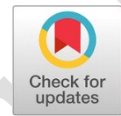




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**Title: Impact of prebiotic fibre on the attributes of probiotic ice cream**



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## Impact of prebiotic fibre on the attributes of probiotic ice cream

### Impacto de la fibra prebiótica en los atributos del helado probiótico

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#### KEYWORDS

Food technology; design; dairy products

Tecnología de alimentos, diseño, productos lácteos

#### ABSTRACT

Ice cream is a complex colloidal food system due to its components. Its complexity makes it an interesting vehicle for bioactive ingredients. In this study, we evaluated the effect of adding inulin (a prebiotic) to a probiotic vanilla-flavoured ice cream on quality parameters. Batches of ice cream were produced with a consistent concentration of the probiotic (*Bifidobacterium*, 10<sup>7</sup> CFU) and inulin concentrations of 3%, 4%, and 6%. Several quality parameters were measured, including physicochemical (pH, total solids, acidity, and density), functional (time to first drip, melting percentage, and overrun), and microbiological (lactic acid bacteria count). These parameters were also sensorially evaluated. Overall, it was found that the concentration of probiotic bacteria is not susceptible to changes in inulin concentration nor to low storage temperatures. The different inulin concentrations did not generate significant changes in pH and acidity. On the contrary, the quality parameters were positively influenced, with a decrease in the melting percentage and first drip, as well as an increase in the percentage of total solids and overrun. In the sensory analysis, it was observed that at these levels of inulin addition, the sensory characteristics of the product remained unchanged.

**RESUMEN:** El helado es un alimento con características coloidales complejas debida sus componentes. Su complejidad lo convierte en un interesante vehículo de ingredientes bioactivos. En este trabajo se evaluó el efecto generado sobre los parámetros de calidad causado por la adición de inulina (prebiótico) en un helado probiótico con sabor a vainilla. Para ello se fabricaron lotes de helado con igual



concentración del probiótico (*Bifidobacterium*,  $10^7$  UFC) y concentraciones de 3%, 4% y 6% de inulina. Se midieron los siguientes parámetros de calidad: fisicoquímicos (pH, sólidos totales, acidez y densidad); funcionales (tiempo de caída de primera gota, porcentaje de derretimiento y *overrun*); microbiológicos (recuento de bacterias ácido-lácticas), y se evaluaron sensorialmente. En general, se encontró que la concentración de las bacterias probióticas no es susceptible a los cambios de concentración de la inulina ni a las bajas temperaturas de almacenamiento. Las diferentes concentraciones de inulina no generaron cambios significativos en el pH y la acidez. Por el contrario, los resultados en los parámetros de calidad son influenciados de manera positiva, observando una disminución del porcentaje de derretimiento y caída de la primera gota, y un aumento en el porcentaje de sólidos totales y *overrun*. En el análisis sensorial se observó que estos niveles de adición de inulina no modifican las características sensoriales del producto.

## 1 Introduction

The pursuit of benefits that contribute to health improvement, physical exercise, and the consumption of healthy foods have become an important part of human life. The food industry is constantly challenged to meet the demand for new food products that are safe, convenient, accessible, palatable, and healthy [1]. The market offers a wide range of products with nutritional advantages, such as fortified, enriched, and functional foods. Currently, products containing beneficial microorganisms for the consumer (probiotics) or nutrients that support the maintenance of intestinal microbiota (prebiotics) have gained popularity. Products that combine both probiotics and prebiotics are termed synbiotic.

Probiotics are microorganisms that beneficially affect the host by modulating and improving intestinal microbial balance. They consist of several species from the *Lactobacillus* and *Bifidobacterium* genera [2]. Various health-related properties have been documented for these microorganisms, including cholesterol level management, anticarcinogenic effects, bacteriocin formation, immune system enhancement, and the production of  $\beta$ -galactosidase with lactose hydrolyzing activity, which is suitable for individuals with lactose intolerance [3]. Probiotics are excellent candidates for functionalizing dairy products, including ice cream, as dairy products represent the largest sector of the functional food market. [4-6].

