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# Polyphenols, carotenoids and flavonoids in an antioxidant probiotic yogurt made with tumbo pulp (*Passiflora tripartita* Kunth)

## *Polifenóis, carotenoides e flavonoides em um iogurte probiótico antioxidante feito com polpa de tumbo (*Passiflora tripartita *Kunth)*

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## Abstract

The species *Passiflora tripartita* Kunth (tumbo) is endemic to South America, whose edible fruits are a rich source of antioxidant metabolites. This study aimed to develop a probiotic yogurt with tumbo fruit pulp, consisting of an adequate antioxidant capacity related to its content in phenolic compounds, flavonoids, and carotenoids, and with good acceptability. Antioxidant capacity was determined by radical scavenging capacity test (DPPH●) and cation radical scavenging capacity test (ABTS+●), total phenol content by Folin Ciocalteu method, total flavonoids, and carotenoids by spectrophotometric method, on days 1, 7, 14 and 21 of storage. The surface plate count method quantified Lactic Acid Bacteria (LAB). The results evidenced that at day 21 of the analysis, the antioxidant capacity presented high values (DPPH●: 8.774 mg GAE/g, 3.386 mg TAE/g, 6.159 mg AAE/g; ABTS+●: 11.630 mg GAE/g, 7.018 mg TAE/g, 9.218 mg AAE/g), the content of phenolic compounds presented high values (3746.389 mg TPGAE/g; 2355.933 mg TPTAE/g), as well as total flavonoids (52.421 mg Quercetin/g) and carotenoids (72.109 µg β-carotene/g). Yogurt presents a value of 3.4 x $10^8$ CFU/g of LAB and it is therefore considered a probiotic. High values were determined as the sensory characteristics such as odor (6.89), color (6.97), texture (6.94), flavor (6.97), and acceptability (6.94), thus being analyzed according to the hedonic scale in 200 panelists. The physicochemical and microbiological quality of the yogurt complies with current regulations. In conclusion, the probiotic yogurt developed with tumbo fruit pulp had a high amount of LAB. It presented high antioxidant capacity correlated with its high content of phenolic compounds, flavonoids and carotenoids, which

remained high during the 21 days of storage. Furthermore, it showed high acceptability and had adequate physicochemical and microbiological quality.

**Keywords:** Acceptability; Antioxidant capacity; Lactic acid bacteria; *Passiflora tripartita* Kunth; Physicochemical quality; Sensory analysis

## Resumo

A espécie *Passiflora tripartita* Kunth conhecida popularmente como tumbo, é endêmica da América do Sul. Seus frutos comestíveis são uma rica fonte de metabólitos antioxidantes. O objetivo do estudo foi desenvolver um iogurte probiótico com polpa de fruta tumbo, com capacidade antioxidante adequada, relacionada ao seu conteúdo em compostos fenólicos, flavonoides e carotenoides, e com boa aceitabilidade. A capacidade antioxidante foi determinada pelo teste de capacidade de absorção do radical DPPH (DPPH●) e pelo teste de capacidade de absorção de cátion-radical (ABTS+●); o conteúdo fenólico total foi determinado pelo método Folin Ciocalteu e os flavonoides totais e carotenoides, pelo método espectrofotométrico, nos dias 1, 7, 14 e 21 de armazenamento. As Bactérias Ácido-Láticas (BAL) foram quantificadas pelo método de contagem de placas de superfície. Os resultados evidenciaram que, no dia 21 da análise, a capacidade antioxidante apresentou valores elevados (DPPH●: 8.774 mg GAE/g, 3.386 mg TAE/g, 6.159 mg AAE/g; ABTS+●: 11.630 mg GAE/g, 7.018 mg TAE/g, 9.218 mg AAE/g) e o conteúdo de compostos fenólicos apresentou valores também expressivos (3746.389 mg TPGAE/g; 2355.933 mg TPTAE/g), bem como flavonoides totais (52.421 mg Quercetina/g) e carotenoides (72.109 µg β-caroteno/g). O iogurte apresentou um valor de 3,4 × $10^8$ CFU/g de BAL e foi, portanto, considerado um probiótico. Valores altos foram determinados em características sensoriais, tais como odor (6,89), cor (6,97), textura (6,94), sabor (6,97) e aceitabilidade (6,94), que foram analisados de acordo com a escala hedônica por 200 provadores. A qualidade físico-química e microbiológica do iogurte está em conformidade com os regulamentos atuais. Em conclusão, o iogurte probiótico desenvolvido com polpa de fruto tumbo teve uma alta quantidade de BAL. Esse iogurte teve uma alta capacidade antioxidante correlacionada com seu alto conteúdo de compostos fenólicos, flavonoides e carotenoides, que permaneceram elevados durante os 21 dias de armazenamento. Além disso, esse iogurte mostrou uma alta aceitabilidade e teve também uma qualidade físico-química e microbiológica adequada.

