Colegio de Postgraduados

Microencapsulation of anthocyanins from *Hibiscus sabdariffa* and the association of stability and phenolic compounds and antioxidant activities

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ABSTRACT

Objective: To design an extraction, characterization, and microencapsulation process for two *Hibiscus* (Sudan and Tecoanapa) cultivars.

Design/Methodology/Approach: The plant material was collected. The samples were first crushed and then optimized with water and alcohol. Afterwards, the extract obtained in this process was microencapsulated. Anthocyanin content and antioxidant activity were evaluated, before and after the treatment. Finally, the efficiency of the preserved extract was determined.

Results: The Sudan cultivar recorded a higher anthocyanin content (1,319.8 mg 100 g^{-1}) than Tecoanapa (557.2 mg 100 g^{-1}). Maltodextrin was the best microencapsulation process for the extracted and encapsulated matrix of Sudan. The matrix had phenols and anthocyanins. The size of the particles recorded a 11.50±0.5 μm diameter. In addition, microencapsulation efficiency reached 85%±5%.

Study Limitations/Implications: The preservation of extracted matrices can be profitable, because it keeps the color and health benefits of the flower; however, the equipment required for the microencapsulation process is very expensive.

Findings/Conclusions: Maltodextrin was the best resin for this procedure, recording a higher percentage of matrix preservation than phenols and anthocyanins.

Keywords: resin, preservation, anthocyanins, maltodextrin, phenols.

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INTRODUCTION

Jamaica belongs to the family Malvaceae (Jung and Joo, 2013). This plant has different names in different countries: roselle (UK, USA), karkadé (Arabia, Sudan), byssap (Senegal), l'oiselle (France), alazán (Caribbean), krachiap daeng (Thailand), and *jamaica* (Mexico and Spain) (Ramírez-Rodrigues *et al.*, 2012). The calyces of *Hibiscus* are used to prepare cold and hot beverages, as well as marmalade and jelly (Mohamed *et al.*,

2012). *Jamaica* is widely consumed by Mexicans, including children and elders (Mercado-Mercado, 2015). In addition, *Hibiscus* is used all over the world to treat different diseases (Picking D., *et al.*, 2011).

Sudan, Thailand, China, Mexico, Egypt, Senegal, and Tanzania are the major producers of *Hibiscus* (Villanueva-Carvajal *et al.*, 2013). In Mexico, *jamaica* is grown in Oaxaca, Michoacan, Nayarit, Puebla, and Guerrero (Pérez-Torres, 2009). According to the Servicio de Información Agroalimentaria y Pesquera (SIAP) of SAGARPA, Guerrero is the major jamaica producer of the country. The municipality of Ayutla de los Libres is the main producer of Guerrero, followed by Tecoanapa, San Luis Acatlán, and Acapulco de Juárez (SIAP, 2023).

During the last few years, researchers worldwide have been focused on discovering the full potential of medicinal plants grown under traditional systems. *H. sabdariffa* is one of several medicinal plants that have been studied and that could be used as a powerful phytochemical agent to treat different diseases (Montaño *et al.*, 2024). The therapeutic effects of *H. sabdariffa* have been associated with bioactive and functional components, such as phenolic acids, flavonoids (Vargas *et al.*, 2018), anthocyanins, organic acids, and dietary fiber (Izquierdo-Vega *et al.*, 2020). Given their biological activities, the bioactive compounds found in the calyces are used as anti-inflammatory, hepatoprotective, antihypertensive, antimicrobial, and antioxidant agents (Montalvo-González *et al.*, 2022; Ghazala-Riaz *et al.*, 2018).

Anthocyanin content is responsible for the characteristic red color of the calyx of *jamaica* flowers (Sumaya *et al.*, 2014). Anthocyanins attract pollinators, which spread the seeds of the plants and protect them from the effect of UV radiation and viral and microbial infections (Garzón, 2008). However, factors such as pH, temperature, light, antioxidants, and metal ions impact anthocyanin stability (Xue *et al.*, 2024). Microencapsulation is a great option for the food industry to preserve bioactive compounds that could be added to the matrices as food supplements in order to develop functional food (Aguilera-Chávez *et al.*, 2022). Therefore, the objective of this research was to design an extraction, characterization, and microencapsulation process for two jamaica cultivars. The hypothesis was that protective resins can be used to preserve the anthocyanins and phenols responsible for biological activities.