Prebiotics are a type of soluble fibre, consisting of low molecular weight, short-chain oligosaccharides and polysaccharides (e.g., oligosaccharides), which are not digested in the body but reach the colon unhydrolyzed, where they are selectively metabolized by beneficial bacteria such as *bifidobacteria* and *lactobacilli*, exerting a positive effect on the host's health. Some examples include inulin, lactulose, lactitol, and oligosaccharides (fructooligosaccharides and galactooligosaccharides). Inulin is a polysaccharide of the form  $\text{Glu } \alpha 1-2 [\beta \text{ Fru} 1-2] n$ , where  $n > 10$ . It has a fully demonstrated prebiotic function and is a fructan-type polysaccharide of great importance in the market. This compound is extracted from chicory root and can also be found in various plant species worldwide [7-9].



This study aimed to investigate the quality parameters of ice cream with synbiotic characteristics. The influence of the added probiotics and prebiotics on the organoleptic characteristics of the ice cream was analysed, and it was assessed whether a change in the concentration of the prebiotic would affect the growth of the microorganism concentration during storage.

## 2 Materials and methods

### 2.1 Raw materials and additives

For the production of the ice cream samples, the following ingredients were used: UHT homogenized whole milk and skimmed milk powder (Colanta, Colombia), homogenized and pasteurized cream with 33% fat content (Alquería S.A., Colombia), white sugar (Manuelita S.A., Colombia), glucose (Productos Jardín), probiotic strain mixtures (YO-MIXTM 207 LYO 250 DCU: *Streptococcus thermophilus*, *Lactobacillus delbrueckii* subsp. *Bulgaricus*, *Lactobacillus acidophilus*, *Bifidobacterium lactis*), vanilla essence (RESPIN, Colombia), commercial stabilizers (Helacrem, composition: E471, E466, E407, E433) and inulin (Tecnas S.A., Colombia), added according to the requirements of each of the proposed experiments. The storage containers used were high-density food-grade polyethylene with a capacity of 2 litres. The ingredients were sourced from local supermarkets and specialized retailers.

### 2.2 Experimental design

To evaluate the effect of adding inulin in different concentrations (Control: 0.0%; H3i: 3.0%; H4i: 4.0%; and H6i: 6.0%) on the response variables (ice cream quality parameters), a completely randomized factorial design was conducted [10]. According to the experimental design, three replicates were made for each treatment (~1 L of ice cream mix per treatment), resulting in a total of 12 ice creams. The control treatment consisted of ice cream without inulin. The statistical model for the experiment design is presented in **Equation 1**.

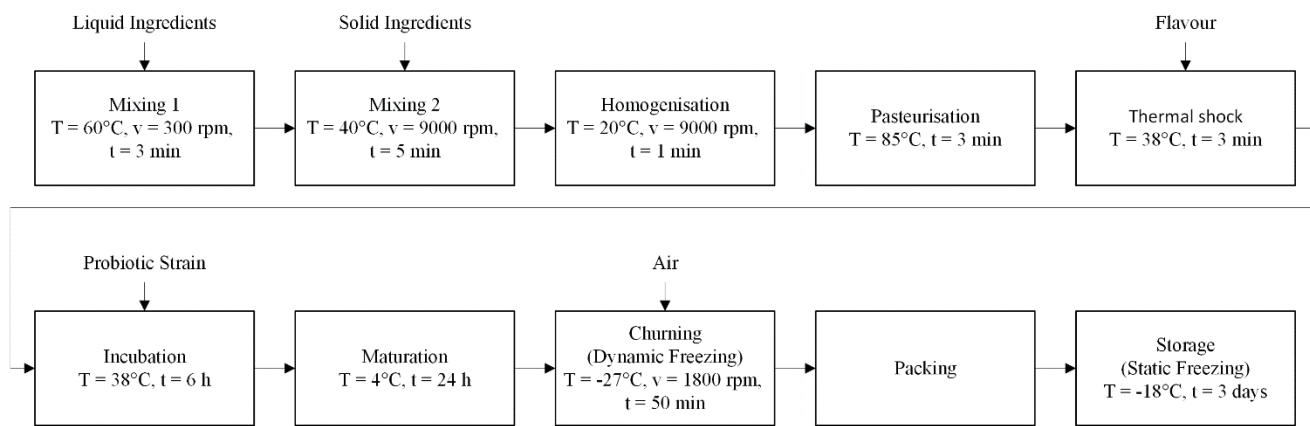
$$y_{ij} = \mu + \tau_i + e_{ij} \quad (1)$$

