, although it began to decline after 13 days. Additionally, figure 3b shows the growth of other bacteria not related to lactobacilli, which increased after 20 days of shelf life.

The product's shelf life ends at 13 days when pathogenic microorganisms are observed. This is a challenge due to the water activity available in the product, as higher water activity leads to increased bacterial development (Wemmenhove *et al.* 2021). The microbial count remained above 10^6 UFC/g, although a decrease was observed after day 13. To achieve the attributed effects of probiotics, microbial counts should be above 10^6 UFC/g, although it is noteworthy that sensorial flavors characteristic of analog cheese without probiotics are expected to be maintained (Karimi *et al.* 2012).

Sensory evaluation of functional cheese. In the just-about-right (JAR) analysis, the laboratory-made cheese showed that the acidic flavor, creaminess, and adhesiveness presented over 60% of Just About Right (JAR=3). Additionally, it was found that the moisture in mouth (JAR=3) was 29%, which means that more is needed because 69% of the consumers (JAR ≤ 3) thought that this parameter was too low; similar results were found in aftertaste. When evaluating sensory characteristics, the prototype cheese was compared with three commercial cheeses. In Figure 4, the just about right (JAR) prototype was analyzed taking into consideration critical attributes for this type of cheese.

The technique uses a scale where the center is just right (JAR =3), in our study's case, referring to the number 3. Below this number, it is considered that the product has a low amount of the evaluated attribute. Conversely, if the evaluation is higher than 3, it denotes that the attribute is present in a higher proportion than necessary. In this way, the ideal product should have all attributes evaluated as "just right". In the food industry, achieving all attributes as just right only sometimes happens, so this test can help establish new formulations by changing certain ingredients or processes. However, when evaluating these methodologies Popper (2014) indicated that if 75% of consumers evaluated that all products within a formulation could be JAR, the decision could be made to accept it as a prototype. However, this parameter could change depending on the type of food being analyzed. In particular, a product is considered suitable for low-fat products when obtaining 60% off "just right" in the evaluated attributes. Taking into consideration the abovementioned information, acidic flavor, creaminess, and adhesiveness were over 60% of JAR.

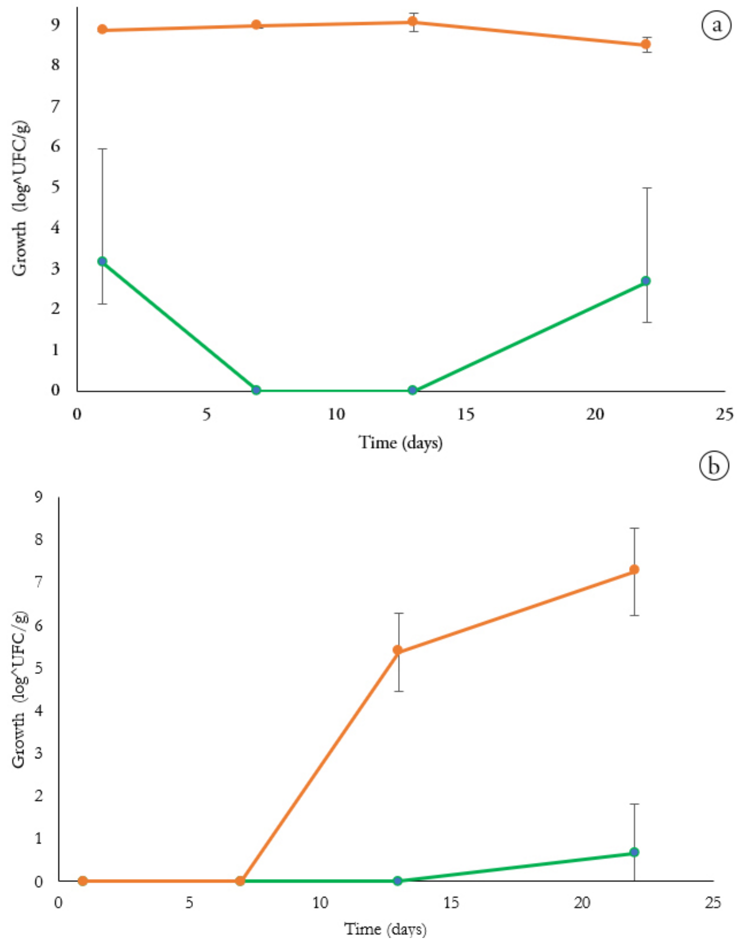

Figure 3. a) Growth of lactobacilli in functional panela cheese. In orange, panela cheese with *Bifidobacterium animalis* subsp. *lactis*, *Lactobacillus delbrueckii* subsp. *bulgaricus* 2772, *Lacticaseibacillus rhamnosus* GG and inulin 4%. In green, control group. b) Growth of bacteria other than lactobacilli in functional panela cheese. In orange, panela cheese with *Bifidobacterium animalis* subsp. *lactis*, *Lactobacillus delbrueckii* subsp. *bulgaricus* 2772, *Lacticaseibacillus rhamnosus* GG and Inulin 4%. In green control group.

