

Antioxidant capacity and phenolic content of a beverage based on jamaica flower (*Jamaica sabdariffa*) enriched with mulberry (*Vaccinium floribundum*) and blackberry (*Rubus glaucus*)

Capacidad antioxidante y contenido fenólico de una bebida a base de flor de Jamaica (*Jamaica sabdariffa*) enriquecida con mortiño (*Vaccinium floribundum*) y mora (*Rubus glaucus*)

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Abstract

The objective of this research was to evaluate the antioxidant capacity and phenolic content of a Jamaica flower-based drink enriched with mulberry and blackberry, for which it was proposed to study two factors: the concentration of Jamaica flower for the infusion and the percentage used of blackberry and mulberry. Treatment 9 made with 5% Jamaica flower and 95% water; 75% mulberry and 25% blackberry and treatment 8 made with 5% Jamaica flower and 95% water; 59% mulberry and 50% blackberry did not differ statistically in the sensory tests, obtaining the highest acceptance by the panel. The content of phenols analysis showed a result of 89.5 mg/L; while the antioxidant capacity presented 1732 mg EAG/L. Based on the results obtained in the microbiological analysis, the absence of pathogens (yeast, mold and mesophilic aerobes)

was evidenced at 0, 15 and 30 days of storage, which implies that the drink will have a useful life of at least 30 days.

Palabras claves: Antioxidant, blackberry, jamaica, mulberry, phenol.

Resumen

El objetivo de esta investigación fue evaluar la capacidad antioxidante y contenido fenólico de una bebida a base de flor de Jamaica enriquecida con mortiño y mora, para lo cual se planteó estudiar dos factores: la concentración de flor de Jamaica para la infusión y el porcentaje empleado de mora y mortiño. El tratamiento 9 elaborado con 5% flor de Jamaica y 95% agua; 75% mortiño y 25% mora y el tratamiento 8 elaborado con 5% flor de Jamaica y 95% agua; 50% mortiño y 50% mora no se diferenciaron estadísticamente en las pruebas sensoriales, obteniendo la mayor aceptación por parte del panel. El análisis de contenido de fenoles totales mostró un resultado de 89.5 %; mientras que en la capacidad antioxidante presentó 1732 mg /L. En base a los resultados obtenidos en el análisis microbiológico se evidenció ausencia de patógenos (levadura, moho y aerobios mesófilos) a 0, 15 y 30 días de almacenamiento, lo cual implica que la bebida tendrá una vida útil de al menos 30 días.

Keywords: Antioxidante, fenoles, jamaica, mortiño, mora.

1. INTRODUCTION

The blueberry is a small fruit that measures between 5 and 8 millimeters in diameter. Its texture is soft and it is blue in color, *Vaccinium floribundum* is its scientific name and it is a berry that has existed for years. It comes from some areas of the highlands of Ecuador such as Carchi, Imbabura, Pichincha, Cotopaxi, Tungurahua, Bolivar, Chimborazo, Cañar, Azuay and Loja (citation). However, Montoya et al. (2009), believe that can be considered a nutraceutical food, because it is rich in polyphenolic compounds, antioxidants and antioxidants, polyphenolic compounds, antioxidants and health-protective pigments.

Mulberry is a very ancient wild fruit that is produced organically in the moorlands of the central part of the country, without any human intervention. The Andean moorlands hold a culinary treasure that has not been exploited as it deserves. It is a fruit that colors the fields with

lilac and purple tones, which combines perfectly in sweet and salty preparations: the mulberry, the true "Pearl of the Andes". Throughout our history, this black pearl was mainly used to prepare the traditional 'Colada Morada' in the month of November, celebrating the 'Day of the Dead' or 'Día de los Difuntos'. The rest of the year, the product stands firm in the moorlands and the surrounding communities hope to have a market to sell this Andean wealth (Molina, 2017).