Where: the subscript **i** is associated with the treatment, and the subscript **j** is associated with the repetition within each treatment.  $y_{ij}$  corresponds to the value of the response variable in repetition **j** of treatment **i**.  $\mu$  is the overall mean of the experiment.  $\tau_i$  is the effect of treatment **i**.  $e_{ij}$  is the experimental error in repetition **j** of treatment **i**.

### 2.3 Ice cream preparation

For the production of the ice cream samples (**Figure 1**), a standard formulation of vanilla-flavoured ice cream was used [11]. The raw materials (liquids and solids) were weighed using an Ohaus Adventurer scale (ARA520, China). The mixing of the ingredients was done in two parts. First, the liquid ingredients (milk at 60 °C, cream, glucose, colouring, and flavouring) were mixed with constant stirring at low speed ( $\pm 300$  rpm) for 3 minutes. Subsequently, the solid ingredients (sugar, powdered milk, and stabiliser) were added to the mixture, which was maintained at 40 °C and stirred at high speed ( $\pm 9000$  rpm,  $\pm 5$  min) until complete dissolution was achieved. The sample was homogenised for one minute ( $\pm 9000$  rpm). The mixture was pasteurised (85°C for 3 minutes) and immediately cooled.

When the mixture reached 45 °C, the probiotic strain was incorporated. The mixture was incubated at 38 °C for 6 hours. Afterwards, it was refrigerated at a temperature ranging between 0 °C and 4 °C for 24 hours (maturation stage). Using an ice cream maker (Princess, Model 282601, Germany), the matured ice cream mix (MICM) was frozen at -27 °C with constant stirring at 1800 rpm for 50 minutes. Finally, it was packaged, labelled, and stored at -18 °C in a freezer (Haceb, N300L SE, Colombia). The order in which the ice creams were made was selected at random. The experiments were conducted in triplicate.



**Figure 1** stages of ice cream formula preparation. (T: temperature, v: velocity, t: time).

### 2.4 Determination of quality parameters

**Physicochemical parameters.** The determination of the physicochemical parameters was carried out on the MICM samples after 24 hours of maturation. All measurements were performed in triplicate. Total solids (TS) were determined according to AOAC 941.08 Gravimetric Method [12], using a Binder drying oven (Model 23 – 115, VD series) at a temperature of 100°C. Titratable acidity was measured using the AOAC 947.01 titration method [13], with 0.1 N sodium hydroxide. The pH was assessed according to AOAC 981.12, using a Mettler Toledo pH meter (Model SevenMulti S40). Density (at 25 °C) was

measured by the gravimetric method with the aid of a pycnometer and a Mettler Toledo analytical balance (model AB204-S/FACT) [11, 14, 15].

**Quality parameters.** The tests for determining the quality parameters were conducted on the ice cream samples after 7 days of storage. The analyses were performed at room temperature (between 25 °C to 27 °C). The following parameters were determined: overrun percentage (OP), time ( $\theta$ ) to first drip (TTFD), melting rate (MR), and melting percentage (MP). For TTFD, MR (see equation 2), and MP (see equation 3), a sample of approximately 70 grams (initial mass, IM) of ice cream stored at -18 °C was placed on a mesh with 56 holes·cm<sup>-2</sup>, the TTFD was measured, and the mass of melted ice cream (melted mass, MM) was weighed every two minutes.