**Palavras-chave:** Aceitabilidade; Capacidade antioxidante; Bactérias ácido-láticas; *Passiflora tripartita* Kunth; Qualidade físico-química; Análise sensorial.

## Highlights

- Theme 1: Probiotic yogurt made with tumbo fruit pulp (*Passiflora tripartita* Kunth)
- Theme 2: Wide general acceptance of probiotic yogurt with tumbo Pulp
- Theme 3: Probiotic food product with quantified antioxidant metabolites

## 1 Introduction

Oxidative stress is associated with the risk of activation of Reactive Oxygen Species (ROS) sources, aiming at generating toxic free radicals (Carvajal, 2019; Viada et al., 2017) at the level of essential organs to maintain homeostasis (Ortiz & Medina, 2020), and decreasing the intestinal microbiota generating a poor absorption of nutrients which does not allow absorption of the required antioxidants (Dumitrescu et al., 2018; Sun et al., 2019).

The excess of these free radicals uncontrolled over time in addition to risk factors of malnutrition such as obesity associated with developing Cardiovascular Diseases (CVD) (Hernández et al., 2020), lipid alterations (Suárez et al., 2021), and Endothelial Dysfunction (ED) may lead to an uncontrollable cycle as part of the Metabolic Syndrome process (Carvajal, 2017; Cavalcante-Silva et al., 2015; Raya-Farías et al., 2018).

A plant species native to South America is *Passiflora tripartita* Kunth, whose edible fruit is called "tumbo" and is consumed by the Andean communities of Peru. The fruit pulp showed higher antioxidant capacity against free radicals than several traditional fruits (Chaparro et al., 2014; Giambanelli et al, 2020; Lopa et al., 2021; Muñoz et al., 2007) due to its high content of phenolic compounds, flavonoids, and carotenoids (Ruiz et al., 2018; Troya et al., 2018).

Currently, phenolic compounds with high antioxidant potential are incorporated in dairy derivatives such as yogurt that add Lactic Acid Bacteria (LAB) in milk, such as Lactobacillus strains that favor the maintenance of the intestinal microbiota, and these strains are being studied (Sánchez et al., 2019). Likewise, in various yogurt formulations, a set of probiotic bacteria are added to improve the absorption of the incorporated antioxidant compounds (Castro et al., 2016; World Gastroenterology Organisation, 2017).

However, one of the drawbacks in the formulation of probiotic yogurts with bioactive compounds derived from fruit pulps is related to the permanence of antioxidant metabolites during storage time (Santos et al., 2019).

For the aforementioned reasons, this study aimed to develop a probiotic yogurt with tumbo fruit pulp with adequate antioxidant capacity related to its content in phenolic compounds, flavonoids and carotenoids, and with good acceptance.

## 2 Materials and methods

### 2.1 Materials

Raw cow's milk was obtained from a local dairy farm in the district of Lurin (Lima, Peru) during morning hours. The color, odor, flavor, and textura were analyzed to determine the quality of raw milk (López et al., 2015). In addition, the physicochemical characterization by evaluating pH, acidity, density and alcohol test was conducted (Instituto Nacional de Defensa de la Competencia y de la Protección de la Propiedad Intelectual, 2003). Instant whole milk powder for milk standardization was purchased from a Peruvian dairy industry company. The commercial lactic cultures Nu-trish *Lactobacillus casei* 431 from Chr. Hansen (Denmark), Dri-Set yogurt 438 and Dri-Set Bioflora ABY 424 from Vivolac Cultures Corporation (USA) were used for milk inoculation. The fruits of tumbo (*P. tripartita* Kunth) were collected in Ancash (Peru), whose species was identified, classified and a voucher specimen was deposited with the registration number USM 281623.

### 2.2 Obtaining the tumbo pulp

The tumbo fruits were washed, peeled, and the epicarp and mesocarp were removed. Then, it was distributed homogeneously in a polyethylene bag, vacuum sealed and stored at 3 °C before use.

### 2.3 Yogurt preparation

Milk was pasteurized (90.0 °C, 10 minutes) (Castro Albarrán et al., 2017), cooled and filtered (48.0 ± 2.0 °C, 180 µm). Dehydrated sugar cane juice (panela) and instant whole milk powder were added, concentrated (85.0 ± 2.0 °C, 10 minutes), cooled and filtered (45.0 ± 2.0 °C, 180 µm). The lactic culture mixture was inoculated (1.0 µg/mL, constant agitation), and tumbo pulp (50.0 mg/mL) was added. It was incubated in a fermenter tank with a temperature regulator (42.0 ± 2.0 °C, 8 hours). It was churned, bottled and cooled to 3.0°C for physicochemical, microbiological and sensory analysis.

