



DEVELOPMENT AND CHARACTERIZATION OF MILK FERMENTED WITH VIILI ADDED OF CURCUMA LONGA

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ABSTRACT

The promotion of health through proper nutrition is a growing concern in daily lives of many people, so the demand for functional foods is increasing. This study aimed to develop a fermented milk with natural dye by Turmeric fermented by mixed culture called Viili, that contains fungus, yeasts and lacto bacteria. The Turmeric was added as a dye and also for its functional and antioxidant properties. Six fermented milk formulations were prepared: Fm (pure fermented milk), Tm (fermented milk added turmeric - 0.6% w/v); Sm (fermented milk added sugar 10% w/v); STm (fermented milk added turmeric - 0.6% w/v and sugar 10% w/v); Scm (fermented milk added salt 1.3% w/v); ScTm (fermented milk added salt 3% w/v and turmeric). The samples were stored for 30 days and subjected to analysis in the 1st, 15th and 30th day of storage. All formulations had *Lactococcus lactis* counts above 10⁸ CFU / mL. Samples without the addition of turmeric in the are lighter than samples that have added turmeric to the formulation. The pH of the samples showed a significant reduction with variation in acidity. Formulations added turmeric presented curcuminoids unchanged in all samples until the end of storage. Samples without the addition of turmeric showed lower amounts of total phenolics. The viscosity varied between the samples. The general sensorial acceptance of the product using the hedonic scale reached greater acceptance in the product with the addition of salt. When with the addition of sugar, there was a reduction in acceptance.

1. Introduction

The concept of functional foods associates to several products the ability to provide physiological benefits and contribute to the health of consumers. The market for products with functional claims has grown significantly, stimulating research and development of new ingredients and products.

Viili is a very viscous fermented milk traditional to the Nordic countries, most commonly found in Finland. Traditionally, it is made from non-homogenized milk, which results in the formation of a cream layer on the surface of the milk (Leporanta, 2003) The

fermentation is carried out by a mesophilic culture, which contains *Lactococcus lactis* subsp. *cremoris*, *Lactococcus lactis* subsp. *lactis* biovar *diacetylactis* and *Leuconostoc mesenteroides* subsp. *cremoris*, along with the fungus *Geotrichum candidum*, which cover the surface of the product (Tamime and Marshall, 1997; Vasilkevici and Shah, 2008). In addition, viili also contains yeasts *Kluyveromyces marxianus* and *Pichia fermentans*, all microorganisms form a unique symbiotic system (López et al., 2010). *Lactococcus lactis* subsp. *cremoris* produces exopolysaccharides

(EPS), thus conferring characteristic intense viscosity.

Several benefits are possible from such microorganisms, including control of intestinal infections, control of cholesterol levels, positive influences on the immune system, improved utilization of lactose in people who do not digest it well, and anticarcinogenic action. This group of bacteria and yeasts that contribute to the regulation of the composition of the intestinal microbiota and offer the possibility of influencing the development of the mucosa and systemic immunity are called probiotics (Gilliland, 2001). Lactic acid bacteria (LAB), of some genera are proven probiotics, can ensure intestinal homeostasis as well as interact with epithelial cells as well as immune cells associated with the gut to induce activation of the immune system (Galdeano et al., 2010). Most administered probiotic cells exert health benefits through adhesion to intestinal cells. However, it has been recommended that oral administration of probiotics be continuous, as cells are constantly eliminated from the digestive tract through feces and host physiological changes and/or administration of antimicrobial agents such as antibiotics (Fung et al., 2011).

Exopolysaccharides (EPS) are long-chain polysaccharides consisting of repetitive units of sugars or sugar derivatives. They are present on the surface of many bacteria, including some lactic acid bacteria (LAB) and may be attached to the bacterial surface forming a capsule, weakly attached, or may be fully secreted into the environment (Ramchandran & Shah, 2010). In general, EPS-producing bacteria exhibit good adhesion properties that may be of interest for transient colonization of the gut. EPS can interact with host cells, and thus are of biological, biotechnological or medical interest (Lopez et al., 2012). In addition EPS can present a selective advantage for probiotic bacteria to survive adverse conditions in the gastrointestinal tract after food ingestion (Salazar et al., 2011; Oerlemans et al., 2021).

EPS-producing LABs have gained considerable attention in the industry, they have traditionally been used for the manufacture of

fermented dairy products due to their ability to confer desirable sensory attributes such as increased viscosity, consistency and improved stability and texture. EPS produced by lactic acid bacteria are a natural alternative as a replacement for commercial additives of plant or animal origin, and the use of EPS can result in a safe, natural and healthy final product with improved texture and stability, which can have an important impact on the development of new products (Derriche et al., 2021).

Some studies with EPS, isolated from lactic acid bacteria, showed that they were able to neutralize the effect of bacterial toxins and enteropathogens, thus conferring a potential benefit to the host (Werning et al., 2022; Thorakkatu et al., 2022). Other benefits attributed to EPS are antitumor and immunostimulant activity and prebiotic effect (Cázares-Vásquez et al., 2021; Prete et al., 2021). In addition, studies associate the consumption of EPS with cholesterol level reduction, in addition to antioxidant, anti-inflammatory, anti-cancer activity and promotion of natural immunity (Bengoa et al., 2021; Jurásková et al., 2022).

Turmeric is also known as saffron or golden ginger, it is a plant with the scientific name *Curcuma longa* L., from the Zingiberaceae family, native to South and Southwest Asia and extensively cultivated in India, China, Taiwan, Japan, Burma, Indonesia, and the African continent (Jyotirmayee & Mahalik, 2022). The crop was introduced in Brazil in the 1980s, the plant is easy to cultivate and has the advantage of not requiring special cultural treatments, developing well in various tropical conditions (Tanwar et al., 2022). Figure 1 shows images of the rhizome and turmeric powder. It is used as a dye in food and beverages, as a condiment, as a flavoring agent, and as medicine (Pelissari et al., 2022). As for its sensory characteristics, turmeric has a weakly aromatic odor, reminiscent of ginger, a pungent and slightly bitter taste (ANVISA, 2010). Interest in turmeric has increased significantly in recent years. This is due to the fact that it is a natural product and has color characteristics similar to those of tartrazine, synthetic yellow dye widely used in

the food and pharmaceutical industry, which can cause adverse reactions to man (Somasundaram et al., 2002).