The melting rate was calculated as the quotient of the melted mass over time [14]. To determine the OP, a cylindrical corer was used to obtain a sample of ice cream with dimensions of 1.13 cm radius (r) and 7.14 cm height (h) (4), corresponding to the initial ice cream volume (initial volume, IV). The sample was placed in a graduated cylinder, and the volume after melting (final volume, FV, air-free mix volume) was recorded. Equation (4) was used to calculate the OP Equation (5) [16].

$MR = \frac{MM}{\theta}$	(2)
$MP(\%) = \frac{MM}{IM} \times 100$	(3)
$IV = \pi r^2 h$	(4)
$Op(\%) = \left( \frac{IV - FV}{IV} \right) * 100$	(5)

**Microbiological determination.** This analysis was conducted on both the MICM and ice cream samples. The following equipment and materials were used: an incubator at 35°C ± 0.5°C (Memmert, Germany), colony counter (Indulab, Ref 007, Colombia), autoclave (MARKET Forge Industries Inc., USA), MRS agar (Merck, Germany), and disposable plastic Petri dishes. The determinations included: counting of mesophilic lactic acid bacteria according to NTC 5034 [17]; fungi and yeasts according to NTC 3954 [18]; aerobic mesophilic bacteria (AMB) according to NTC 3908 [19]; enterobacteria according to NTC 5652 [20]; and *E. coli* according to NTC 4306 [21].

**Organoleptic properties:** Individuals who voluntarily agreed to serve as sensory evaluators participated in two consumer-oriented tests. The same evaluation form presented both the nine-point hedonic test information and the preference test information. These tests were conducted from 9:30 to 10:30 am at the Universidad Santiago de Cali (Coordinates: 03° 27'0" N 76° 32'0" W, Altitude 995 m.a.s.l.). The



sensory acceptance and preference of ice cream samples were evaluated. Thirty-five untrained consumers sensorially evaluated the ice cream samples. The ages of the consumers ranged from 17 to 30 years.

The participants included both men and women of various occupations, socioeconomic backgrounds, and regional origins. Additionally, the participants were selected from the students at the University. The criteria for panelist selection were regular ice cream consumption and good health. A few minutes before the test, they completed a brief survey about their health and consumption habits. The selected panelists had no allergies or sensitivities to the ingredients of the ice creams. They were given a brief explanation of the research objectives and the importance of their participation. The samples were presented in cups ( $\pm 4$  g) marked with random numbers (179  $\equiv$  C, 485  $\equiv$  H3i, 760  $\equiv$  H4i, 923  $\equiv$  H6i). Each panelist evaluated each sample only once. The duration of the test did not exceed one hour [22].

## 2.5 Statistical analysis

The data obtained were statistically analysed using the free version of PSPP® software, version 0.7.9 B20120320. Graphs were generated using Excel 2010 (Microsoft Office Excel 2010). Analysis of variance (ANOVA) was used to evaluate significant differences in the physicochemical analysis data among the ice cream batches produced with different concentrations of inulin. As a post hoc analysis, the Tukey test was used with a significance ( $\alpha$ ) of 0.01. Sensory analysis and its correlation with instrumental results were performed using Friedman and Wilcoxon tests [22].

## 2.6 Ethical parameters

**Informed consent.** For the execution of consumer surveys for sensory analysis, an introduction and clear explanation about the reasons, purposes, and defined objectives of these tests were provided. A brief description of the product was given, detailing each of the ingredients, to ensure that any participant with allergies or intolerances would be excluded from the test. Additionally, written consent was obtained from all participants to use the information collected in the surveys for research purposes.

## 3 Results and discussion

### 3.1 Physicochemical parameters

**Table 1** presents the average values ( $\bar{X} \pm SD$ ) of the physicochemical parameters of probiotic ice cream samples enriched with inulin (C: 0.0%; H3i: 3.0%; H4i: 4.0%; and H6i: 6.0%). The control ice cream sample (C) did not contain inulin. Averages with a similar superscript in a row do not differ significantly ( $p < 0.01$ ).