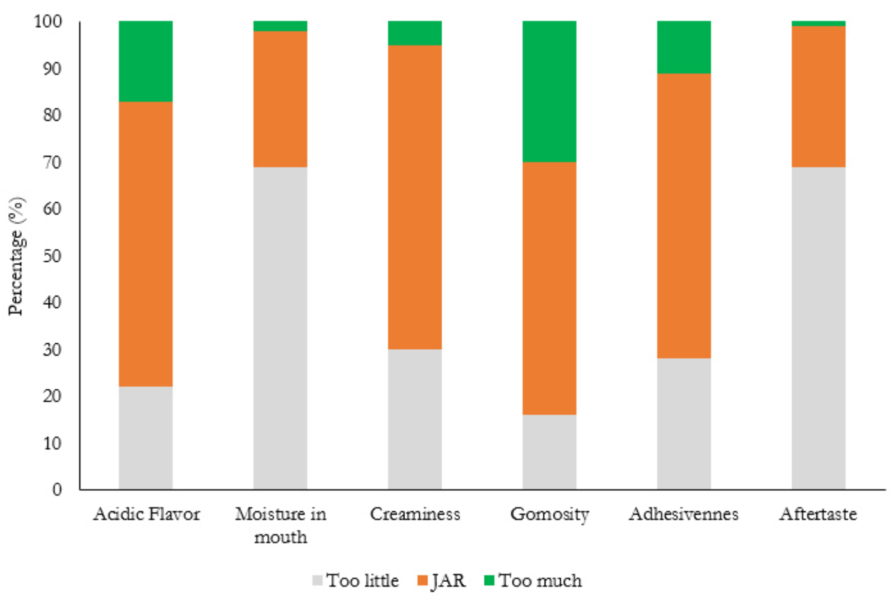

Figure 4. Just-about-right JAR of the prototype developed in the laboratory

The attributes that could be considered in the case of making a new formulation are moisture in the mouth and aftertaste, where JAR is around 30%. This could be because the flavor development had yet to occur at the testing point, as the lactic acid bacteria would be expected to acidify the product over time. The creaminess found was like the percentage defined by other researchers as Jiménez-Guzmán *et al.* (2009) who produced panela cheese with EPS, in that case, with *Streptococcus thermophilus*.

A sensory technique called CATA (check all that apply) was applied, where consumers were asked to mark all attributes detected in the prototype panela cheese. The results reveal that the prototype of panela cheese with lactic acid bacteria can be described as a product with a dairy flavor (87%), a fresh milk smell (85%), white color (84%), smooth texture (86%), and when tasted, it generates moisture in the mouth (82%).

The Check-All-That-Apply (CATA) analysis revealed that the prototype cheese had dairy flavor (87%), smelled of fresh milk (85%), was white (84%), had a soft texture (86%), and generated mouth moisture upon tasting (82%). The results are consistent with expectations, as its white color and dairy flavor characterize panela cheese (IDFA, 2023). Similarly, the soft texture and mouth moisture can be attributed to the presence of EPS. The EPS produced by *Lactobacillus delbrueckii* subsp. *bulgaricus* NCFB 2772 aids water retention, creating a sensation of moisture and perceived smoothness, preventing syneresis and granularity, and providing the product with a natural thickness (Oberg *et al.* 2022).

On the other hand, the acceptance level of the generated prototype was 5.03 ± 1.23, indicating a slight liking, but that was in the range of the other commercial brands analyzed. Moreover, the purchase intention would be 69%.

In conclusion, it can be established that a functional panela cheese was developed. This product incorporated three probiotic strains: *Bifidobacterium animalis* subsp. lactis BB-12® (3.33%), *Lactobacillus delbrueckii* subsp. *bulgaricus* NCBF 2772 (3.33%), and *Lacticaseibacillus rhamnosus* GG (3.33%) for a final probiotic concentration of 10%. In addition, the prototype included a probiotic Orafti® inulin (4%).