MATERIALS AND METHODS

Samples from the Sudan and Tecoanapa varieties were collected in the community of Tecoanapa, Guerrero. The samples of the two *jamaica* (*Hibiscus sabdariffa* L.) varieties were transported in bags. The *jamaica* calyces were dried in the shade for four days. During the evenings, the samples were left in the open, covered with a waterproof coat. Afterwards, a hammer mill was used to crush the samples until they reached the desired particle size. Finally, the samples were placed in amber glass jars. A thin-layer chromatography (TLC) was used for the preliminary analysis. In order to determine the presence of flavonoids, a mobile phase was conducted with 100 ethyl-acetate: 26 formic acid: 26 acetic acid: 1 water. For this purpose, 0.5g of dry and crushed plant material and seeds of the Sudan and Tecoanapa varieties were weighted. The sample was placed in a test tube with 15

mL of distilled water. Subsequently, it was subjected to a bain-marie for 20 minutes at 60 °C. Afterwards, the content was filtered, in order to obtain a first extract of the calyces and the seeds of *H. sabdariffa*. Three-mL of this extract were poured into three test tubes. Three drops of olive oil were added to the first test tube; the test tube was then stirred to observe the foam and to detect saponin content. Three drops of hydrochloric acid were added to the second test tube to establish the presence of flavonoids. Finally, three drops of ferric chloride (3%) were added to the third test tube to detect tannin content. Based on the preliminary analysis, the following extracts were then obtained from the calyces: cold water, methanol, and ethanol.

Subsequently, 4 g of dry and crushed plant material were added to the three extraction solvents (30 mL of methanol, ethanol, and cold water). The 6-hour extraction process was carried out at room temperature. Four g of dry and crushed plant material were used to prepare boiling aqueous extracts, with different extraction times (3, 5, and 10 min). Once the boiling time of each extraction was over, they were filtered and stored at 4 °C.

Quantification of phenolic content

The Folin-Ciocalteu method described by Waterman and Mole (1994) was used to determine the total phenolic content. One g of calyx sample of each of the two varieties and 5 mL of 80% methanol were weighted. The sample was centrifuged for 20 minutes. Subsequently, 250 μL of the sample and 750 μL of distilled water were poured into test tubes. Afterwards, 250 μL of this solution were placed in test tubes, where 15.75 mL of deionized water and 1 mL of Folin reagent were added. After one minute (but not longer than 8 minutes), 3 mL of 20% Na_2CO_3 were added to the mixture. The mixture was rested for two hours in the dark. The readings were taken at 760 nm, with a medium sensitivity and visible light. Deionized water was used as blank. Based on the calibration curve, the phenolic concentration of the sample was calculated and expressed in mg L^{-1} of gallic acid.

Determination of anthocyanin content

The method described by Nakata (2014) was used to determine anthocyanin content. Ten mL of HCl 0.1 N were added to a test tube in which 0.1 g of dry and crushed *jamaica* calyx had been placed. The mixture was stirred for 10 minutes. Afterwards, it was centrifuged at 3,500 rpm, for 30 minutes, to clarify the solution. Absorbance was taken at 516 nm. The molar extinction coefficient was used for the calculations.

Determination of antioxidant activity (DPPH)

The DPPH method described by Brand-Williams (1995) was used to determine antioxidant activity. In order to determine antioxidant activity, 30 μL of methanol extract were added to each sample, along with 2 mL of DPPH. The mixture was rested for two hours in the dark. Subsequently, an Unico® 2800 UV/VIS spectrophotometer was used to measure absorbance at 517 nm. Antioxidant activity was expressed as an inhibition percentage. The results were expressed in IC50.