**Table 1.** Average values of physicochemical parameters of probiotic ice cream samples enriched with inulin

Parameter	Concentration			
	C	H3i	H4i	H6%i
pH	5.6033 ± 5.77E-03 <sup>a</sup>	5.6100 ± 0.0E+00 <sup>a</sup>	5.6033 ± 5.77E-03 <sup>a</sup>	5.6100 ± 0.00E+00 <sup>a</sup>
Acidity	0.3767 ± 5.77E-03 <sup>a</sup>	0.3700 ± 6.8E-17 <sup>a</sup>	0.3767 ± 5.77E-03 <sup>a</sup>	0.3733 ± 5.77E-03 <sup>a</sup>
Density	1.0895 ± 4.73E-04 <sup>b</sup>	1.0997 ± 1.5E-04 <sup>a</sup>	1.1004 ± 2.89E-04 <sup>a</sup>	1.0998 ± 1.53E-04 <sup>a</sup>
Total Solids	33.08 ± 4.04E-02 <sup>d</sup>	33.96 ± 6.0E-02 <sup>c</sup>	34.50 ± 2.08E-02 <sup>b</sup>	35.02 ± 6.43E-02 <sup>a</sup>

**pH.** No significant differences ( $p > 0.01$ ) were observed between treatments in the obtained pH values. On average, a pH of 5.61 was obtained with a maximum variation of 0.1 pH units. The variation in inulin concentration does not significantly affect the pH. Values ranging from 4.18 to 4.94 were reported for yogurt ice cream containing microencapsulated *Bifidobacteria* BB-12, which were significantly lower than the values obtained in our research [23]. These differences in acidity levels are attributed to variations in the fermentation time of the milk before ice cream production. On the other hand, researchers developed prebiotic ice creams with three different levels of inulin addition (0%, 2.5%, and 5%). They found that incorporating inulin didn't lead to noteworthy alterations in the ice creams' pH and titratable acidity [24].

**Acidity.** Expressed as a percentage of lactic acid, it showed an average value of 0.38% with a variation between treatments of 0.3%. The results obtained are within the specification suggested by the Colombian technical standard (NTC 1239) for ice creams and ice cream mixes (yogurt ice creams with a minimum of 0.25%) [25]. Additionally, it was observed that the results did not differ significantly between the four treatments ( $p > 0.01$ ). Although the ice cream studied is not categorized as a yogurt ice cream, it shares similar characteristics due to the incorporation of probiotic microorganisms that produce acidity in the mix by transforming the present lactose.

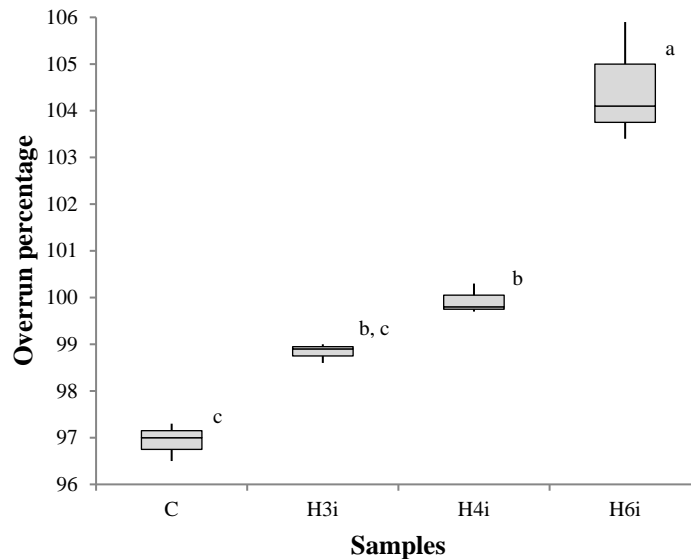
**Density.** Table 1 presents the average density values of the ice cream samples. The control sample shows a significant difference ( $p < 0.01$ ) compared to the samples containing inulin; however, no significant differences are observed among the inulin-containing samples. The density value remains constant despite changes in the inulin content. In contrast, the total solids (TS) content was significantly different among all treatments ( $p < 0.01$ ).