The presence of inulin demonstrated a prebiotic effect, promoting the LAB growth, which reached a high microbial count exceeding 8 x 10^8 CFU/g; surpassing the minimum requirement of 10^6 CFU/g according to Mexican norm NOM 181, which establishes that the range should be between 10^6 and 10^8 CFU/g to consider it a functional product. The interaction of LAB within the food matrix was confirmed through SEM.

The resulting functional product contained BAL and inulin (prebiotic); therefore, it is possible to consider it functional, demonstrating excellent microbial and structural properties and offering desirable sensory characteristics such as creaminess and mouth moisture.

Ethical considerations: Chat GPT was used to translate from Spanish to English. Grammarly was employed to check the document's style. After using these tools/services, the authors reviewed and edited the content as needed. Conflicts of interest: The manuscript was prepared and revised with the participation of all authors, who declare that no conflict of interest would jeopardize the validity of the results presented. Authors' contribution: Karina Tiscareño-Ortega: Validation, formal analysis, investigation, original draft preparation, and review and editing; Norma Angélica Chávez-Vela and Julieta Domínguez-Soberanes: Conceptualization, methodology, validation, formal analysis, investigation, resources, original draft preparation, review and editing, visualization, supervision, project administration, and funding acquisition; Gabriela Mariana Rodríguez-Serrano: Conceptualization, methodology, validation, formal analysis, investigation, resources, original draft preparation and review and editing.

REFERENCES

AĞAGÜNDÜZ, D.; COCOZZA, E.: CEMALI, Ö.; BAYAZIT, A. D.; NANÌ, M. F.; CERQUA, I.; SAYGILI, S. K.; CANANI, R. B.; AMERO, P.; CAPASSO, R. 2023. Understanding the role of the gut microbiome in gastrointestinal cancer: A review. Frontiers in Pharmacology. 14:1130562. https://doi.org/10.3389/fphar.2023.1130562

BANCALARI, E.; GATTI, M.; BOTTARI, B.; MORA, D.; ARIOLI, S. 2022. Disclosing *Lactobacillus delbrueckii* subsp. *bulgaricus* intraspecific diversity in exopolysaccharides production. Food Microbiology. 102:103924. https://doi.org/10.1016/j.fm.2021.103924

CASTRO, J.M.; TORNADIJO, M.E.; FRESNO, J.M. SANDOVAL, H. 2015. Biocheese: A food probiotic carrier. BioMed Research International. 2015(1):723056. https://doi.org/10.1155/2015/723056

CEA, J.L.A. 2018. Importancia del lenguaje: Información y vocabulario del análisis sensorial en la cata de quesos. ILE: Industrias lácteas españolas. 40(460):30-46.

ESCOBAR, M.C.; VAN TASSELL, M.L.; MARTÍNEZ-BUSTOS, F.; SINGH, M.; CASTAÑO-TOSTADO, E.; AMAYA-LLANO, S.L.; MILLER, M.J. 2012. Characterization of a Panela cheese with added probiotics and fava bean starch. Journal of Dairy Science. 95(6):2779-2787. https://doi.org/10.3168/jds.2011-4655

FOX, P.F.; GUINEE, T.P.; COGAN, T.M.; MCSWEENEY, P.L. 2017. Fundamentals of cheese science. Ed. Springer. Boston. 271p.

FUENTES-BERRIO, L.; ACEVEDO-CORREA, D.; GELVEZ-ORDOÑEZ, V.M. 2015. Alimentos funcionales: Impacto y retos para el Desarrollo y bienestar de la Sociedad Colombiana. Biotecnología en el Sector Agropecuario y Agroindustrial. 13(2):140-149. https://doi.org/10.18684/BSAA(13)140-149

GIBSON, G.R.; HUTKINS, R.; SANDERS, M.E.; PRESCOTT, S.L.; REIMER, R.A.; SALMINEN, S.J.; SCOTT, K.; STANTON, C.; SWANSON, K.S.; CANI, P.D.; VERBEKE, K.; REID, G. 2017. Expert consensus document: The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. Nature Reviews Gastroenterology & Hepatology. 14(8):491-502. https://doi.org/10.1038/nrgastro.2017.75