The yogurt made with the tumbo pulp (Yt) was stored for 21 days in glass containers with airtight lids, under refrigerated conditions (5.0±1.0°C), taking samples in a sterile environment for the determination of antioxidant capacity, total phenol, flavonoid and carotenoid content, after 1, 7, 14 and 21 days. Yogurt made

with the same formulation, but without tumbo pulp, control (Yc) was also stored for 21 days and evaluated with the same determinations and the same period.

### 2.4 Analysis of the physicochemical and microbiological quality of the yogurt

The physicochemical quality was determined by evaluating pH (potentiometric method), milk fat (IDF, 1987), protein (Instituto Nacional de Defensa de la Competencia y de la Protección de la Propiedad Intelectual, 1998a), relative density (Instituto Nacional de Defensa de la Competencia y de la Protección de la Propiedad Intelectual, 1998b), titratable acidity (Instituto Nacional de Defensa de la Competencia y de la Protección de la Propiedad Intelectual, 2008), syneresis (Wang et al., 2010), total solids (International Diabetes Federation, 1991) and non-fat solids by calculating the difference between total solids and fat.

Microbiological quality was determined by evaluating total coliforms (Instituto Nacional de Defensa de la Competencia y de la Protección de la Propiedad Intelectual, 2014), molds and yeasts according to ICMSF (International Commission on Microbiological Specifications for Foods, 2000); and LAB count on MRS agar (Azefor et al., 2015).

### 2.5 Sensory analysis of the probiotic yogurt

Yogurt sensory characteristics such as odor, color, texture, flavor and acceptability were evaluated using a seven-point hedonic scale according to Greis et al. (2020) and ICONTEC (Instituto Nacional de Defensa de la Competencia y de la Protección de la Propiedad Intelectual, 2013), with a hedonic scale from "strongly dislike it (1)" to "strongly like it (7)". The tests were conducted with a sample of 200 panelists consumers of probiotic yogurts, in the districts of Ate Vitarte and San Juan de Lurigancho in Lima, Peru.

### 2.6 Chemical analysis of probiotic yogurt

The sample and the control (Yt, Yc) were divided into two treatments to analyze the antioxidant capacity and determine the total phenols and flavonoid content. The first treatment was a dilution (1:10) with double distilled water (Ytw, Ycw) and the second treatment was a dilution (1:10) with 96% ethanol (Yte, Yce). Both treatments were centrifuged (2500 rpm, 5 minutes), and the supernatant was filtered through Whatman No. 4 paper (Maidstone, UK).

#### 2.6.1 Antioxidant capacity

Antioxidant capacity was evaluated by radical scavenging capacity assay and cation radical scavenging capacity test.

The radical scavenging capacity estimation of each sample was performed using 2,2-diphenyl-1-picrylhydrazyl radical (DPPH ●, Sigma Aldrich) according to the method of Brand-Williams et al. (1995), Noh et al. (2020) and Ganoza-Yupanqui et al. (2021). From each sample, 500 µL was taken, mixed with DPPH● reagent (0.1 mM, 1000 µL) for 30 min. Each mixture was read at 517 nm in a double-beam Ultraviolet-Visible (UV-Vis) spectrophotometer (Thermo Scientific, AQ8000, USA).

Estimation of the cation radical scavenging capacity of each sample was performed using the cation radical 2,2'-azinobis (3-ethyl benzothiazoline 6-sulfonic acid) (ABTS+●) according to the method of Re et al. (1999), Noh et al. (2020) and Ganoza-Yupanqui et al. (2021). From each sample, 30 µL was taken, mixed with the reagent ABTS+● (Aλ-754: 0.7, 1470 µL) for 5 min. Each mixture was read at 754 nm in the spectrophotometer.

From a stock solution (10 mg/mL) with standards (Sigma-Aldrich) of Gallic Acid (GA) using different types of calibration solutions (5.0-15.0 mg/mL), of Tannic Acid (TA) (2.0-10.0 mg/mL) and Ascorbic Acid (AA) (2.5-12.5 mg/mL) were prepared and evaluated as described above. The radical scavenging capacity

and cation radical scavenging capacity (related to antioxidant capacity) of each sample were expressed as mg GA, TA and AA equivalent per gram of yogurt (mg GAE, TAE, AAE/g).

#### 2.6.2 Qualitative screening for phenolic compounds

A qualitative screening was performed to identify compounds potentially responsible for the antioxidant power in the samples. Chemical reactions were performed with $FeCl_3$, $AlCl_3$/NaOH, vanillin/$H_2SO_4$, HCl/NaOH and Mg/HCl to determine the presence of flavonoids, phenols, anthocyanins and/or betalains (Lock, 2016).

#### 2.6.3 Total Phenolic Content (TPC)

The evaluation of the Total Phenolic Content (TPC) in each sample was carried out according to the procedure described by Sánchez-Rangel et al. (2013), Hernández-Carranza et al. (2019) and Kim et al. (2019), using Folin Ciocalteu's reagent and UV-visible spectrophotometry.