**Total Solids.** The results show that the increase in total solids (TS) was linear and proportional to the amount of inulin added in each treatment. This behaviour was similar to that reported by other authors [26-28]. Furthermore, according to NTC 1239, the TS should be between 33% and 36%, which was achieved in all the samples produced [25].



### 3.2 Functional Parameters

Significant differences ( $p < 0.01$ ) were observed in the OP (overrun percentage) values between the different treatments (**Figure 2**).

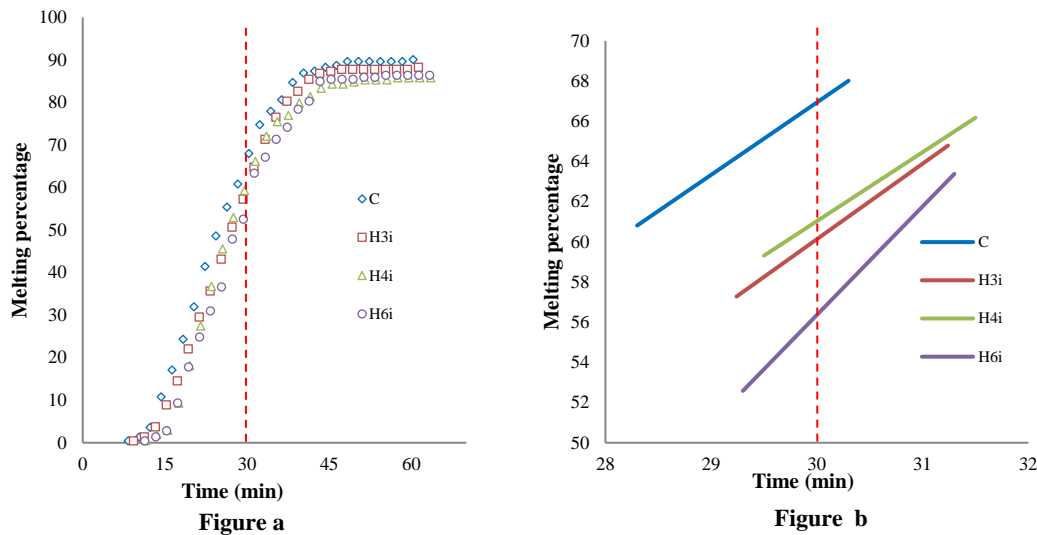


**Figure 2** Box and whisker plot of results for probiotic ice cream samples enriched with inulin

According to the results, the OP increases with higher insulin concentration. Air incorporation depends on the composition of the mix, primarily the fat content, as well as the type and amount of stabilizer and other solid components. The addition of inulin enhances air retention leading to a higher OP in the ice cream. This may be attributed to the better distribution of air bubbles in the network formed by proteins and stabilizers. However, excessive air incorporation air results in low-quality ice cream, which lacks body and melts quickly. Conversely, ice cream with minimal air incorporation creates a dense, heavy sensation that is equally undesirable [29].

The TTFD was recorded as follows: C: 8.3 min; H3i: 9.2 min; H4i: 11.5 min; and H6i: 11.3 min. The TTFD increased with the addition of inulin, indicating that the ice cream takes longer to melt. **Figure 3a** presents the melting curves for the different concentrations of inulin, and **Figure 3b** shows the melting percentage at 30 minutes. The curves demonstrate that as the concentration of inulin increases, the melting percentage decreases. The H3i and H4i treatments exhibited a similar behaviour. Regarding the melting rate, the average values recorded were C: 0.50 g/min; H3i: 0.45 g/min; H4i: 0.43 g/min; and H6i: 0.43 g/min, showing that as the concentration of inulin increases, the melting rate decreases. Augmenting solids concentration decreases melting and prolongs TTFD [14]. Composition alteration affects thermal diffusivity, possibly delaying/accelerating heat penetration [30]. Elevated inulin concentration

substantially extends TTFD, which indicates melting time [31]. Similarly, inulin incorporation significantly boosts overrun [24].