According to Carvajal, Waliszewski and Infanzón (2006): Jamaica sabdarifa is native to Africa and intensively cultivated in tropical and subtropical regions of India, Thailand, Senegal, the United States, Panama and Mexico. In Ecuador the cultivation of this flower occurs in certain areas of the Amazon, specifically in the provinces of Napo, Morona Santiago and Pastaza where there are small areas of

production. Organic jamaica flower production in Ecuador needs to find innovative tools that can increase yields and flower quality.

Blackberry (*Rubus glaucus* Benth) is native to the high tropical zones of the Americas, and is grown mainly in Ecuador, Colombia, Panama, El Salvador, Honduras, Guatemala, Mexico, and the United States (citation). In Ecuador, blackberry production is distributed along the entire inter-Andean alley, especially in the provinces of Tungurahua, Cotopaxi, Bolivar, Chimborazo, Pichincha, Imbabura and Carchi. The province with the highest production is Bolívar, contributing 34,209 t/year, equivalent to 39% of the national production of the fruit. This province registers a yield of 6.90 t/ha. The second blackberry producing province is Tungurahua, contributing 33% of the national production. In addition, this province has a yield of 8 t/ha which is the highest of all producing provinces (INIAP, 2016).

In the Ecuadorian market today, several ventures linked to mulberry have been identified, including products such as colada morada, ice cream and jams. However, one of the highlights is the recognized and valued artisanal wine made from this fruit. The mulberry is native to the cold regions of the Ecuadorian highlands. Despite its potential, it is not currently being exported due to the perception that it lacks added value and its distinctive flavor that differentiates it from conventional wines (Cevallos, 2019).

It is essential that the Ecuadorian population reconsiders its perspective and promotes the consumption of mulberry both domestically and internationally. This could open new opportunities to take advantage of the unique qualities of this fruit and its potential in the food

and beverage industry. Jamaica flower has several medicinal aspects for health, as it is known to be very useful for humans such as: "Cardiac tonic, has diuretic, antiseptic, analgesic, anti-inflammatory, antimicrobial, astringent, healing, digestive, depurative, emollient, sedative, mild laxative, weight reducer, detoxifying, antioxidant, toning, stimulant, aphrodisiac and vasodilator and vitamin".

In addition, Jamaica has a significant content of vitamins A and C, a large amount of minerals, citric and malic acid. The antioxidants found in Jamaica make it a food that can help fight parasite problems, is useful for weight loss, controls cholesterol and normalizes triglycerides" (Cardenas, 2015).

2. MATERIALS AND METHODS

2.1. Location

The research was carried out at the Food Processing Laboratory of the Agrarian University of Ecuador, University City "Dr. Jacobo Bucaram Ortíz" Milagro Campus.

2.2. Materials

Jamaica flower, blackberry, mulberry, panela, stainless steel pots, volumetric jars, stainless steel knife, homemade strainer, stainless steel spoons, 500 mL beakers, digital balance, Ph-meter, industrial blender, digital refractometer, thermometers, spectrophotometer.

2.3. Treatments

For the development of this research, two factors were studied: the concentration of jamaica flower for the infusion and the percentage of blackberry and mulberry used (Table 1 and 2).

The combinations of these factors allowed the development of nine treatments (Table 3), and formulation for Jamaica flower drink (Table 4).

Table 1. Concentration of jamaica flower in infusion

Factor A	Concentration of jamaica flower
a1:	1% jamaica flower – 99% water
a2:	3% jamaica flower – 97% water
a3:	5% jamaica flower – 95% water

Table 2. Percentages of blackberry and mulberry

Factor B	Concentration of blackberry and mulberry
b1:	25% mulberry – 75% blackberry
b2:	50% mulberry – 50% blackberry
b3:	75% mulberry – 25% blackberry