500 µL of each sample was taken, placed and shaken with Folin-Ciocalteu reagent (0.1 M, 500 µL), at 45°C for 10 min. After this time, $Na_2CO_3$ (0.5%, 500 µL) was added, and the mixture was allowed to stand for 30 min at room temperature. After incubation, it was read at 765 nm. The results were expressed as mg Total Phenols Gallic Acid Equivalent (TPGAE) and Total Phenols Tannic Acid Equivalent (TPTAE) per gram of yogurt (mg TPGAE/g, mg TPTAE/g). A calibration curve for GA and TA (0.1-1.0 mg/mL) was prepared and processed in the same way as the samples.

#### 2.6.4 Total Flavonoid Content (TFC)

The TPC was evaluated according to Hernández-Carranza et al. (2016, 2019). First, 500 µL of each sample was taken, mixed with $NaNO_2$ (1.5%, 500 µL) in a vortex and allowed to stand for 10 min. Then, $AlCl_3$ (3%, 1000 µL) was added, mixed for 2 minutes, 1000 µL NaOH 1N was added, allowed to stand for 2 minutes and read at 490 nm. Finally, a quercetin standard curve was performed. The result was expressed as mg quercetin per 100 g of yogurt.

#### 2.6.5 Carotenoid content

Carotenoid extraction was carried out according to Xavier et al. (2012) with the direct sample of yogurt with tumbo pulp (Yto) and control (Yco). Extraction was a dilution (1:10) with 96% ethanol was also performed for both samples (Yte, Yce).

For carotenoid quantification, β-carotene (Sigma Aldrich) was used as standard compared to a calibration curve starting from 100 µg/mL in dilutions of 2.0-15.0 µg/mL, according to the methodology described by Rutz et al. (2016).

### 2.7 Data processing

All experiments were performed in triplicate. Results were expressed as mean ± standard deviation, and data were evaluated by parametric test (one-way Analysis of Variance (ANOVA), Tukey's post hoc test) and nonparametric test (Friedman´s test, Wilcoxon signed ranks test). The value $p < 0.05$ was considered statistically significant. All data obtained were analyzed using IBM SPSS Statistic 26 (2021) software.

## 3 Results and discussion

The evaluation results of the quality of the raw milk expressed in its organoleptic and physicochemical characteristics are within the acceptable ranges for consumption according to current regulations (Table 1).

**Table 1.** Organoleptic and physicochemical quality of raw milk.

| | Results |
|---|---|
| **Organoleptic Characteristics** | |
| Color | White |
| Odor | Characteristic |
| Flavor | Sligthtly sweet |
| Texture | Slightly watery |
| **Physicochemical Characteristics** | |
| pH | 6.53 |
| Acidity (g/100 g) | 0.12 |
| Density (g/mL) | 1.032 |
| Alcohol test | Negative |

The evaluation results of the physicochemical and microbiological quality of the yogurt project showed a food product that combines good indicators. Furthermore, the evaluation of the microbiological quality of the probiotic yogurt with tumbo pulp showed sanitary characteristics following current Peruvian regulations, and within the parameters according to Codex Alimentarius (Comisión del CODEX Alimentarius, 2002) (Table 2).

The physicochemical parameters determined for the probiotic yogurt with tumbo pulp to consolidate the quality agreed with the study of Hossain et al. (2012). They evaluated these parameters in a yogurt preparation with strawberry, orange, and grapefruits, with similar results to the yogurt with tumbo pulp. The most notable parameter was associated with a lower acidity which favored acceptability and allowed maintaining the organoleptic characteristics of the probiotic yogurt with tumbo pulp.

The results of the LAB count evaluation of the yogurt with tumbo pulp were 3.4 x $10^8$ CFU/g (Table 2), exceeding the recommended values as CFU greater than $10^7$ is considered a probiotic (Castro et al., 2015). However, in comparison with the yogurt with cactus pulp developed by Barat & Ozcan (2017), its vegetable matrix did not allow adequate development of probiotics obtaining a decrease in the growth of LAB, accompanied by competitiveness between *Streptococcus thermophillus* and *Lactobacillus acidophilus* by the milk matrix, so the author did not achieve a yogurt with an adequate LAB count to be probiotic.

The increase of CFU in the yogurt developed with tumbo (Table 2) could be due to higher fiber content, increasing the amount of prebiotics necessary for the selective growth of LAB probiotics. Probiotics are considered microorganisms that provide beneficial effects on the intestinal flora, generating an adequate balance between beneficial and pathogenic bacteria that invade the intestinal walls (Floch, 2018).

**Table 2.** Physicochemical and microbiological quality of probiotic yogurt.

| | Results |
|---|---|
| **Physicochemical Quality** | |
| Milk fat (♦) | 3.51 |
| Non-fat solids (♦) | 19.45 |
| Total solids (♦) | 22.96 |
| Proteins (♦), factor: 6.38 | 4.86 |
| Acidity (♦) | 0.79 |
| pH | 3.94 |
| Density (g/mL) | 1.06 |
| Syneresis (♦) | 8.81 |
| **Microbiological Quality** | |
| Lactic Acid Bacteria (LAB) count | 3.4 x $10^8$ CFU/g |
| Mold and yeast count | < 10 CFU/g |
| Total coliforms | Absent |

(♦) = expressed in g/100 g; CFU = Colony Forming Units for grame.