Microencapsulation

Aqueous extracts

Based on the total phenolic and monomeric anthocyanin content and the antioxidant activity of 10 g of Sudan and 40 g of Tecoanapa, the extract was analyzed in a boiling aqueous system for 25 minutes.

Control extract

Five mL of aqueous extract were placed in an amber glass jar and kept in the dark, at room temperature, for a month.

Emulsion preparation

Core Gum E0 gum arabic (Gomas Naturales, S.A. de C.V., México) and maltodextrin (with a 10 dextrose) were used. Sixty g of gum arabic and 40 g of maltodextrin were weighted in a beaker for the C1, S1, C2, and S2 extracts. All the extracts were gauged at 1,000 ml in the appropriate aqueous extract. The final arrangement for Tecoanapa (C) and Sudan (S) was as follows: two concentrations for C1 and S1 (10 g L^{-1}) and C2 and S2 (40 g L^{-1}) and two resins (R1 and R2) for the microencapsulates (maltodextrin:gum arabic). The R1 and R2 ratios were 60M:40GA and 40M:60GA, respectively. In addition, three types of extracts were used: aqueous extract (E), microcapsules (M), and control extract (T).

Microencapsulation

The spray drying process was carried out with a Niro Mobile Minor® R&D Spray Dryer (Niro Atomizer, Denmark). The feed volumetric flux reached 2.77 mL min^{-1}, with an air pressure of 2.8 bars, inlet air of 160 °C, and outlet air of 60 °C.

Statistical analysis

The data of each measurement was used to develop a database in the R 4.0.3 statistical package, which was also used to conduct a statistical analysis. Parameters such as means, standard deviation, variances, and ranges were determined based on the distribution of each variable. ANOVA and Tukey's Test were used to compare groups. A $P<0.01$ value was statistically significant in the design for the two varieties of *jamaica*, the two resins, and the type of extract.

RESULTS AND DISCUSSION

The preliminary phytochemical analysis showed a higher tannin content in the Sudan variety than in the Tecoanapa variety. The anthocyanin, saponin, flavonoid, and potential antioxidant contents were similar in both varieties. The content of the seeds did not record variations; however, they did have saponins (Table 1).

The phytochemical composition of the calyces of the Sudan and Tecoanapa varieties recorded an anthocyanin content difference in preliminary analysis. Table 1 shows that the Sudan variety recorded a higher anthocyanin content (1,319.8±265.8 mg 100 g) than the Tecoanapa variety (557.2±5.5 mg L^{-1}). Nevertheless, both cultivars recorded a 63% antioxidant activity (minimum difference). Meanwhile, consumers prefer the Sudan variety

Table 1. Comparison between the organs of two *jamaica* varieties (Sudan and Tecoanapa).

Plant material	Saponins	Tannins	Flavonoids	Anthocyanins (mg L^{-1})	Antioxidant potential (%)
Cáliz Sudan	(+)	(+++)	(+)	1319.8±265.8	63.7±3.5
Cáliz Tecoanapa	(+)	(++)	(+)	557.2±5.5	63.2±3.4
Semilla Sudan	(+)	(−)	(−)	ND	ND
Semilla Tecoanapa	(+)	(−)	(−)	ND	ND

ND=Not determined. Mean ± Standard deviation (n=5).

due to its intense color and flavor, which are a result of its high anthocyanin concentration. Ramírez-Cortés *et al.* (2011) reported concentrations ranging from 205.93 to 1,110.74 mg 100 g, while Salinas-Moreno *et al.* (2012) registered a concentration of 1,488 mg 100 g in dry calyces of *H. sabdariffa*. Meanwhile, Cid-Ortega *et al.* (2012) pointed out that the percentage or number of nutritional compounds are different in each *jamaica* variety, depending on the part of the plant where they are found. Consequently, the percentage is different in calyces, dry leaves, and even in seeds.