Table 3. Treatments to be evaluated

Treatments	Combination	Description (infusion + blackberry and mulberry extract)
1	a1b1	99 % wáter to 1% of jamaica flower + 25% mulberry – 75% blackberry
2	a1b2	99 % water to 1% of jamaica flower + 50% mulberry – 50% blackberry
3	a1b3	99 % water to 1% of jamaica flower + 75% mulberry – 25% blackberry
4	a2b1	97 % water to 3% of jamaica flower + 25% mulberry – 75% blackberry
5	a2b2	97 % water to 3% of jamaica flower + 50% mulberry – 50% blackberry
6	a2b3	97 % water to 3% of jamaica flower + 75% mulberry – 25% blackberry
7	a3b1	95 % water to 5% of jamaica flower + 25% mulberry – 75% blackberry
8	a3b2	95 % water to 5% of jamaica flower + 50% mulberry – 50% blackberry
9	a3b3	95 % water to 5% of jamaica flower + 75% mulberry – 25% blackberry

Table 4. Formulation for Jamaica flower drink

Ingredients	Percentages	Formulation 1 L
Jamaica flower infusión	50%	500 mL
Blackberry and mulberry mix	45%	450 mL
Panela	4%	4 g
Xanthan Gum	1%	1g
TOTAL	100%	1000 mL
Potassium sorbate	0.05%	0.5 g

2.4. Experimental design

A completely randomized block design (DBCA) was used, in which the blocking source was made up of the 30 untrained judges of a sensory panel who rated each sample based on a hedonic criterion, with 1 being the lowest rating equivalent to “I don't like it” and 5 is the highest which corresponds to “I like it a lot”. This criterion was applied to each of the attributes: color, smell, flavor and appearance.

For the development of the research, the size of the experimental unit was 1000 mL, and each panelist was provided with 30 mL for each sample to taste.

2.5. Methods and techniques

In the Figure 1 details the flow chart for obtaining Jamaica flower infusion, the Figure 2 shows the flow chart for obtaining the mulberry and blackberry extract; and the figure 3 shows the scheme for the production of the Jamaica beverage.

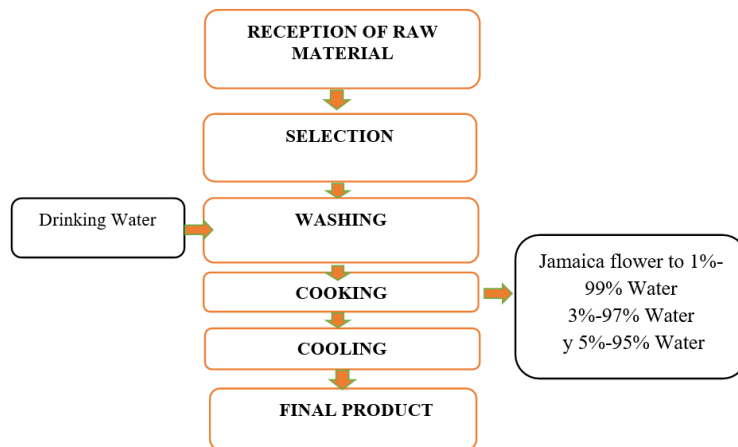


Figure 1. Flow chart to make Jamaica flower infusion.

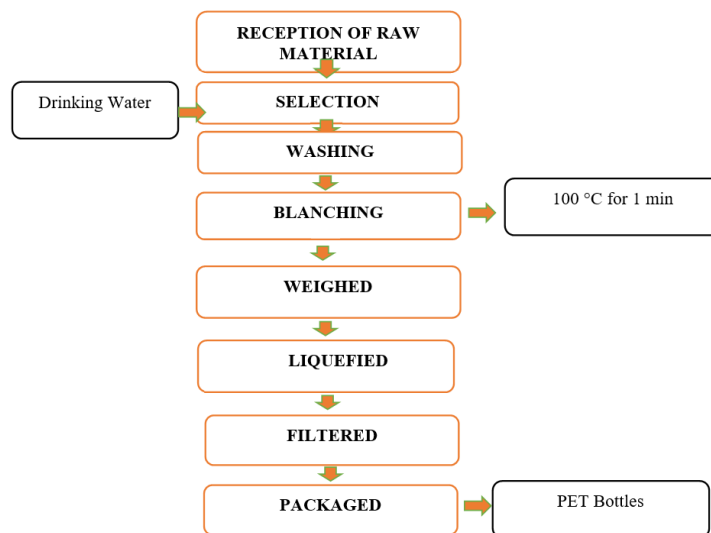


Figure 2. Flow chart for the production of mulberry and blackberry extract.