In the sensory evaluation of the probiotic yogurt with tumbo regarding its acceptability, color, flavor, smell, and texture, carried out by the panelists, results were obtained, with a score higher than 6.8, which guarantees an acceptable product and conforming quality. The hedonic scale applied to the panelists showed a score concordant with the description "strongly like it", valuing the acceptance of the probiotic with tumbo pulp (Figure 1). The yogurt with tumbo obtained a higher score scale, compared to the score of 6.0 of the fermented beverage from buttermilk with *L. acidophilus* and *S. thermophilus* elaborated by Miranda et al. (2014), in addition to the LAB count of the buttermilk beverage (1.2 x $10^7$ CFU/mL) that was lower than the yogurt with tumbo.

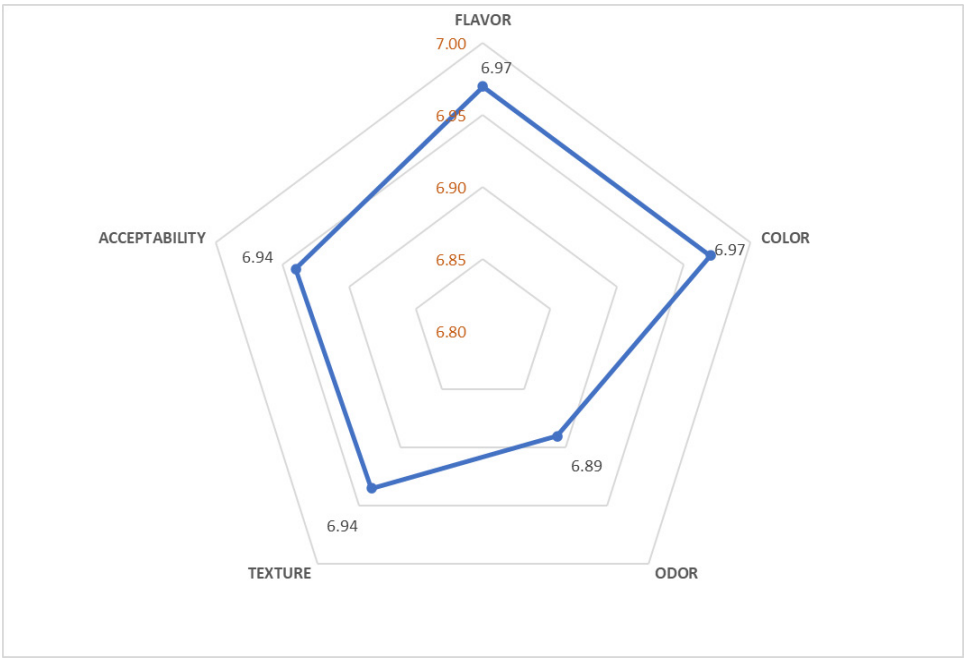


**Figure 1.** Sensory analysis of the probiotic yogurt. Values are calculated as mean (n = 200). Seven-point hedonic scale: strongly dislike it, dislike it, slightly dislike it, neither like nor dislike it, like it slightly, like it, strongly like it.

The sample was treated with two solvents, bidistilled water that extracts polar chemical compounds and 96% ethanol that extracts medium polar chemical compounds due to its carbon chain to evaluate the antioxidant capacity and determine the TPC and total flavonoids of the probiotic yogurt with tumbo pulp. The developed procedure is established in the study owing to a diversity of polar and medium polar phenolic compounds available (Abarca-Vargas & Petricevich, 2020; Gharaati Jahromi, 2019).

Then, the antioxidant capacity of the probiotic yogurt with tumbo pulp was evaluated by radical scavenging capacity assay (DPPH●) and cation radical scavenging capacity test (ABTS+●), comparing the results with three standards, TA, GA, and AA related to the presence of phenolic compounds contained in tumbo pulp (Giambanelli et al, 2020; Lopa et al., 2021; Ruiz et al., 2018).

The antioxidant capacity was evaluated considering the median inhibitory concentration ($IC_{50}$) of the calibration curve of GA (DPPH●: 11.123 mg/mL; ABTS+●: 9.282 mg/mL), TA (DPPH●: 5.323 mg/mL; ABTS+●: 4.939 mg/mL) and AA (DPPH●: 8.229 mg/mL; ABTS+●: 6.892 mg/mL) that was also obtained by mathematical and statistical calculations and the results are shown in Table 3. Several studies evaluated the antioxidant activity of yogurts by radical scavenging capacity assay using the Trolox standard or expressing in percentage inhibition (Silva et al., 2022; Nguyen & Hwang, 2016; Zapata et al., 2015). However, it was not considered applicable in the present study because Trolox is an analog of vitamin E that has not been found in tumbo pulp. Moreover, its prooxidant activity is described due to the stability and concentration of the reagent (Giordano et al., 2020).