Besides being known for its coloring and flavoring properties, it is well known and exploited by traditional Asian medicine as an anti-inflammatory, anti-arthritic, bile function regulator and cholesterol level reducer, carminative, antispasmodic, antioxidant, anti-diarrheal, and diuretic (Abd El-Hack et al., 2021; Jyotirmayee & Mahalik, 2022). Through studies developed using extracts of turmeric rhizome, antioxidant, antimicrobial, anti-inflammatory and anticancer activities were identified (Shi et al., 2021; Lee et al., 2003). In addition to antioxidant effects, several benefits have been attributed to turmeric, including anticarcinogenic activity. Fuloria et al. (2022) showed that the addition of turmeric ethanolic extract at concentrations of 0.5 to 1%, in the diet of mice, significantly inhibited tumor multiplicity, tumor burden, and tumor incidence, when the administered at early tumor stage. Curcumin, a dietary polyphenol found in turmeric, has been shown in studies to have an anti-adipogenic function. Curcumin inhibits the synthesis of fatty acids, suppressing the accumulation of lipids. Through its interaction with diverse signal transduction pathways, curcumin can reverse insulin resistance, hyperglycemia, and other inflammatory symptoms associated with obesity and metabolic diseases (Boaz, 2011).

Due to the increasing expansion of dairy products in the functional food segment, the

objective of this work was to develop different formulations of a fermented milk, named viili, with exopolysaccharides (EPS) and containing probiotic *Lactococcus lactis*, added of a natural dye, an ingredient that presents functional and antioxidant property. To characterize and analyze the viability of microorganisms and constituent compounds of the formulations, over 30 days of storage at 4°C, in order to enable health benefit claims with the ingestion of the products. To also perform a sensory analysis to verify the acceptance of the fermented milks by potential consumers.

2. Materials and methods

2.1. Materials

2.1.1. Viili Culture

To promote milk fermentation, a culture of Mesophilic viili powder contained *Lactococcus lactis* subsp. *cremoris*, *Lactococcus lactis* subsp. biovar *diacetylactis*, *Leuconostoc citrovorum*, *Kluyveromyces marxianus* and *Geotrichum candidum*.

The culture was reactivated by three successive fermentations in skimmed milk (Molico, Néstle) reconstituted at 13% (w/v) and frozen (-18 °C) until use with 20% glycerol (v/v) in 5mL portions (inoculum).

2.1.2. *Curcuma longa*

The turmeric used in the preparation of the formulations was acquired in the local market, in the form of powder, of the same brand (Kirin) and same batch.



Figure 1. Images of turmeric in rhizome and powder form (Himesh et al., 2011)

2.2. Methods

2.2.1. Fermented milk

Powdered milk was used for the production of fermented milk. skimmed, reconstituted at 13% (w/v) in distilled water. The milk was heated (95°C), in a water bath with thermostat, for 5 minutes, cooled to 20°C. 4%(v/v) of the mesophilic starter culture inoculum added.

The fermentation was carried out in 1L glass flasks at 20°C for 24h. Six different formulations were produced, being them Fm - natural fermented milk without any addition, Tm - milk fermented with the addition of turmeric, Sm - fermented milk with the addition of sucrose, STm - fermented milk with addition of sucrose and turmeric, ScM - milk fermented with addition of sodium chloride, ScTm- fermented milk with addition sodium chloride and turmeric. After the fermentation, the products were refrigerated to 4°C for up to 30 days. The amount of turmeric powder was determined according to the maximum concentration of turmeric, as a dye, allowed by Brazilian legislation in fermented milk (BRASIL, 2000).

2.2.2. Lactic Acid Bacteria Count

The quantification of lactic acid bacteria was performed in MRS agar (Man Rogosa and Sharp, pH 6.5 ± 0.2), with addition of cycloheximide (200 mg.L⁻¹), to inhibit fungus. The plates were incubated at 30°C for 72 hours, in anaerobiosis. The count was in CFU.mL⁻¹ of the milk fermented (Irigoyen et al, 2005).

2.2.3. *Lactococcus lactis* count

The count of *Lactococcus lactis* was performed using the medium M17 added with cycloheximide (200mg.L⁻¹) to inhibit fungi (pH 7.2 ± 0.2) with incubation at 30°C under anaerobic conditions for 48 hours, at methodology was performed according to Irigoyen (2005).

2.2.4. Total Fungi count

The fungi count was performed in PDA medium added with acid 10% tartaric acid (pH 3.5 ± 0.2), with incubation temperature of 25°C, in aerobic conditions, for 120 hours. The result was expressed in CFU.mL⁻¹ of the fermented milk (BRASIL, 2003).

2.2.5. Color

Color measurements were made with digital colorimeter (Konica Minolta Sensing, Inc., Tokyo, Japan) in CIELab system (L*, a* and b*) with illuminant D65.

2.2.6. Viscosity

The viscosity of the samples was determined using Brookfield digital viscometer, with spindle 4, speed of 12 rpm, in 600mL of a sample kept under refrigeration (Hassan et al., 2022).

2.2.7. Curcuminoids

Curcuminoids present in turmeric powder and samples added turmeric were determined qualitatively by analysis of Thin Layer Chromatography. As support for the stationary phase, it was A silica plate was used to apply and run the samples. the phase mobile consisted of chloroform, ethyl alcohol and glacial acetic acid, mixed in the ratio of 95:5:0.5. The chromatogram obtained was examined under light ultraviolet at a wavelength of 365nm (ANVISA, 2010).

2.2.8. Total phenolic content

The extracts for the analyzes were obtained with 1 gram of the sample lyophilized and addition of 80% ethanol in the proportion 1:10, with stirring for 20 min. The mixture was centrifuged at 3500 rpm, following the supernatant to concentration on a rotary evaporator at 70°C to 10 mL. The solution was then stored at -22°C until used. The analysis followed the methodology described by Adom and Liu (2002).

The determination of phenolic compounds was performed using 2.5 mL of Folin-Ciocalteu reagent (10%), 2.0 mL of sodium carbonate 7.5% and 0.5 mL of sample extracts. The phenolic compounds were determined with reading in spectrophotometer at 760 nm. The quantification was performed by the standard gallic acid curve and the results were expressed in mg equivalents of gallic acid/100g on a dry basis (Swain and Hills, 1959).

2.2.9. Antioxidant activity

The antioxidant capability of the extracts with respect to the ABTS+• free radical (2,2-azino-bis-[3-ethylbenzthiazoline-6-sulfonic acid], Sigma Aldrich Chemie, Steinheim,

Germany) was determined using the method described by Sanchez-Gonzales et al. (2005). The absorbance was read at 730 nm in a UV-vis spectrophotometer (model Libra S22, Biochrom, Cambridge, UK). The quantification was based on a standard curve of Trolox (100–2000 mM), and the results were expressed in TEAC as $\mu\text{mol Trolox/g}$ sample on a dry basis. The antioxidant activity by scavenging activity of DPPH• (2,2-diphenyl-1-picrylhydrazyl, Sigma Aldrich Chemie, Steinheim, Germany) was performed according to Brand-Williams et al. (1995), with absorbance was read at 517 nm in a UV-vis spectrophotometer (model Libra S22, Biochrom, Cambridge, UK). The quantification of the extracts was performed using a standard curve of Trolox (100–1000 mM, Sigma Aldrich Chemie, Steinheim, Germany), and the results were expressed in Trolox equivalent antioxidant capacity (TEAC) as $\mu\text{mol Trolox/g}$ sample on a dry basis.