Figure 2 shows that the Sudan (1.39±0.22 mg gallic acid 100 g) and Tecoanapa (31±0.17 mg gallic acid 100 g) varieties did not record differences in the total phenolic content. These results can be the consequence of the quantification of the total phenolic fraction using the Folin-Ciocalteu method. This method includes anthocyanins and flavonoids (flavone, isoflavonoids, flavonols, and tannins), which also have antioxidant activities. Mercado-Mercado *et al.* (2015) associated the values of the antioxidant capacity with the molecular structure of the phenolic compound found in *jamaica* calyces.

Most of the studies agree that the beneficial effects of *jamaica* are mainly a consequence of its anthocyanin, phenolic acid, and flavonoid content (Izquierdo-Vega *et al.*, 2020). However, the antioxidant activity recorded in this study is not directly associated with anthocyanin content, but rather with other metabolites or a mixture of metabolites. Cid-Ortega and Guerrero-Beltran (2012) pointed out that the content of bioactive compounds (such as phenols and anthocyanins) depends on the *jamaica* variety and the extraction method. The solvents used to obtain metabolites were cold and hot water, along with

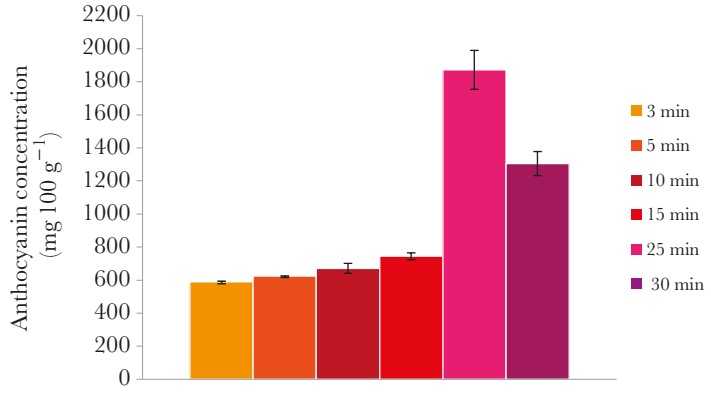

Figure 1. Anthocyanin concentrations of the Sudan variety, based on different extraction times and using boiling water. Mean values ± standard deviation (n=3).

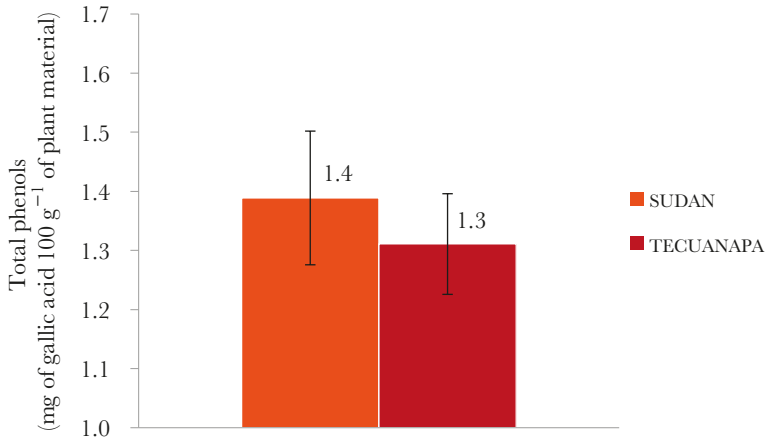

Figure 2. Comparison of the total phenolic content between the Sudan and Tecoanapa cultivars. Means ± standard deviation (n=3).

methanol and ethanol. Hot water was the best solvent for the extraction. Hot water is the most polar of the solvents used in this study, because its temperature produces a higher membrane permeability and increases the exit of secondary metabolites to the extraction medium. In addition, increasing the exposure time of the plant material to boiling water increases the concentration of the anthocyanins extracted. Although anthocyanins are thermolabile, no reduction in their concentration was recorded. This phenomenon suggests that the exposure time could be extended until a significant reduction of the anthocyanin content takes place. This method will establish the appropriate exposure time for the extraction (Table 2). Meanwhile, ethanol was less efficient than methanol (Table 3). However, methanol is toxic and its use is not recommended in food (Salinas-Moreno *et al.*, 2005). Nevertheless, Mercado-Mercado *et al.* (2015) used methanol-acetone extracts to obtain antioxidant activity from *H. sabdariffa*. Izquierdo-Vega *et al.* (2020) explained that, since *Jamaica* calyces are mostly used to prepare cold, warm (tea), and fermented beverages, most researchers work with these aqueous extracts for their analyses. In addition, Salinas-

Table 2. Comparison of the extraction time of anthocyanins using water.