**Table 3.** Antioxidant capacity of the probiotic yogurt.

| | Treated Probiotic Yogurt | DAYS OF STORAGE | | | |
|---|---|---|---|---|---|
| | | 1 | 7 | 14 | 21 |
| DPPH (mg GAE/g) | Ytw | 11.129 ± 0.017$^{a}$ | 10.133 ± 0.035$^{b}$ | 9.438 ± 0.089$^{c}$ | 8.774 ± 0.070$^{d}$ |
| | Ycw | 5.407 ± 0.034$^{a}$ | 5.351 ± 0.051$^{a}$ | 5.586 ± 0.054$^{b}$ | 5.401 ± 0.086$^{a}$ |
| | Yte | 10.989 ± 0.108$^{a}$ | 10.139 ± 0.044$^{b}$ | 9.451 ± 0.045$^{c}$ | 8.511 ± 0.061$^{d}$ |
| | Yce | 5.049 ± 0.064$^{a}$ | 5.066 ± 0.054$^{a}$ | 5.105 ± 0.060$^{a}$ | 4.697 ± 0.051$^{b}$ |
| ABTS (mg GAE/g) | Ytw | 13.748 ± 0.085$^{a}$ | 12.965 ± 0.046$^{b}$ | 12.309 ± 0.034$^{c}$ | 11.630 ± 0.036$^{d}$ |
| | Ycw | 3.801 ± 0.020$^{a}$* | 3.675 ± 0.046$^{a}$* | 3.727 ± 0.035$^{a}$* | 3.836 ± 0.010$^{a}$* |
| | Yte | 13.622 ± 0.017$^{a}$ | 12.637 ± 0.046$^{b}$ | 12.097 ± 0.026$^{c}$ | 10.991 ± 0.036$^{d}$ |
| | Yce | 3.819 ± 0.027$^{a}$* | 3.709 ± 0.030$^{a}$* | 3.774 ± 0.030$^{a}$* | 3.686 ± 0.026$^{a}$* |
| DPPH (mg TAE/g) | Ytw | 5.328 ± 0.014$^{a}$ | 4.507 ± 0.029$^{b}$ | 3.930 ± 0.070$^{c}$ | 3.386 ± 0.057$^{d}$ |
| | Ycw | 0.610 ± 0.028$^{a}$ | 0.564 ± 0.042$^{a}$ | 0.758 ± 0.044$^{b}$ | 0.605 ± 0.071$^{a}$ |
| | Yte | 5.212 ± 0.089$^{a}$ | 4.512 ± 0.036$^{b}$ | 3.994 ± 0.036$^{c}$ | 3.169 ± 0.050$^{d}$ |
| | Yce | 0.315 ± 0.052$^{a}$ | 0.329 ± 0.044$^{a}$ | 0.361 ± 0.049$^{a}$ | 0.025 ± 0.042$^{b}$ |
| ABTS (mg TAE/g) | Ytw | 8.893 ± 0.075$^{a}$ | 8.200 ± 0.041$^{b}$ | 7.619 ± 0.031$^{c}$ | 7.018 ± 0.032$^{d}$ |
| | Ycw | 0.090 ± 0.018$^{a}$* | -0.023 ± 0.040$^{a}$* | 0.023 ± 0.030$^{a}$* | 0.120 ± 0.009$^{a}$* |
| | Yte | 8.780 ± 0.016$^{a}$ | 7.880 ± 0.084$^{b}$ | 7.431 ± 0.023$^{c}$ | 6.452 ± 0.032$^{d}$ |
| | Yce | 0.105 ± 0.023$^{a}$* | 0.008 ± 0.027$^{a}$* | 0.039 ± 0.027$^{a}$* | -0.012 ± 0.023$^{a}$* |
| DPPH (mg AAE/g) | Ytw | 8.235 ± 0.015$^{a}$ | 7.357 ± 0.031$^{b}$ | 6.741 ± 0.074$^{c}$ | 6.159 ± 0.061$^{d}$ |
| | Ycw | 3.190 ± 0.030$^{a}$ | 3.141 ± 0.045$^{a}$ | 3.348 ± 0.048$^{b}$ | 3.186 ± 0.076$^{a}$ |
| | Yte | 8.111 ± 0.095$^{a}$ | 7.362 ± 0.039$^{b}$ | 6.755 ± 0.039$^{c}$ | 5.927 ± 0.053$^{d}$ |
| | Yce | 2.875 ± 0.056$^{a}$ | 2.889 ± 0.048$^{a}$ | 2.924 ± 0.053$^{a}$ | 2.564 ± 0.045$^{b}$ |
| ABTS (mg AAE/g) | Ytw | 11.316 ± 0.084$^{a}$ | 10.541 ± 0.045$^{b}$ | 9.891 ± 0.034$^{c}$ | 9.218 ± 0.035$^{d}$ |
| | Ycw | 1.465 ± 0.020$^{a}$* | 1.339 ± 0.046$^{a}$* | 1.391 ± 0.035$^{a}$* | 1.499 ± 0.001$^{a}$* |
| | Yte | 11.191 ± 0.017$^{a}$ | 10.216 ± 0.045$^{b}$ | 9.680 ± 0.026$^{c}$ | 8.585 ± 0.035$^{d}$ |
| | Yce | 1.482 ± 0.026$^{a}$* | 1.370 ± 0.030$^{a}$* | 1.408 ± 0.030$^{a}$* | 1.350 ± 0.026$^{a}$* |