2.2.10 pH, titratable acidity and Centesimal

Acidity by titration with 0.1M NaOH solution, expressing the results in g of lactic acid/100 g fresh weight (IAL, 2008). pH measurements were made with digital potentiometer (Hanna, HI 223) and the centesimal composition of the fermented milk samples was determined through analysis of lipids, ash, moisture, protein and carbohydrates per difference (AOAC, 2006).

2.2.11. Exopolysaccharides (EPS)

EPS quantification was performed following Schiavão-Souza et al. (2007), with modifications. The procedure consisted of weighing 10g of the samples in 50mL centrifuge tubes, added with 0.25 mL of acid 80% trichloroacetic acid, the samples were stirred under refrigeration (4°C) for 30 minutes. Afterwards, they were centrifuged at 3000 rpm for 10 minutes at 4°C. The EPS were precipitated by addition of cold ethanol in the supernatants obtained. The EPS were separated through a new centrifugation under the same conditions as above.

Total carbohydrates were determined with the precipitate obtained diluted in 2mL of distilled water. 1mL of the diluted precipitate was added to 1mL of 5% phenol plus 5 mL of

concentrated sulfuric acid. The tubes were left to rest for 10 minutes and then warmed up in a water bath at 30°C for 20 minutes. After this procedure, reading in spectrophotometer at 490nm. The total carbohydrates were determined from the glucose calibration curve at concentrations of 10 to 100 $\mu\text{g/mL}$.

2.2.12. Sensory analysis

The project was submitted and approved by the Ethics Committee in Research Involving Human Beings (Process No. 117.625) of State University of Londrina.

A sensory test was carried out for each product, with the addition of sucrose with the participation of 99 potential consumers and added sodium chloride with 74 potential consumers to evaluate product attributes. The judges were instructed to indicate how much they liked or disliked the products in relation to the attributes of color, aroma, texture, flavor and overall acceptance, through a structured nine-point hedonic scale ranging from “I disliked extremely (1)” to “Like Extremely (9)” (Stone and Sidel, 2004).

After, the judges were also asked to indicate their intention to purchase the product, using a seven-point scale ranging from “Certainly not would buy (1)” to “Certainly would buy (7)” (Stone and Sidel, 2004).

2.2.13. Statistical Analysis

The physical, chemical and microbiological analyzes followed the completely randomized design. The analysis was carried out in triplicate and the results were submitted to the Analysis of Variance (ANOVA) and Tukey's test, for comparison of means at the 5% level of significance. The experimental designs for sensory evaluation were randomized block design, where the treatments were the formulations and the blocks the potential consumers. The results were submitted to ANOVA and Tukey's test.

3. Results and discussions

3.1. Content of curcuminoids

The qualitative analysis during the 1st, 15th and 30th day was performed in fermented milk samples added with 0.6% (w/v) of turmeric and kept under refrigeration at 4°C.

The 3 main curcuminoids present in yellow turmeric and that contribute to its functional properties are: curcumin, dimethoxycurcumin and bisdemethoxy curcumin (Reddy et al., 2019). For comparisons, the curcuminoids present in white curcuma were also analyzed qualitatively by TLC.

From a thin layer chromatogram read under ultraviolet light at a wavelength of 365nm, it was possible to observe and compare the bands of the components in yellow turmeric powder and those present in the samples. In Figure 2 it can be verified for turmeric powder in three distinct bands.

When examined under ultraviolet light (365 nm), turmeric powder presented in the middle part of the silica plate, a green spot fluorescent, which corresponding to demethoxycurcumin, corresponding in figure to point 2. In the upper third, the stain referring to the curcumin, point 3, also fluorescent green and in the lower third, the stain with the same color as the previous ones referring to the bisdemethoxycurcumin, point 1. The fluorescent bands that appear in the figure before point 1, are the sample application sites (ANVISA, 2010).

After reading the plate under UV light, the Retention Factor calculations (Rf) were performed at each of the points to certify that each point actually belonged to the above mentioned compounds. From the descriptions of identification and calculation of Rf for curcuminoids provided by the Pharmacopoeia Brazilian (ANVISA, 2010), the presence of the 3 main curcuminoids in yellow turmeric used for milk formulations fermented. The analysis also allowed to observe that the 3 compounds did not degraded or underwent changes over the 30 days of storage. In Figure 3 one can observe differences in the bands between the white and yellow turmeric.

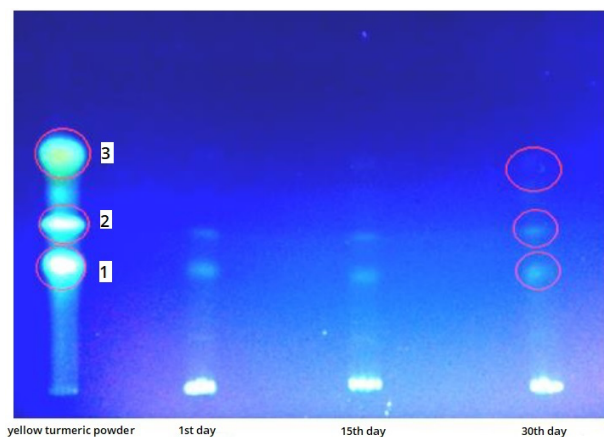


Figure 2. Curcuminoids present in yellow turmeric powder and fermented milk samples with turmeric added during the 30 days of storage. Bisdemethoxycurcumin (1); demethoxycurcumin (2); curcumin (3).

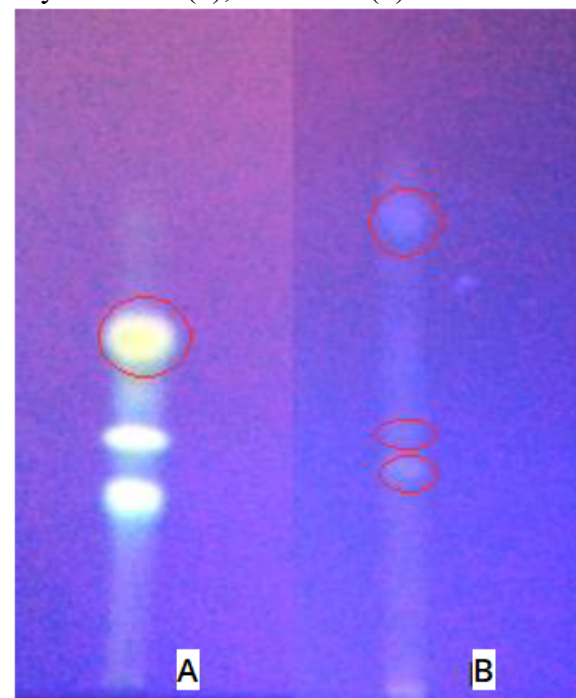


Figure 3. Curcuminoids present in yellow turmeric powder (A) and white turmeric (B).