**Figure 3.** Melting percentage of probiotic ice cream samples enriched with inulin.

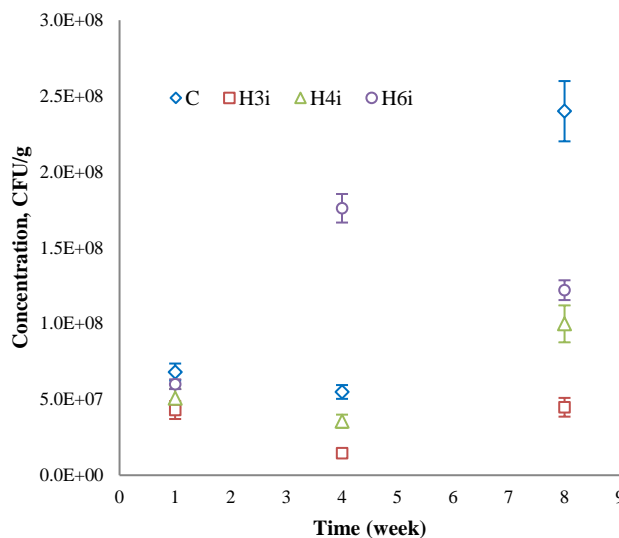
Melting resistance in ice cream is influenced by various factors such as overrun percentage (OP), type of stabilizer used, composition (proteins, fats), and size of ice crystals [30]. The melting rate is influenced by the penetration of heat into the ice cream, with a direct relationship existing between thermal diffusivity and melting rate [16]. Inulin acts as a stabilizer, and along with the commercial stabilizers present in the formulation, it helps to immobilize water molecules and reduce their freedom of movement among other molecules in the mix, thus delaying the melting of the product [32, 33]. Additionally, inulin can form microcrystals that interact creating small aggregates that trap a large amount of water, resulting in a creamy and smooth texture that mimics the mouthfeel of fat [34], making it an effective fat replacer in such products.

### 3.3 Microbiological Analysis

For quality control of the raw materials (powdered milk, liquid milk, cream, and inulin) and the finished product (C, H3i, H4i, H6i), microbiological analyses were conducted for moulds and yeasts, enterobacteria, *E. coli*, and aerobic mesophilic bacteria (AMB). Values lower than  $10^1$  were observed for each of the microbiological parameters, both in the raw materials and the finished product. Quality control was also performed on the finished product during its shelf life at weeks 1, 4, and 8, with the

same result: values lower than  $10^1$ . The values found in the quality control comply with the specifications described in the Colombian technical standard NTC 1239 Ice Creams and Ice Cream Mixes [25].

The count of LAB (Lactic Acid Bacteria) was conducted throughout the proposed shelf life of the ice cream (8 weeks) to verify the stability of the probiotic strain mix under storage conditions ( $-18^{\circ}\text{C}$ ), with three independent analyses performed at week 1, 4, and 8. The probiotic content was analyzed before and after the partial freezing and air incorporation treatment performed in the ice cream machine at an average temperature of  $-20^{\circ}\text{C}$ . This was done to ensure that this treatment did not affect the initial concentration of probiotics in the sample. Before freezing, a value of  $5.20 \times 10^7$  CFU/g was obtained. According to the results, there was no evident decrease in the concentration of LAB after this process. **Figure 4** presents the behaviour of the LAB during the shelf life of the probiotic ice cream samples enriched with inulin.