Ytw = probiotic yogurt with tumbo pulp treated with bidistilled water; Ycw = control of Ytw without tumbo pulp treated with bidistilled water; Yte = probiotic yogurt with tumbo pulp treated with 96% etanol; Yce = control of Yte without tumbo pulp treated with 96% ethanol. GAE = Gallic Acid Equivalent; TAE = Tannic Acid Equivalent; AAE = Ascorbic Acid Equivalent. All values are expressed as Mean ± Standard Deviation, evaluations performed in triplicate. a, b, c, d indicate significant difference ($p < 0.05$); * Friedman's test and Wilcoxon signed-rank test were applied.

Qualitative assays were previously performed to detect the presence of phenolic compounds in the yogurt with tumbo, the results of which could denote the presence of phenolic compounds ($FeCl_3$, blue-green color) and flavonoids ($AlCl_3$/NaOH, yellow color; Mg/HCl, red color), which were related to the results of Giambanelli et al. (2020).

According to IRAM (Instituto Argentino de Normalización, 2004), to be considered a natural product with adequate levels of phenols and flavonoids, a product must be found in proportions greater than 50.0 mg GAE/g. Yogurt with tumbo pulp treated with double distilled water (Ytw) presented a content of total phenols higher than recommended (Table 4) from day 1 (4218.882 ± 21.227 mg TPGAE/g) to day 21 (3746.389 ± 28.929 mg TPGAE/g), *i.e.*, superior to yogurt made from *Aronia melanocarpa* juice, the result of which was 54.05 ± 1.43 mg GAE/g (Nguyen & Hwang, 2016).

**Table 4.** Total Phenolic Content (TPC) in probiotic yogurt.

| | Treated Probiotic Yogurt | DAYS OF STORAGE | | | |
|---|---|---|---|---|---|
| | | 1 | 7 | 14 | 21 |
| TPC (mg TPGAE/g) | Ytw | 4218.882 ± 21.227$^{a}$ | 3968.739 ± 28.929$^{b}$ | 3862.196 ± 27.794$^{c}$ | 3746.389 ± 28.929$^{d}$ |
| | Ycw | 378.717 ± 32.093$^{a}$* | 401.879 ± 36.768$^{a}$* | 378.718 ± 28.928$^{a}$* | 313.866 ± 8.023$^{a}$* |
| | Yte | 3945.577 ± 36.780$^{a}$ | 3764.918 ± 36.768$^{b}$ | 3667.640 ± 41.691$^{b}$ | 3394.335 ± 42.456$^{c}$ |
| | Yce | 383.350 ± 28.929$^{a}$* | 387.982 ± 27.794$^{a}$* | 364.820 ± 8.023$^{a}$* | 332.394 ± 27.794$^{a}$* |
| TPC (mg TPTAE/g) | Ytw | 2678.691 ± 14.500$^{a}$ | 2507.819 ± 19.761$^{b}$ | 2435.040 ± 18.986$^{c}$ | 2355.933 ± 19.761$^{d}$ |
| | Ycw | 55.491 ± 21.922$^{a}$* | 71.312 ± 25.116$^{a}$* | 55.491 ± 19.761$^{a}$* | 11.191 ± 5.481$^{a}$* |
| | Yte | 2491.997 ± 25.116$^{a}$ | 2359.097 ± 9.493$^{b}$ | 2302.140 ± 28.479$^{b}$ | 2115.446 ± 29.002$^{c}$ |
| | Yce | 58.655 ± 19.761$^{a}$* | 61.819 ± 18.986$^{a}$* | 45.998 ± 5.481$^{a}$* | 23.848 ± 18.986$^{a}$* |

Ytw = probiotic yogurt with tumbo pulp treated with bidistilled water; Ycw = control of Ytw without tumbo pulp treated with bidistilled water; Yte = probiotic yogurt with tumbo pulp treated with 96% etanol; Yce = control of Yte without tumbo pulp treated with 96% ethanol. TPGAE = Total Phenols Gallic Acid Equivalent; TPTAE = Total Phenols Tannic Acid Equivalent. All values are expressed as Mean ± Standard Deviation, evaluations performed in triplicate. a, b, c, d indicate significant difference ($p < 0.05$); * Friedman's test and Wilcoxon signed-rank test were applied.