3.2. Lactic Acid Bacteria and *Lactococcus lactis* Counts

The table shows the results during the 1st, 15th and 30th day of storage for total lactic bacteria count and *Lactococcus lactis* in the 6 tested formulations. All formulations showed results above 8 Log CFU.mL⁻¹ during the 30 days of storage, maintaining high counts, being considered a source of probiotics.

The analysis for fungi was also performed in this work, although *Geotrichum candidum* was described as a of the constituent microorganisms in the culture, none of the treatments identified the presence of the fungus, which in traditional viili grows on the surface of the product and forms a velvety covering, but there is also a type of viili without the fungus (LEPORANTA,

2003). The absence of this microorganism was probably due to the form of fermentation, since pasteurized milk and inoculated with the mixed culture of viili was fermented in 1L closed flasks, which resulted on a reduced contact surface and oxygen limitation, essential for the fungal development.

Table 1. Counts of Lactic Bacteria and *Lactococcus lactis* during 30 days of storage.

Samples**	Time (days)	Lactic Bacteria* (Log CFU.mL ⁻¹)	<i>Lactococcus lactis</i> * (Log CFU.mL ⁻¹)
Fm	1	9.18±0.10 ^a	9.32±0.01 ^a
	15	9.08±0.03 ^a	9.00±0.11 ^{abc}
	30	9.04±0.01 ^{ab}	9.11±0.02 ^{abcd}
Tm	1	9.30±0.14 ^a	9.30±0.17 ^a
	15	8.70±0.01 ^b	9.18±0.01 ^{abc}
	30	8.30±0.02 ^c	8.72±0.01 ^c
Sm	1	9.08±0.01 ^a	9.23±0.02 ^a
	15	8.80±0.23 ^{bc}	9.20±0.01 ^{ab}
	30	8.30±0.11 ^c	8.96±0.04 ^{de}
STm	1	9.28±0.01 ^a	9.30±0.13 ^a
	15	9.00±0.01 ^{ab}	9.00±0.02 ^{bcd}
	30	9.00±0.10 ^{ab}	9.30±0.01 ^a
Scm	1	9.15±0.05 ^a	9.11±0.01 ^a
	15	8.70±0.04 ^b	9.18±0.01 ^{abc}
	30	8.41±0.02 ^{bc}	8.87±0.16 ^c
ScTm	1	9.15±0.02 ^a	9.30±0.02 ^a
	15	9.60±0.00 ^a	9.00±0.00 ^{abc}
	30	8.48±0.00 ^{bc}	8.85±0.07 ^d

*Means in the same column accompanied by equal lowercase letters do not differ from each other at the level of $p \leq 0.05$. *Lactococcus lactis* counts in M17 pH 7.2 ± 0.2 at 30°C for 48 hours, total lactic acid bacteria in MRS pH 6.5 ± 0.2 at 30°C for 72h, both with addition of cycloheximide (200mg L⁻¹) with counts under anaerobic conditions.

** Fm (pure fermented milk), Tm (fermented milk added turmeric - 0.6% w/v); Sm (fermented milk added sugar 10% w/v); STm (fermented milk added turmeric - 0.6% w/v and sugar 10% w/v); Scm (fermented milk added salt 1.3% w/v); ScTm (fermented milk added salt 3% w/v and turmeric).

After fermentation, on the first day of storage all samples showed counts of total lactic acid bacteria and *Lactococcus lactis* close to 10⁹ CFU.mL⁻¹, however over the 30 days of storage this value was reduced in Tm, Sm, Scm and ScTm samples.

The Fm and STm formulations maintained the counts at 9 log CFU.mL⁻¹, until the last day of storage. Maintaining the original number of microorganisms, in samples of pure viili, added only with turmeric and added sugar and turmeric, it probably happened because these

formulations presented more favorable conditions for the maintenance of bacteria. According to Donkor et al. (2007) the presence of inhibitors, such as sodium chloride, sucrose, hydrogen peroxide, metabolites, nutrients and buffers, are factors that affect the survival of the microorganism throughout from storage.

In a study carried out by Fadaei, Mortazavi & Pourahmad, (2012), the growth average of *Lactococcus lactis* in fermented milk with Viili culture was 8.39 log CFU.mL⁻¹ 5 days after fermentation. As reported, the count of

microorganisms decreased significantly until the 15th day of storage, which did not happen in the present study.

3.3. Centesimal composition and Color

The centesimal composition of the fermented milks was stable during the storage at 4°C for the same formulation, showing no significant difference at a 5% significance level over the 30 days.

The percentage of moisture between the different formulations showed a significant difference ranging between 85.00 and 90.16%, with the product without any additive showed the highest humidity, and the one added of turmeric and sugar to less. With the values presented, it can be seen that as other components are added to the fermented milk its moisture decreases, due to the greater amount of total solids that these present.

On average, the products presented the following compositions for Moisture, Ash, Proteins, Lipids and Carbohydrates respectively. Fm (90.08%, 0.81%, 3.14%, 0.04%, 5.92%). Tm (89.90%, 0.85%, 3.71%, 0.04%, 5.48%). Sm (85.59%, 0.70%, 3.35%, 0.02%, 10.61%). STm (85.33%, 0.80%, 3.67%, 0.04%, 12.64%). Scm (87.10%, 1.93%, 3.31%, 0.01%, 7.63%). ScTm (85.25%, 2.15%, 3.80%, 0.05%, 9.31%). As expected, the added turmeric samples presented the highest value of proteins and those with addition of sucrose, the highest carbohydrate content. Among the parameters, the formulations were similar in most aspects analyzed. With the variation of additives, the variations were concentrated in moisture and carbohydrates on a larger scale.

Table 2 shows the results of the different formulations of the fermented milk with and without added turmeric for color parameters in the CIELAB system (L^* , a^* and b^*). The samples without the addition of turmeric in the formulation presented, on average, values for the parameter L^* of 79.10; 80.92 and 81.50 respectively for samples Fm, Sm and Scm, these samples being clearer when compared to the Tm samples, STm and ScTm that had addition of turmeric in its formulation having an average for L^* values of 75.52; 77.57 and 77.90

respectively. According to Rein and Heinonen (2004), the increase in pigments causes a decrease in luminosity, a fact that can be observed when comparing the L^* values for the formulations that had the addition of turmeric, with a reduction in the values for the parameter L^* , thus being darker.