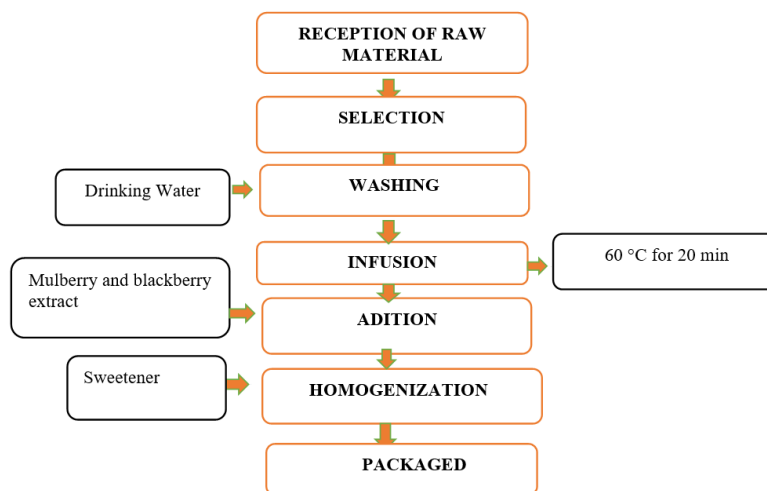


Figure 3. Flow chart to the production of the Jamaica beverage.

2.6. Variables to be evaluated

Determination of phenol content

The samples were homogenized in a laboratory blender; from each homogenized test portion, 5.0 ± 0.1 g were weighed in 50 ml centrifuge tubes, adding 25 ml of 50 % v/v ethanol as extraction solvent. The extraction stage was carried out in the centrifuge tubes themselves using the Ultra-Turrax model T-21 homogenizer at $11\,000\text{ min}^{-1}$ for 2 min. Once the extraction was completed, the extracts were centrifuged at $1\,000\text{ min}^{-1}$ and aliquots were taken from the liquid phase, suitably diluted, to carry out the analytical determinations.

The total polyphenol content was determined using the Folin-Ciocalteu reagent, according to the method proposed by Slinkard and Singleton (1977), and the results were expressed as gallic acid (EGA) in mg/100 g.

Sensory characteristics

Using a hedonic scale, the sensory panel evaluated the attributes of odor, smell, taste and appearance of the samples of each of the treatments under study. The ratings used are described below: 5 Very good, 4 Good, 3 Fair, 2 Bad, 1 Very bad

Shelf life

The microbiological analysis of molds, yeasts and mesophilic aerobes was carried out in accordance with INEN Standard 1529-10. For this reason, the sample with the highest sensory acceptance was taken to an external laboratory for analysis at 0, 15 and 30 days.

Determination of soluble solids (°Brix)

The analysis of soluble solids was carried out by means of the pictometric method, in accordance with INEN Standard 2172.

Determination of pH

The analysis for the determination of pH will be carried out by means of the potentiometric method, according to INEN 1842:2013 Standard.

Test method

This method is based on the cultivation between $22\text{ }^{\circ}\text{C}$ and $25\text{ }^{\circ}\text{C}$ of the propagating units of molds and yeasts, using the plate count technique by deep seeding and a medium containing yeast extract, glucose and mineral salts.

Materials and culture methods

The glassware must be repeatedly sterilized and all material must be perfectly clean and sterile. Petri dishes; wide mouth serological pipettes 1; 5 cm^3 and 10 cm^3 graduated in 1/10 of unit; spreaders; culture media; davis salt-yeast agar or similar.