Table 5 shows the flavonoid content of the probiotic yogurt with tumbo pulp (Ytw and Yte) during the 21 days of storage, the results of which were higher than the yogurt fortified with noni juice described by Kwon et al. (2021). Likewise, the results could also denote a better stability of flavonoids over time.

**Table 5.** Total Flavonoid Content (TFC) in probiotic yogurt.

| | Treated Probiotic Yogurt | DAYS OF STORAGE | | | |
|---|---|---|---|---|---|
| | | 1 | 7 | 14 | 21 |
| TFC (mg Quercetin/g) | Ytw | 73.300 ± 1.395$^{a}$ | 60.366 ± 0.554$^{b}$ | 56.855 ± 0.847$^{c}$ | 52.421 ± 1.154$^{d}$ |
| | Ycw | 1.608 ± 0.554$^{a}$* | 0.684 ± 0.320$^{a}$* | 1.238 ± 0.320$^{a}$* | 0.868 ± 0.320$^{a}$* |
| | Yte | 59.627 ± 0.847$^{a}$* | 50.203 ± 0.320$^{a}$* | 42.812 ± 0.850$^{a}$* | 36.345 ± 0.320$^{a}$* |
| | Yce | 4.010 ± 0.320$^{a}$* | 1.423 ± 0.320$^{a}$* | 0.684 ± 0.320$^{a}$* | 1.238 ± 0.320$^{a}$* |

Ytw = probiotic yogurt with tumbo pulp treated with bidistilled water; Ycw = control of Ytw without tumbo pulp treated with bidistilled water; Yte = probiotic yogurt with tumbo pulp treated with 96% etanol; Yce = control of Yte without tumbo pulp treated with 96% ethanol. All values are expressed as Mean ± Standard Deviation, evaluations performed in triplicate. a, b, c, d indicate significant difference ($p < 0.05$); * Friedman's test and Wilcoxon signed-rank test were applied.

Table 6 shows the carotenoid content of the probiotic yogurt with tumbo pulp (Yto and Yte) during the 21 days of storage, whose results were higher than the yogurt enriched with encapsulated red bell pepper residues described by Šeregelj et al. (2019); and presented a higher amount of β-carotene than the probiotic yogurt enriched with Spirulina biomass developed by Patel et al. (2019). In addition, ethanol allowed the extraction of carotenoids from food products (Saini & Keum, 2018), so its values were close to the extraction method, according to Xavier et al. (2012).

**Table 6.** Carotenoid content of probiotic yogurt.

| | Treated Probiotic Yogurt | DAYS OF STORAGE | | | |
|---|---|---|---|---|---|
| | | 1 | 7 | 14 | 21 |
| Carotenoids (µg β-carotene/g) | Yto | 95.136 ± 1.953$^{a}$ | 86.120 ± 1.612$^{b}$ | 81.164 ± 1.185$^{c}$ | 72.109 ± 0.777$^{d}$ |
| | Yco | 26.054 ± 0.448$^{a}$* | 24.502 ± 0.449$^{a}$* | 26.572 ± 0.448$^{a}$* | 23.467 ± 0.448$^{a}$* |
| | Yte | 85.822 ± 1.950$^{a}$ | 79.353 ± 1.186$^{b}$ | 77.346 ± 1.721$^{b}$ | 65.382 ± 1.616$^{c}$ |
| | Yce | 32.782 ± 0.448$^{a}$* | 28.900 ± 0.448$^{a}$* | 24.761 ± 0.777$^{a}$* | 22.432 ± 0.776$^{a}$* |

Yto = probiotic yogurt with tumbo Pulp, according to Xavier et al. (2012); Yco = control of Yto without tumbo Pulp, according to Xavier et al. (2012); Yte = probiotic yogurt with tumbo pulp treated with 96% etanol; Yce = control of Yte without tumbo pulp treated with 96% ethanol. All values are expressed as Mean ± Standard Deviation, evaluations performed in triplicate. a, b, c, d indicate significant difference ($p < 0.05$). *Friedman's test and Wilcoxon signed-rank test were applied.

## 4 Conclusion

The probiotic yogurt developed with *P.a tripartita* (tumbo) fruit pulp had a high amount of LAB, thus exceeding the recommended values as CFU greater than $10^7$ is considered a probiotic. Furthermore, the probiotic yogurt presented high antioxidant capacity through the radical scavenging capacity test and the cation radical scavenging capacity test, which correlated with its high content of phenolic compounds, flavonoids, and carotenoids that could remain high during the 21 days of storage. In addition, it showed high acceptability and had an adequate physicochemical and microbiological quality.

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