Regarding parameters a^* (red (+) and (-) green) and b^* (yellow (+) and (-) blue), it is observed that all formulations showed negative values for the parameter a^* and positive for b^* , that is, all formulations have yellowish-green coloration, with differences in their intensities. To the samples without added turmeric (Fm, Sm and Scm) the values of a^* and b^* are lower than those of the formulations with the addition of turmeric (Tm, STm and ScTm), having on average presented values respectively for a^* and b^* of -3.21 and 6.36 in formulations without turmeric and -8.85 and 39.81 for formulations containing turmeric. These differences are the result of the presence of turmeric, which has curcumin (CC) and two other methoxy compounds (DMC and BDMC), being these the compounds that give to turmeric the yellow color. According to Yan et al., (2021), curcumin has a green-yellow in acidic conditions, which is possible to observe in the samples containing turmeric, which showed lower values for a^* and higher to b^* .

Almeida et al., (2018) evaluated in his work the color parameters of the three different curcuminoids. In this work it is possible verify that each curcuminoid contributes specifically to the parameters L^* , a^* and b^* , that is, although it is not possible to differentiate in the present work of the curcuminoid fractions, it is possible to conclude that is possible that color presented by the formulations containing turmeric is not due exclusively to its addition, but also to the concentration of each fraction of curcuminoids, thus existing difference during storage due to possible variations of these fractions. According to the authors, curcumin presents for the parameters L^* (72.84), a^* (16.84) and b^* (110.06). For DMC the L^* (72.15), a^* (1.96) and b^* (82.73). For BDMC the L^* (81.54), a^* (-4.72) and b^* (49.44).

Table 2. Color parameters for the formulations during 30 days of storage.

Samples**	Time (days)	L*	a*	b*
Fm	1	80.76±0.00 ^b	-3.16±0.00 ^{bc}	6.40±0.04 ⁱ
	15	78.40±0.22 ^d	-3.20±0.04 ^{bc}	7.35±0.02 ^{ij}
	30	78.14±0.24 ^d	-3.59±0.01 ^d	7.08±0.53 ^{jk}
Tm	1	76.22±0.06 ^c	-7.32±0.00 ^{ef}	34.30±0.42 ^g
	15	74.80±0.54 ^g	-8.27±0.05 ^{ef}	45.87±2.32 ^a
	30	75.55±0.51 ^g	-10.09±0.21 ^e	39.73±0.04 ^e
Sm	1	79.43±0.43 ^c	-3.08±0.00 ^b	6.20±0.02 ^l
	15	81.62±0.39 ^b	-3.28±0.00 ^c	5.13±0.03 ^m
	30	81.72±0.11 ^b	-2.91±0.04 ^a	6.05±0.21 ^l
STm	1	76.71±0.29 ^e	-8.82±0.05 ^h	38.17±0.04 ^f
	15	77.78±0.08 ^d	-9.51±0.02 ^j	41.09±0.06 ^c
	30	78.23±0.11 ^d	-9.38±0.03 ^j	40.52±0.04 ^d
Scm	1	81.64±0.35 ^b	-3.21±0.00 ^{bc}	7.59±0.08 ⁱ
	15	79.66±0.19 ^c	-3.26±0.01 ^{bc}	4.72±0.04 ^m
	30	83.22±0.19 ^a	-3.27±0.02 ^{bc}	6.80±0.31 ^k
ScTm	1	78.77±0.45 ^d	-8.45±0.05 ^{fg}	37.31±0.07 ^g
	15	76.56±0.27 ^e	-9.20±0.02 ^g	39.69±0.17 ^e
	30	78.34±0.45 ^d	-8.62±0.06 ⁱ	41.68±0.01 ^b

* Means in the same column accompanied by equal lowercase letters do not differ from each other at $p \leq 0.05$. Parameters L* black (0) – white (100), a* red (+) – green (-), b* yellow (+) blue (-).

** Fm (pure fermented milk), Tm (fermented milk added turmeric - 0.6% w/v); Sm (fermented milk added sugar 10% w/v); STm (fermented milk added turmeric - 0.6% w/v and sugar 10% w/v); Scm (fermented milk added salt 1.3% w/v); ScTm (fermented milk added salt 3% w/v and turmeric).

3.4. pH and acidity

After fermentation, viili has a pH of 4.43 and an acidity content of 0.9% (Kalkan & Balpetek, 2022). The values found for the pH of the samples on the first day of storage ranged from 4.41 for the fermented milk with no addition, to 4.58 for the sample with added salt (Figure 4). Due to the accumulation of lactic acid during storage, after 30 days, the values obtained for the pH of the samples showed a significant reduction.

In the work of Thamer and Penna (2006), the pH of dairy beverages probiotics, fermented with skimmed milk powder, varied between 4.72 and 4.83. Casarotti et al. (2014), found values between 4.38 and 4.13 when analyzing milk fermented with probiotic culture over 28 days of storage.

The products presented an acidity variation between 0.90 and 1.4% of lactic acid during storage. The values found are within those

established by current legislation, which determines the value minimum of 0.6 and maximum of 1.5 g of lactic acid/100mL of fermented milk (Brasil, 2000). The increase in acidity and decrease in pH value can be justified by the accumulation of lactic acid, because even after fermentation, under cold storage, fermenting bacteria hydrolyze lactose (Ugidos-Rodríguez, Matallana-González & Sánchez-Mata., 2018). In samples with turmeric, even if added in a small amount, 0.6%, there was a slight increase in acidity.

The acidity found by Vélez-Ruiz (2019) in yogurts with probiotic characteristics ranged from 0.45 to 0.63 g.100mL⁻¹. Celik & Temiz, (2022) found values in yogurts that varied between 0.80 and 0.93 g.100mL⁻¹, while Hakimi, Zahraee, & Rohani (2018), when analyzing yogurts from commercial brands obtained acidity values of 0.90 to 0.95 g of lactic acid in 100mL.

3.5. Phenolic Compounds and Antioxidant Capacity

The phenolic compounds present in samples are presented in Figure 5. The addition of turmeric powder promoted a significant increase in total phenolics, even in small quantity (0.6%). For the total phenolic compounds, the samples without the addition of turmeric: Fm, Sm and Scm, ranged from 66.15 to 92.39 mg gallic acid equivalents. 100g^{-1} . Samples with added turmeric powder ranged from 90.79 to 130.88 mg gallic acid equivalents. 100g^{-1} . With the increase in the levels of phenolic compounds, there was also an increase in the antioxidant capacity of the samples, as shown in Figure 5, in the DPPH and ABTS tests. Naturally, due to the greater amount of phenolics, there was a greater availability for antioxidant action.