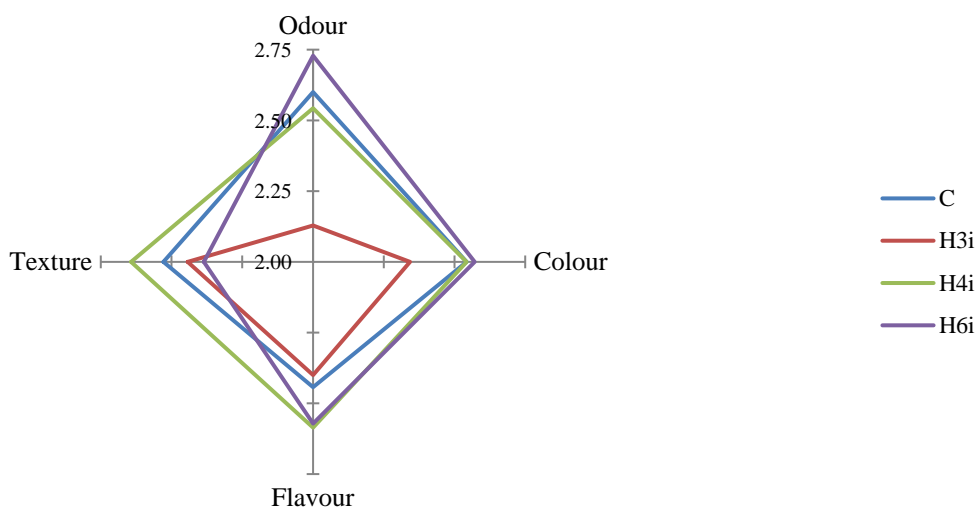
**Figure 4.** Behaviour of lactic acid bacteria during the shelf life of probiotic ice cream enriched with inulin

After conducting an analysis of variance among the average values of each batch, it was determined that the results were not significantly different ( $p > 0.05$ ). This indicates that the concentration of inulin does not positively or negatively affect the LAB concentration in the samples. However, it is known that the addition of prebiotics, especially those based on carbohydrates, has been widely used as ingredients in functional foods to promote the survival of probiotic bacteria over time [4]. To achieve the positive physiological effects exerted by probiotics, it has been suggested that a sufficient amount of viable bacteria, approximately  $10^9$  CFU/g, should be ingested [2]. In this research, values between  $5.0 \times 10^7$  CFU/g and  $2.5 \times 10^8$  CFU/g were obtained at eight weeks of storage.



### 3.4 Organoleptic Properties

In the analysis of the preference test results, it was found that the preference percentage for the ice cream samples was C: 13.6%, H3i: 22.7%, H4i: 36.4%, H6i: 27.3%. The sample most preferred by the participants was the one containing 4% inulin. On the other hand, the results of the hedonic test, summarized using the average ranks from the Friedman test, show no significant differences in consumer acceptance. Although there are no significant differences ( $p>0.05$ ) between the ice cream samples, the highest scores for colour and odour were given to the H6i sample. Meanwhile, the H4i treatment achieved the highest values for flavour and texture. As seen in **Figure 5**, odour was the attribute with the greatest dispersion, with the H3i treatment receiving a lower value for this parameter.



**Figure 5.** Radar charts showing the representation of the average ranges from the Friedman Test

## 4 Conclusions

It can be concluded that the addition of inulin to a probiotic ice cream yielded positive results, with a decrease in Melting Percentage (MP) and Time to First Drip (TTFD), as well as an increase in Total Solids (% TS) and Overrun Percentage (OP). When evaluating physical properties such as pH and acidity, it was found that the addition of different amounts of inulin does not cause significant changes in these parameters. The addition of inulin improved the incorporation of air into the mix (OP), achieving optimal percentages for good quality ice cream, and increasing the product's profitability.

The TTFD and MP were also positively influenced as the concentration of inulin increased, delaying the TTFD and, consequently, the MP of the ice cream. The concentration of probiotic bacteria was not susceptible to changes in inulin concentration or to low storage temperatures. Microbiological control is an important quality parameter for assessing the manufacturing practices used in the food product.

## 5 Declaration of competing interest

We declare that we have no significant competing interests including financial or non-financial, professional, or personal interests interfering with the full and objective presentation of the work described in this manuscript.

## 6 Acknowledgment

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## 8 Author contributions

The authors contributions to this research are as follows: Contributed to conceptualization, bibliographic investigation, project administration, experimental methodology development, data collection, and writing of the paper: J. S. Ramírez, Y. Diez and L. M. Grisales; played a role in initially conceptualizing the study, establishing the research methodologies and contributed to data organization and analysis: J. S. Ramírez.

## 9 Data availability statement

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

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