GONZÁLEZ-CÓRDOVA, A.F.; YESCAS, C.; ORTIZ-ESTRADA, Á.M.; DE LA ROSA-ALCARAZ, M.D.L.Á.; HERNÁNDEZ-MENDOZA, A.; VALLEJO-CORDOBA, B. 2016. Invited review: artisanal Mexican cheeses. Journal of Dairy Science. 99(5):3250-3262. https://doi.org/10.3168/jds.2015-10103

HAMMAM, A.R.; AHMED, M.S. 2019. Technological aspects, health benefits, and sensory properties of probiotic cheese. SN Applied Sciences. 1:1113. https://doi.org/10.1007/s42452-019-1154-4

HARUTOSHI, T. 2013. Exopolysaccharides of lactic acid bacteria for food and colon health applications. En: Kongo, M. Lactic acid bacteria-R & D for food, health and livestock purposes. IntechOpen. https://doi.org/10.5772/50839

HERNÁNDEZ-CERVANTES, M.; LÓPEZ-VELÁZQUEZ, J.; GÓMEZ-ALVARADO, T.; SANTIAGO-CABRERA, R.; RAMÓN-CANUL, L.G.; DELGADO-VIDAL, F.K.; SHAIN-MERCADO, A.J.; HUANTE-GONZÁLEZ, Y.; DE JESÚS RAMÍREZ-RIVERA, E. 2010. Comparación de la descripción sensorial del queso fresco “cuajada” mediante el análisis descriptivo cuantitativo y el perfil flash. Ciencia y Mar. 14(42):3-12

HUGENHOLTZ, J.; SMID, E.J. 2002. Nutraceutical production with food-grade microorganisms. Current Opinion in Biotechnology. 13(5):497-507. https://doi.org/10.1016/S0958-1669(02)00367-1

HUGHES, R.L.; ALVARADO, D.A.; SWANSON, K.S.; HOLSCHER, H.D. 2022. The prebiotic potential of inulin-type fructans: A systematic review. Advances in Nutrition. 13(2):492-529. https://doi.org/10.1093/advances/nmab119

HUTCHINSON, N.T.; WANG, S.S.; RUND, L.A.; CAETANO-SILVA, M.E.; ALLEN, J.M.; JOHNSON, R.W.; WOODS, J.A. 2023. Effects of an inulin fiber diet on the gut microbiome, colon, and inflammatory biomarkers in aged mice. Experimental Gerontology. 176:112164. https://doi.org/10.1016/j.exger.2023.112164

INTERNATIONAL DAIRY FOODS ASSOCIATION - IDFA. 2023. Pasteurization. International Dairy Foods Association. Washington, D.C. Disponible desde internet en: https://www.idfa.org/pasteurization

JIMÉNEZ-GUZMÁN, J.; FLORES-NÁJERA, A.; CRUZ-GUERRERO, A.E.; GARCÍA-GARIBAY, M. 2009. Use of an exopolysaccharide-producing strain of *Streptococcus thermophilus* in the manufacture of Mexican Panela cheese. LWT-Food Science and Technology. 42(9):1508-1512. https://doi.org/10.1016/j.lwt.2009.04.009

JUNGERSEN, M.; WIND, A.; JOHANSEN, E.; CHRISTENSEN, J.E.; STUER-LAURIDSEN, B.; ESKESEN, D. 2014. The Science behind the Probiotic Strain *Bifidobacterium animalis* subsp. lactis BB-12(®). Microorganisms. 2(2):92-110. https://doi.org/10.3390/microorganisms2020092

KARIMI, R.; AZIZI, M.H.; GHASEMLOU, M.; VAZIRI, M. 2015. Application of inulin in cheese as prebiotic, Fat Replacer and Texturizer: A Review. Carbohydrate Polymers. 119:85-100. https://doi.org/10.1016/j.carbpol.2014.11.029

KARIMI, R.; SOHRABVANDI, S.; MORTAZAVIAN, A. M. 2012. Sensory article: Sensory characteristics of probiotic cheese. Comprehensive Reviews in Food Science and Food Safety. 11(5):437-452. https://doi.org/10.1111/j.1541-4337.2012.00194.x

MACHADO, M.; SOUSA, S.C.; RODRÍGUEZ-ALCALÁ, L.M.; PINTADO, M.; GOMES, A. M. 2023. Functional lipid enriched probiotic cheese: Gastrointestinal stability and potential health benefits. International Dairy Journal. 144:105700. https://doi.org/10.1016/j.idairyj.2023.105700