3. RESULTS AND DISCUSSION

Variables such as pH and °Brix corresponding to the physicochemical analysis were evaluated, where it is shown that the hibiscus flower has a pH of 3.40 and °Brix of 12, in the blackberry was obtained a pH of 3.17 with a °Brix of 11, the mulberry has a pH of 4.50 and °Brix of 14, likewise, in treatment 1 the pH was 4.01 and °Brix of 11, in treatment 2 the pH was 4.55, while the °Brix was 12, in treatment 3 the pH was 5.02 with °Brix of 15, treatment 4 the pH was 4.35 with °Brix of 13, treatment 5 the pH was 4.35 with °Brix of 13, treatment 6 the pH was 4.05 with °Brix of 11, treatment 7 pH was 4.01 with °Brix of 11, treatment 8 pH was 5.02 with °Brix

of 15, and finally treatment 9 pH was 4.50 with °Brix of 14 (Table 5).

Table 6, shows the results of the sensory evaluation carried out by the 30 untrained judges, who rated the color, smell, flavor, and texture of the beverage based on hedonic criteria.

Table 5. *Contenido de fenoles y capacidad antioxidante de la bebida*

Sample	pH	° Brix
Jamaica Flower	3.40	12
Mulberry	3.17	11
Blackberry	4.50	14
Treatmet 1	4.01	11
Treatmet 2	4.55	12
Treatmet 3	5.02	15
Treatmet 4	4.35	13
Treatmet 5	4.35	13
Treatmet 6	4.05	11
Treatmet 7	4.01	11
Treatmet 8	5.02	15
Treatmet 9	4.5	14

Table 6. *Sensory Analysis Results*

No	Factor A	Factor B	Color	Smell	Flavor	Texture
T ₁	a1: 1% Jamaica flower and 99% water	b1: 25% mulberry and 75% blackberry	4.03bcd	3.77bcd	3.17cd	3.53cd
T ₂	a1: 1% Jamaica flower and 99% water	b2: 50% mulberry and 50% blackberry	3.57d	3.33d	2.90cd	3.53.cd
T ₃	a1: 1% Jamaica flower and 99% water	b3: 75% mulberry and 25% blackberry	3.60d	3.43cd	3.13cd	3.50cd
T ₄	a2: 3% Jamaica flower and 97% water	b1: 25% mulberry and 75% blackberry	3.60d	3.53cd	2.87cd	3.23b
T ₅	a2: 3% Jamaica flower and 97% water	b2: 50% mulberry and 50% blackberry	3.97cd	3.87bcd	3.10cd	3.70bcd
T ₆	a2: 3% Jamaica flower and 97% water	b3: 75% mulberry and 25% blackberry	4.03 bcd	3.67cd	3.60bc	4.07abc
T ₇	a3: 5% Jamaica flower and 95% water	b1: 25% mulberry and 75% blackberry	4.20bc	3.90bc	3.47bcd	3.97abc
T ₈	a3: 5% Jamaica flower and 95% water	b2: 50% mulberry and 50% blackberry	4.53ab	4.23ab	4.17ab	4.27ab
T ₉	a3: 5% Jamaica flower and 95% water	b3: 75% mulberry and 25% blackberry	4.73 ^a	4.57 ^a	4.50 ^a	4.43 ^a
Coefficient of variation (%)			16.29	17.86	25.93	19.60

Means with a common letter are not significantly different ($p > 0.05$).

After applying the analysis of variance, all sensory variables showed significant effects in the interaction of the factors. The results of the coefficients of variation explain that the analyses have an acceptable precision, according to the coefficients of variation

obtained, given that for color 16.29% was obtained; smell 17.86%; for flavor 25.93% and texture 19.60%.

In the evaluation of color, it was evident that treatment T9 (5% jamaica flower and 95% water;

75% mulberry and 25% blackberry) obtained the highest sensory acceptance with an average of 4.73 equivalent to "I LIKE IT VERY MUCH" based on the hedonic scale used, which is not statistically different from treatment 8 (5% jamaica flower and 95% water; 50% mulberry and 50% blackberry) with an average of 4.53.