Plant material	Anthocyanin content (mg L^{-1})		
	3 min	5 min	10 min
Sudán	590.1±7.7	624.7±4.9	768.9±4.3
Tecoanapa	137.3±3.1	146.8±2.4	153.1±1.4

Mean ± standard deviation (n=5).

Table 3. Comparison of the solvents used to extract anthocyanins.

Cultivar	Extraction solvent and concentration of anthocyanins (mg L^{-1})			
	H_2O (boiling 10 min)	H_2O (cold 1 h)	Methanol	Ethanol
Sudán	768.9±4.3	560.2±4.3	164.7±0.5	92.7±0.6
Tecoanapa	153.1±1.4	95.2±2	86.9±0.6	59.9±0.5

Mean ± standard deviation (n=5).

Moreno *et al.* (2012) mentioned that increasing the exposure time of the plant material to boiling water increases the concentration of anthocyanins.

According to Escobar-Ortiz *et al.* (2020), the main effect on the extraction yield is a consequence of the solid-liquid ratio. Cissé *et al.* (2012) showed that increasing the solid-liquid ratio improves yield extraction of the anthocyanins found in *jamaica*. These results match the principles of mass transfer. The evaluation of the extraction time recorded an increase of 60.2% in anthocyanin concentration from 15 to 25 minutes. This increase suggests that the total permeability or rupture of the cell membrane occurred at 25 minutes. Afterwards, a falling in the anthocyanin concentration took place (Figure 3). This phenomenon suggested that degradation (diminished concentration) started when anthocyanins were directly released and exposed to a hot aqueous medium.

In descending order of importance, the four types of anthocyanins found in *jamaica* calyces are: delphinidin-3-sambubioside (D3S), cyanidin-3-sambubioside (C3S), delphinidin-3-glucoside (D3G), and cyanidin-3-glucoside (C3G) (Riaz *et al.*, 2018). D3S recorded a total anthocyanin content of 85%. It is the main source of the antioxidant capacity of the extracts of *H. sabdariffa* L. (Da-Costa-Rocha *et al.*, 2014).

According to Hopkins *et al.* (2013), anthocyanins —particularly D3S and C3S— are responsible for some biological effects and they can be found in great quantities in aqueous extracts. Meanwhile, Lo CW *et al.* (2007) associated them with anti-cancer activities, as a result of their capacity to stimulate the apoptosis.

Abubakar *et al.* (2019) pointed out that, 4 hours after consuming polyphenol-rich *H. sabdariffa* beverages, the antioxidant activity of 25 apparently healthy individuals was higher than the control group, who only drank water. These results were possibly the consequence of the antioxidant properties of gallic acids (4-O- and 3-O-methyl gallic acids). In addition, Vargas *et al.* (2018) mentioned that the extract of both Tecoanapa and Sudan varieties have flavonols and flavones.

According to the analysis of variance, the average size of the spheres of Teconapa was 11.50±1.62 μm for C1M (R1) and 11.38±1.38 μm for C2M (R2); meanwhile, Sudan