According to the literature, *Curcuma longa* has a high content of total phenolic compounds, the turmeric rhizome contains from 4 to 8 mg. 100g^{-1} of curcuminoid pigments. In some cases, the concentration of curcuminoid pigments can reach up to 11.80 mg. 100g^{-1} . These variations can be due to several factors such as different varieties or

cultivars, period of bulb development and cultural practices (Mathai, 1976; Nguyen et al., 2021).

Phenolic compounds are also present in the formulations of viili without the addition of turmeric. The occurrence of phenolic compounds in milk and dairy products can be a consequence of several factors, such as example, the consumption of certain forage crops by livestock, the protein catabolism by bacteria, contamination with sanitizing agents, process-induced incorporation or its deliberate addition (O'Connell & Fox, 2001).

3.6. Exopolysaccharides (EPS)

Table 3 contain the results obtained for glucose and an estimation of viili exopolysaccharide sugar fractions and total EPS in the samples evaluated during 30 days of storage.

In this study were found for the 6 formulations of viili analyzed, different values referring to the amount of EPS produced. In between the samples without added sugar the lowest amounts of EPS were found in Fm and Tm, with 75.71 and 74.48 mg/L and the highest for Scm and ScTm with 90.46 and 93.19mg/L respectively. For Nguyen et al., (2020), for some strains of EPS-producing lactic acid bacteria, such as those of *Lactococcus*, through variations in physiological conditions it can be increase EPS biosynthesis.

Viili is characteristic for having a sticky texture and high viscosity due to exopolysaccharides (EPS) produced by *Lactococcus lactis* ssp. *cremoris* (Gotoh et al., 2021). The yield of EPS produced by different LAB can usually vary between 50-2700mg/L (Yusra et al., 2022). The EPS of this fermented milk is described as being a pentasaccharide composed of galactose, glucose, rhamnose in a similar proportion 02:02:01 (Kumar et al., 2022). Through this proportion, the amount of EPS present in the viili can be estimated by analysis of total carbohydrates, using glucose as standard.

Studies indicate that glucose was more efficient than fructose, lactose or galactose as a carbon source for growth and biosynthesis of EPS to *Lactobacillus fermentum* F6 (Zhang et al., 2011). Other works also found similar results using glucose as a source of carbon for the production of EPS by many bacteria (Kumar et al. 2019). Midik et al., (2020) concluded that *Lactococcus delbrueckii* subsp. *bulgaricus* NCFB 2772, produced three times more EPS with glucose as the source of sugar. Gotoh et al., (2021) found that *Lactococcus lactis* subsp. *cremoris* NIZO B40, produced about nine times more EPS with glucose as a sugar source under acidifying conditions.

Yang et al., (1999), found a variation between 164 and 263 mg/L of EPS produced by strains of *Lactococcus lactis* ssp. *cremoris* grown in skimmed milk.

3.7. Viscosity

The values obtained for the viscosity analysis, performed with Brookfield viscometer, were between 810.70 and 923.76 centipoise (Table 4). The viscosity of fermented milk is largely due to by the presence of EPS. According to Damodaran, Parkin, Fennema

(2010), soluble polymers such as proteins also promote increased viscosity, being greater the higher the protein concentration.