In the evaluation of the smell, T9 and T8 obtained the highest preference from the panel of judges, with mean values of 4.57 and 4.23, respectively. In the flavor attribute, the treatment that showed the greatest acceptance

by the panelists was T9 with a mean of 4.50, which did not differ statistically from the T8 treatment (4.17) (Table #).

As for texture, a similar behavior to other attributes was found, T9 (4.43), T8 (4.27) and T6 (4.07) did not differ significantly from each other (Table #).

In general terms, treatments 8 and 9 had the highest sensory acceptance by the panel of evaluating judges. These treatments have the highest percentage of jamaica flower (5%), compared to T8 (4.27) and T6 (4.07).

Table 7. Phenol content and antioxidant capacity of the beverage

Parameters	Méthods	Results	Units
Total Phenols	Singleton and Rossi (1965) (Spectrophotometry)	89.5	%
Antioxidant Capacity	(FRAP) (Spectrophotometry)	1732	mg/L

The analysis of antioxidant capacity and phenol content was carried out on treatment 9 prepared with 5% hibiscus flower and 95% water; 75% mulberry and 25% blackberry. The phenol content was 89.5% while the antioxidant capacity was 1732 mg/L.

The results of the analysis of antioxidant capacity and phenolic content are within the range established by the standards. The

antioxidants are divided into vitamin C, vitamin E, glutathione, lipoic acid, uric acid, carotenoids and phenolics in flavonoids, phenolic acid and polyphenols, this amount our body assimilates without causing damage, so it is a great benefit yan these components of antioxidants and phenolics block free radicals preventing diseases such as cancer, heart attacks and diseases such as cancer, heart attacks and protects the immune system.

Table 8. Useful life Analysis

Parameters analyzed	0 days	15 days	30 days	Units
Yeast plate count	<10	<10	<10	UFC/mL
Mold plate count	<10	<10	<10	UFC/mL
Mesophilic Aerobes	<10	<10	<10	UFC/mL

Table 8 shows the results of the shelf-life analysis carried out on the sample with the highest sensory acceptance of the beverage based on jamaica flower enriched with mulberry and blackberry. The absence of pathogens (yeast, mold and mesophilic aerobes) was evidenced at 0, 15 and 30 days of storage, which implies that the beverage made with 5% jamaica flower and 95% water; 75% mulberry and 25% blackberry, will have a shelf life of at least 30 days.

In the study carried out by Cornejo and Párraga (2021), the capacity antioxidant and phenolic content of a drink based on Jamaica flower, with three concentrations that were 0.5, 1% and 1.5%, at which physical analysis was carried out chemical and microbiological. For the sensory evaluation, a test was applied with a 7-point hedonic scale with 30 untrained tasters evaluating the smell, flavor, color and taste parameters. Analysis of variance was applied parametric, using Kruskal Wallis contrast test, it was possible to verify that Treatment T1 was selected as the best according to the tasters.

Subsequently, the antioxidant capacity and phenolic content were analyzed to the best treatment (T1) where averages of 50.45 $\mu\text{mol ET}/100\text{g}$ and 671 mg were shown EGA/100g respectively, concluding that the dehydrated Jamaica flower influences the physical and chemical characteristics of the drink, thus favoring way to the industrialization of the flower. In the study carried out the analysis of phenol content at treatment 9 made with 5% Jamaica flower and 95% water; 75% mulberry and 25% default was 89.5%; while the capacity antioxidant presented 1732 mg/L, these results vary with those presented in other investigations.

In the study carried out by Sánchez and Bravo(2021), results of 3.8 were observed ± 0.10 (on a 4-point scale) for the most optimal treatment (T1), which was evaluated more favorably by tasters. This treatment involved the incorporation of 10% of Jamaica flower in the preparation of the drink.