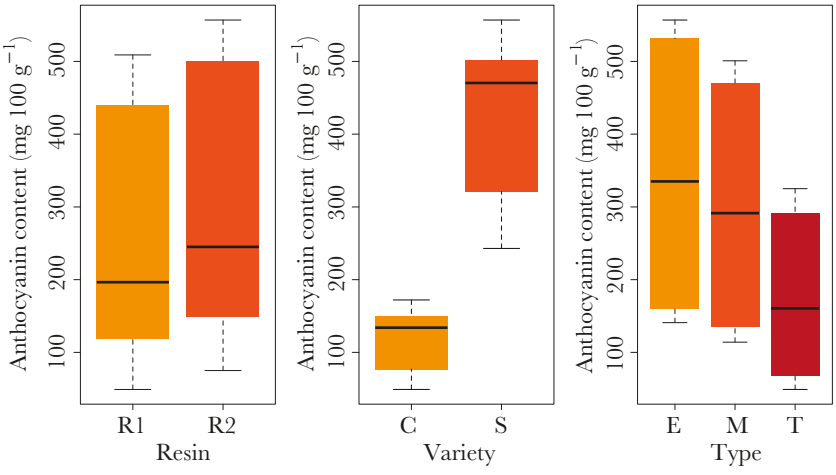

Figure 3. Anthocyanin concentration of the Sudan variety, at different boiling water extraction times. Mean values ± standard deviation (n=3).

obtained 11.59±1.38 μm for S1M (R1) and 11.28±1.33 μm for S2M (R2) (Figure 4).The effectiveness of the spray drying encapsulation method for *jamaica* aqueous extracts reached 81.15, 87.37, 85.95, and 89.95% for C1M (R1), S1M (R1), C2M (R2), and S2M (R2), respectively (Figure 5).

For its part, the total phenolic encapsulation method recorded a 71.02, 73.83, 76.0, and 81.25% efficiency for C1M (R1), S1M (R1), C2M (R2), and S2M (R2), respectively (Figure 6). After a month of microencapsulation, the encapsulation on the antioxidant activity recorded an 87.86, 88.13, 92.32, and 92.58% efficiency for C1M (R1), S1M (R1), C2M (R2), and S2M (R2), respectively. A control kept in a refrigerator was compared with a recently extracted product (Figure 7). Carneiro *et al.* (2013) researched the efficiency of encapsulation and the oxidative stability of flaxseed oil and recorded an encapsulation efficiency of 63-95.7%. These authors used maltodextrin mixed with gum arabic and a buttermilk protein concentrate. Chatterjee *et al.* (2014) reported lower encapsulation efficiency percentages (60-65%) for the dry extract encapsulation of *Phormidium valderianum* with maltodextrin and gum arabic. This variation in the results

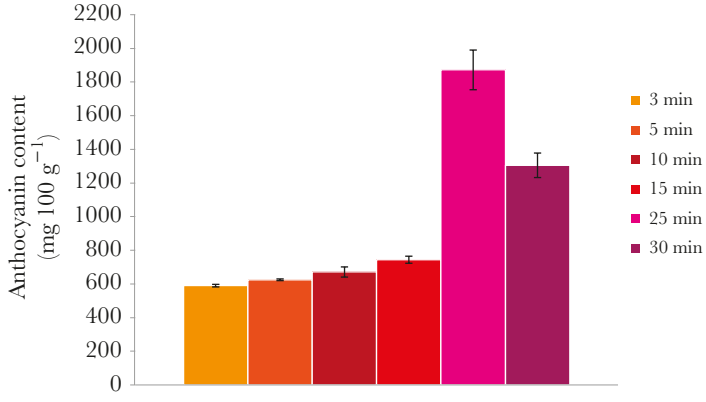

Figure 4. Distribution of the anthocyanin content means, depending on resin, variety, and type variables.