OBERG, T.S.; MCMAHON, D.J.; CULUMBER, M.D.; MCAULIFFE, O.; OBERG, C.J. 2022. Invited review: Review of taxonomic changes in dairy-related lactobacilli. Journal of Dairy Science. 105(4):2750-2770. https://doi.org/10.3168/jds.2021-21138

PANDEY, K.R.; NAIK, S.R.; VAKIL, B.V. 2015. Probiotics, Prebiotics and symbiotic- A Review. Journal of Food Science and Technology. 52(12):7577-7587. https://doi.org/10.1007/s13197-015-1921-1

POPPER, R. 2014. Use of just-about-right-scales in consumer research. En: Novel Techniques in Sensory Characterization and Consumer Profiling. Varela, P., y Ares, G. (Eds). CRC Press. Estados Unidos. p.137-156.

PRANCKUTĖ, R.; KAUNIETIS, A.; KUISIENĖ, N.; ČITAVIČIUS, D.J. 2016. Combining prebiotics with probiotic bacteria can enhance bacterial growth and secretion of bacteriocins. International journal of biological macromolecules. 89:669-676. https://doi.org/10.1016/j.ijbiomac.2016.05.041

RANI, V; YADAV, U.C. 2018. Functional food and human health. Ed. Springer. New York. 698p.https://doi.org/10.1007/978-981-13-1123-9

RAWI, M.H.; ZAMAN, S.A.; PA'EE, K.F.; LEONG, S S.; SARBINI, S.R. 2020. Prebiotics metabolism by gut-isolated probiotics. Journal of Food Science and Technology. 57(8):2786-2799. https://doi.org/10.1007/s13197-020-04244-5

ROLIM, F.R.; NETO, O.C.F.; OLIVEIRA, M.E.G.; OLIVEIRA, C.J.; QUEIROGA, R.C. 2020. Cheeses as food matrixes for probiotics: In vitro and in vivo tests. Trends in Food Science & Technology. 100:138-154. https://doi.org/10.1016/j.tifs.2020.04.008

SHOAIB, M.; SHEHZAD, A.; OMAR, M.; RAKHA, A.; RAZA, H.; SHARIF, H. R.; SHAKEEL, A.; ANSARI, A.; NIAZI, S. 2016. Inulin: Properties, health benefits and food applications. Carbohydrate polymers. 147:444–454. https://doi.org/10.1016/j.carbpol.2016.04.020

STACHELSKA, M.A.; FOLIGNI, R. 2018. Development of a time-effective and highly specific quantitative real-time polymerase chain reaction assay for the identification of *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* in artisanal raw cow's milk cheese. Acta Veterinaria Brno. 87(3):301-308. https://doi.org/10.2754/avb201887030301

TALBOT-WALSH, G.; KANNAR, D.; SELOMULYA, C. 2018. A review on technological parameters and recent advances in the fortification of processed cheese. Trends in Food Science & Technology. 81:193-202. https://doi.org/10.1016/j.tifs.2018.09.023

TEGEGNE, B.A.; KEBEDE, B. 2022. Probiotics, their prophylactic and therapeutic applications in human health development: A review of the literature. Heliyon. 8(6):e09725. https://doi.org/10.1016/j.heliyon.2022.e09725

THOMPSON, M.S.; YAN, T.H.; SAARI, N.; SARBINI, S.R. 2022. A review: Resistant starch, a promising prebiotic for obesity and weight management. Food Bioscience. 101965. https://doi.org/10.1016/j.fbio.2022.101965

TRESINA, P.S.; SELVAM, M.S.; DOSS, A.; MOHAN, V.R. 2022. Antidiabetic bioactive natural products from medicinal plants. Studies in Natural Products Chemistry. 75:75-118. https://doi.org/10.1016/B978-0-323-91250-1.00004-5

VILLENA, J.; VIZOSO-PINTO, M.G.; KITAZAWA, H. 2016. Intestinal innate antiviral immunity and immunobiotics: beneficial effects against rotavirus infection. Frontiers in immunology. 7:563. https://doi.org/10.3389/fimmu.2016.00563

WEMMENHOVE, E.; WELLS-BENNIK, M.H.J.; ZWIETERING, M.H. 2021. A model to predict the fate of Listeria monocytogenes in different cheese types–A major role for undissociated lactic acid in addition to pH, water activity, and temperature. International Journal of Food Microbiology. 357:109350. https://doi.org/10.1016/j.ijfoodmicro.2021.109350