Likewise, in a similar work, Cedeño (2021) achieved good acceptance by part of the judging panel when creating an alcoholic beverage based on 20% Jamaica flower and rose petals.

However, in this study a more marked preference was evident for sensory panel for drinks with 5% Jamaica flowers. These drinks presented an intense red tone due to the inclusion of jamaica flower, and the blackberries provided dark and translucent nuances. Small blackberry and mulberry seeds in the composition. The smell of the drink constituted an amalgam of floral and citrus notes from the jamaica flower, together with the sweetness of blackberries and mulberry, which resulted in a fresh and fruity with acidic touches. The flavor was characterized by a balance between the flavors sweet and sour. The texture of the drink presented a certain consistency due to the presence of seeds and pieces of fruit.

The content analysis of phenols carried out in the present investigation is performed treatment 9, made with 5% Jamaica flower and 95% water; 75% mulberry and 25% mulberry was 89.5%; while the antioxidant capacity presented 1732 mg/L, these results vary with those presented in other investigations, as in the study developed by Cedeño (2021) who attributes the variation to the amount of moringa used as fining agents. The antioxidant capacity of control group stood at 1327.6 mg/L,

while in treatment 3 (which incorporated 6% moringa) a value of 1781.1 mg/L was observed. Moringa is recognized for its abundance of antioxidants, such as vitamin C, vitamin E and a variety of phenolic compounds. The inclusion of moringa in treatment 3 possibly contributed to a higher antioxidant content, which translated in a higher antioxidant capacity compared to the control group.

However, this value is close to the result obtained in this research, in which is attributed to mulberry (the main component) an antioxidant capacity of 1373 mg/L, according to the analyzes carried out by Montoya *et al.* (2009).

Herrera (2022) evaluated the antioxidant capacity and phenolic content of a wine of mulberry and blackberry using different concentrations of ammonium salts, the results obtained indicated that the values of the antioxidant capacity and phenolic content were close in the different treatments, which led to the conclusion that the provision of nutrients for the yeast did not influence significantly in the antioxidant capacity (640.9 to 673.4 mg/L) and the content of phenols (739 to 764 mg/L) of the final product. The antioxidant capacity value was shown to be superior to values found in this research, however, in the phenolic content were lower in phenolic content.

In the useful life analysis, the absence of pathogens (yeast, mold and mesophilic aerobes) at 0, 15 and 30 days of storage, which implies that the drink made with 5% Jamaica flower and 95% water; 75% mulberry and 25% blackberry, will have a shelf life of at least 30 days, it should be noted that in this drink it was treated with 0.05% potassium sorbate as a preservative. On the other hand, Cedeño (2021) carried out the

microbiological test on the sample of Jamaica flower wine and roses with 6% moringa, evidencing the absence of pathogenic microorganisms (total coliforms, molds and yeasts), up to 30 days of storage. In based on the results obtained in their research, they attribute effects to moringa antimicrobial, which influences the useful life of this product.

4. CONCLUSIONS

Treatment 9 made with 5% Jamaica flower and 95% water; 75% mulberry and 25% blackberry and treatment 8 made with 5% Jamaica flower and 95% water; fifty% mulberry and 50% blackberry did not differentiate statistically in the tests sensory, obtaining the greatest acceptance by the judges panel in each one of the parameters evaluated (color, smell, taste and flavor), however for subsequent tests were taken into account to treatment 9 as winner, for being the one that presented the highest average in each of the attributes.

The phenols content analysis made to treatment 9 prepared with 5% Jamaica flower and 95% water; 75% mulberry and 25% blackberry showed a result of 89.5 %; while the antioxidant capacity presented 1732 mg/L.

Based on the results obtained in the microbiological analysis, it was evident absence of pathogens (yeast, mold and mesophilic aerobes) at 0, 15 and 30 days storage, which implies that the drink made with 5% Jamaica flower and 95% water; 75% mulberry and 25% blackberry, it will have a useful life of at least 30 days.

Declaración de intereses

Ninguna.

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