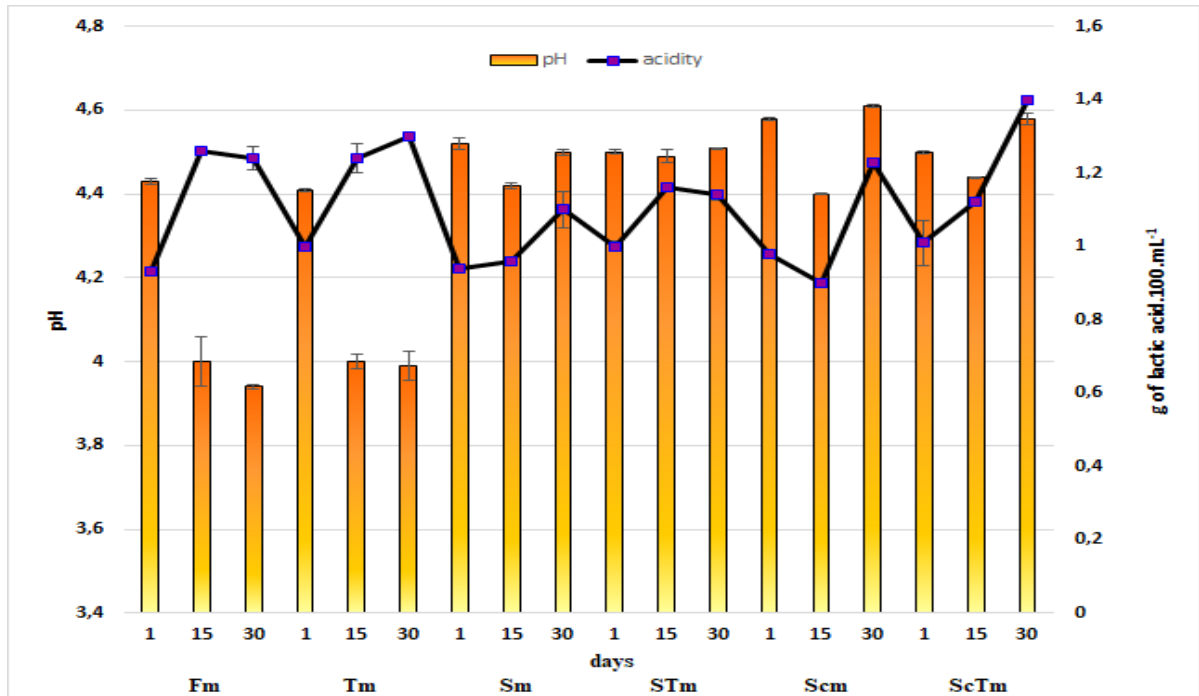


Figure 4. pH and acidity during 30 days of storage

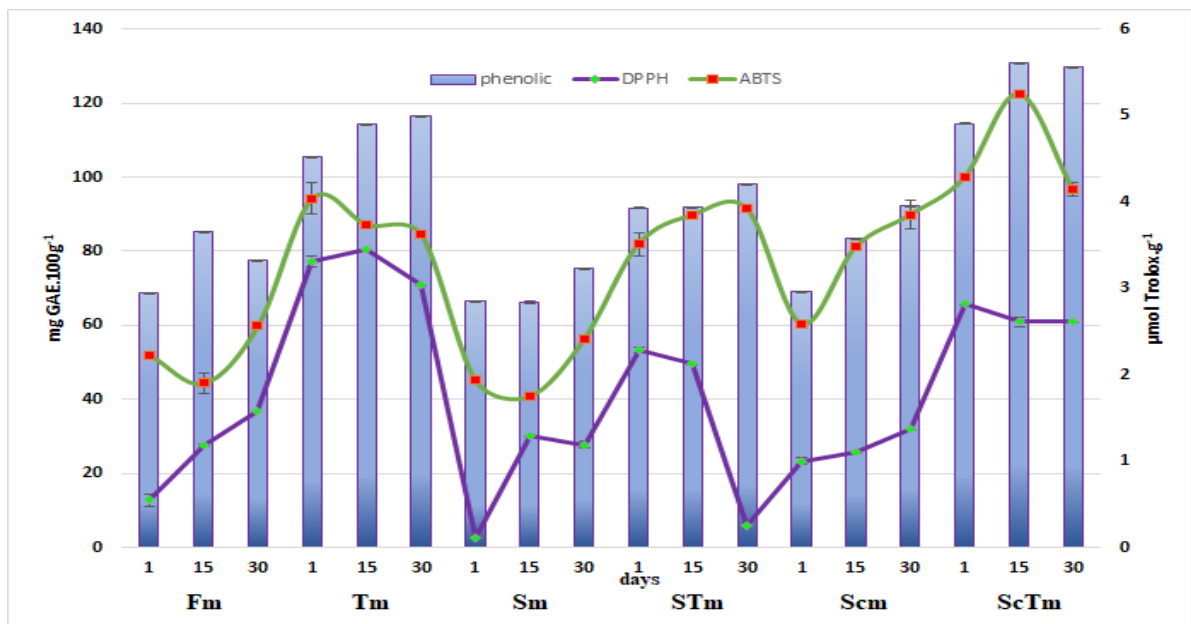


Figure 5. pH and acidity during 30 days of storage

EPS is produced by lactic acid bacteria gradually during acidification and during changing environmental conditions, the interactions between polysaccharides and proteins will continue (Yang et al., 2021). The increase in viscosity during the storage it is attributed

to the interactions that occur between the EPS and the protein over time, as can be seen in Table 4.

The viili formulations that showed the highest viscosity were Sm and STm. The viscosity of samples without added sugar ranged from 845.11 for ScTm to 896.76 cP for Fm.

Table 3. Analysis of glucose (phenol-Sulfuric) of the formulations and estimation of the values of EPS fractions of viili and total EPS during 30 days of storage.

** Sample	Time (days)	Phenol-sulfuric glucose (mg/L)*	mean	EPS fractions			Total EPS mg/L
				Glucose (2)	Galactose (2)	Rhamnose (1)	
Fm	1	32.27±0.037 ^{def}	30.28	30.28	30.28	15.14	75.71
	15	28.29±0.007 ^f					
	30	30.28±0.033 ^{def}					
Tm	1	30.25±0.017 ^{def}	29.79	29.79	29.79	14.90	74.48
	15	30.99±0.004 ^{def}					
	30	28.14±0.004 ^f					
Sm	1	61.00±0.002 ^g	67.68	67.68	67.68	33.84	168.21
	15	63.46±0.006 ^g					
	30	78.59±0.014 ^{ab}					
STm	1	65.63±0.004 ^{bc}	75.29	75.29	75.29	37.65	188.24
	15	81.17±0.107 ^a					
	30	78.77±0.008 ^{ab}					
Scm	1	41.44±0.077 ^{def}	36.18	36.18	36.18	18.09	90.46
	15	29.08±0.018 ^{def}					
	30	38.03±0.022 ^{def}					
ScTm	1	43.56±0.083 ^{de}	37.27	37.27	37.27	18.64	93.13
	15	37.28±0.021 ^{def}					
	30	44.40±0.045 ^d					

* Means in the same column accompanied by equal lowercase letters do not differ from each other at $p \leq 0.05$.

** Fm (pure fermented milk), Tm (fermented milk added turmeric - 0.6% w/v); Sm (fermented milk added sugar 10% w/v); STm (fermented milk added turmeric - 0.6% w/v and sugar 10% w/v); Scm (fermented milk added salt 1.3% w/v); ScTm (fermented milk added salt 3% w/v and turmeric).

3.8. Sensory analysis

The sensorial analysis of the fermented milks was carried out by evaluating the acceptance of the samples through the 9-point hedonic scale. For the product added with sucrose, presented in the form of fermented milk, the test had the participation of 99 volunteers where 53.5% were women aged 17 to 50 and 46.5% men aged 17 to 35 years. The test with the added product of chloride of sodium, presented in the form of salad dressing, had the participation of 74 volunteers, 63.5% women

aged 17 to 50 and 36.5% men aged 17 to 35 years.

Of the total number of participants, 76% stated that they liked and consumed fermented milk, 54% reported the same in relation to the sauce for salad and 89% said they liked and consumed probiotic products.

In the acceptance test, the attributes appearance, aroma, flavor, texture and overall acceptance of four milk formulations fermented (Sm, STm, Scm, ScTm).

For products with added sucrose, presented in the form of fermented milk, there was greater

acceptance of the attributes for formulation Sm, without the turmeric (Table 5). Products presented in the form of salad dressing, with addition of sodium chloride, had a greater acceptance than those with addition of sucrose, with no significant difference between the two formulations with added salt, Scm and ScTm (Table 5).

The results obtained for acceptability, approval, indifference and rejection referring to each of the 4 formulations are found in Figure 6A. The Acceptability between formulations ranged from 4.47 to 7.13, with that the formulations with sugar (Sm 5.32 and STm 4.47) presented lower significant acceptability compared to those with salt (Scm and ScTm).

There was difference significant for the acceptability among the added samples of sucrose, with and without the addition of turmeric. The sample Sm, with the addition of sucrose and without turmeric was the most accepted compared to STm. Among the samples with salt, which had very close average acceptability values, there was no difference significant. The standard deviation between acceptability values was quite high, mainly for the first two formulations (Sm and STm), this shows the divergence in the values provided by each taster.

Table 4. Viscosity of formulations after 30 days of storage, expressed in centipoise

Sample**	Viscosity (cP)		
	1*	15*	30*
Fm	808.04±10.50 ^f	865.83±14.89 ^{bcd}	896.76±11.47 ^{abc}
Tm	866.91±20.71 ^{bcd}	887.56±9.91 ^{bcd}	893.07±17.28 ^{abc}
Sm	889.96±17.29 ^{abc}	906.66±11.55 ^{ab}	920.53±7.33 ^a
STm	891.44±17.96 ^{abc}	915.29±15.68 ^a	923.76±6.43 ^a
Scm	810.70±2.04 ^f	827.16±19.01 ^{ef}	856.17±16.81 ^{cde}
ScTm	812.03±8.68 ^f	814.56±9.15 ^{def}	845.11±20.78 ^{def}

* Days. Means in the same column accompanied by equal lowercase letters do not differ from each other at $p \leq 0.05$.

** Fm (pure fermented milk), Tm (fermented milk added turmeric - 0.6% w/v); Sm (fermented milk added sugar 10% w/v); STm (fermented milk added turmeric - 0.6% w/v and sugar 10% w/v); Scm (fermented milk added salt 1.3% w/v); ScTm (fermented milk added salt 3% w/v and turmeric).

Table 5. Acceptance of sensory attributes of fermented milk with viili culture*

Sample**	Appearance	Aroma	Flavor	Texture	Global Acceptance
Sm	6.8±1.8 ^a	5.3±1.9 ^a	5.5±2.2 ^a	5.2±2.3 ^a	5.3±2.1 ^a
STm	5.6±2.3 ^b	4.7±2.1 ^b	5.4±2.2 ^a	4.1±2.4 ^b	4.5±2.2 ^b
Scm	7.6±1.6 ^a	7.2±1.5 ^a	7.0±1.6 ^a	7.2±1.8 ^a	7.2±1.7 ^a
ScTm	7.7±1.1 ^a	7.2±1.3 ^a	7.0±1.6 ^a	7.2±1.8 ^a	7.2±1.4 ^a

** Sm (fermented milk added sugar 10% w/v); STm (fermented milk added turmeric - 0.6% w/v and sugar 10% w/v); Scm (fermented milk added salt 1.3% w/v); ScTm (fermented milk added salt 3% w/v and turmeric).

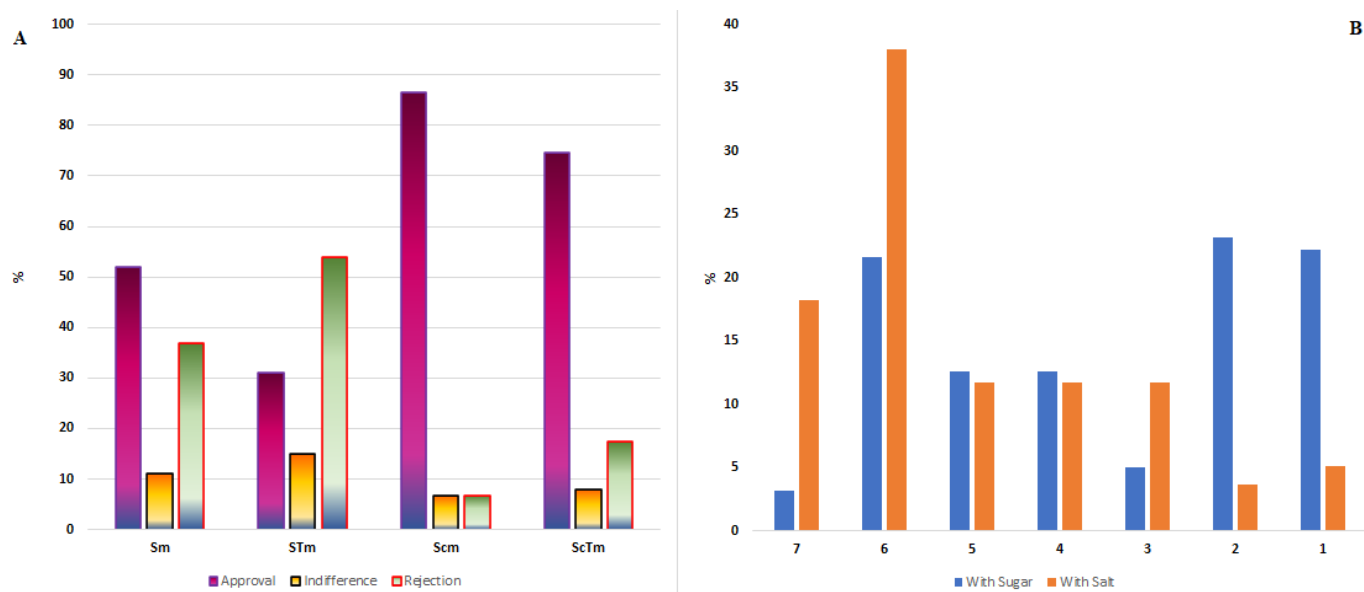


Figure 6. A: Percentage of approval, indifference and rejection of products (Approval, percentage of grades from 6 to 9), (Indifference, percentage of grades 5) and (Rejection, percentage of scores from 1 to 4). **B:** Tasters' purchase intention for milk fermented with sugar or salt. (7 – Would certainly buy), (6 – would possibly buy), (5 – maybe buy), (4 – Maybe buy it, maybe not), (3 – Maybe not by), (2 – I might not buy) and (1 – I certainly wouldn't buy).

The approval, indifference and rejection percentages allow observe the perception of the panelists in relation to each formulation (Figure 6A). The sample with added sugar only was approved by 52% of the panelists who gave scores between 6 (I liked it slightly) and 9 (I liked it extremely), 11% if were indifferent to it with a score of 5 (I neither liked nor disliked it) and 37% of the judges rejected the formulation As, with the attribution of grades from 1 (dislike extremely) to 4 (dislike slightly).

For the formulation with added sugar and turmeric (STm) an approval was 31%, indifference 15% and rejection 54%.

The product with addition of salt, served in the form of salad dressing was the one with the highest acceptability 7,13 and also the highest approval rating 86.6%. The formulation with salt and turmeric had high approval by judges 74.7% and low levels of indifference and rejection, 8% and 17.3%, respectively.

For the acceptability of probiotic low-fat yogurt, Karaca et al., (2019) obtained values between 4.4 and 7.6. In similar work, Boukria et

al., (2020), found values for the acceptability of fermented skimmed milk per mixed culture between 6.18 and 6.65. The viscous fermented milks typical of the Nordic countries are very widespread and appreciated in northern Europe. Finland and Denmark present the highest consumption of the Nordic group with an average of 41 kg per person year, with an average consumption of 100 g per day/person (Fondén et al., 2006; Ganina & Krasnova, 2021). Nonetheless in Brazil, viili is not known, and its sensory characteristics, such as high viscosity and acidity, are a novelty for the palate of its potential consumers.

Figure 6B contains the percentages obtained with the application of the purchase intention test, the two products developed, fermented milk (with sugar) and salad dressing (with salt) were evaluated.

4. Conclusions

The addition of turmeric, salt or sugar to milk fermented with the mixed culture viili, did not cause a decrease in microbial viability, being

thus, the claim of a probiotic product can be maintained for up to 30 days refrigerated storage.

The addition of turmeric to the viili fermented product contributed to the increase in the content of phenolic compounds in the product, causing an increase in antioxidant activity

The product viili added with turmeric and salt can be used as a functional salad dressing, as in addition to maintaining the aforementioned benefits, it was well accepted by potential consumers.

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