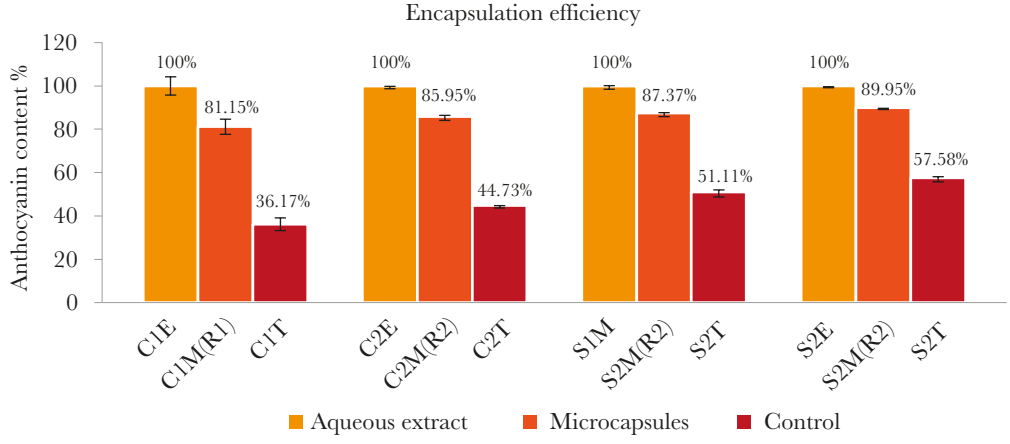

Figure 5. Preservation of anthocyanins in a fresh and microencapsulated extract and control, a month after the spray drying process.

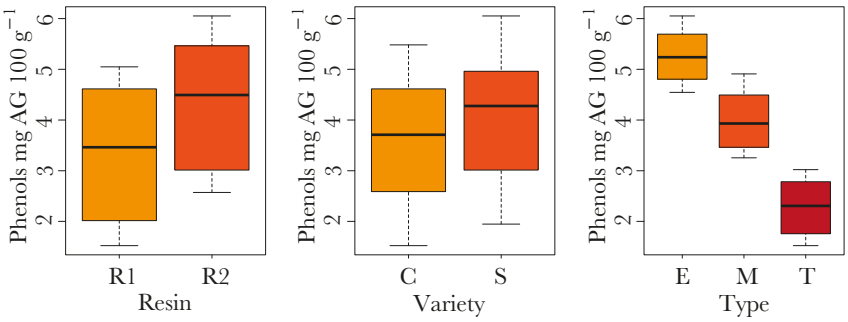

Figure 6. Distribution of the phenol means depending on resin, variety, and type variables. E: recently extracted extract; M: microencapsulated; T: control (extracted extract, kept in the refrigerator for the same period).

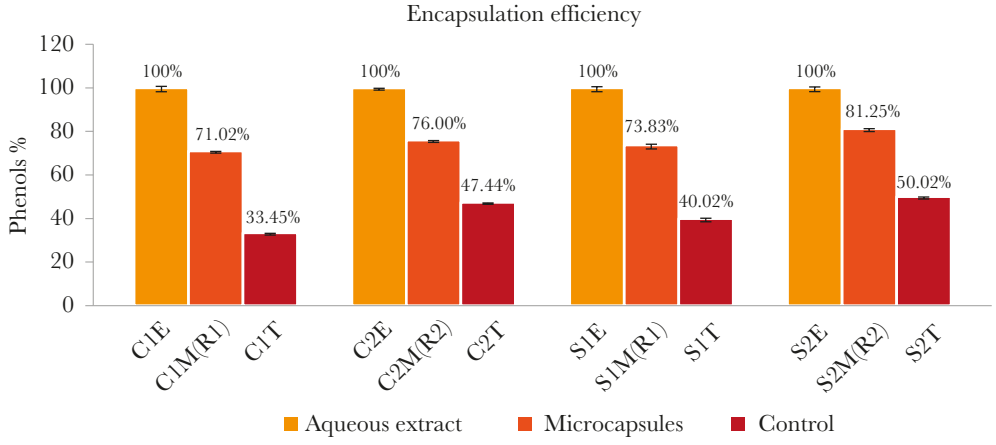

Figure 7. Percentage of phenol preservation in a microencapsulation with two resins, a month after the spray drying process.

can be the consequence of the type of metabolites responsible for the antioxidant activity and the encapsulation process used in the experiments.

Based on the means and variances of the microencapsulation process (anthocyanins, phenols, and antioxidant activity (DPPH)), R2 (60M:40GA) was the best resin for encapsulation. The Sudan variety recorded the best anthocyanin preservation (429.0 mg 100 g^{-1}), followed by Tecoapana (119.03 mg 100 g^{-1}). Based on the type of treatment, S2M (R2) recorded the best anthocyanin preservation (499.6 mg 100 g^{-1}), followed by S1M (R1) (438.8 mg 100 g^{-1}), C2M (R2) (147.8 mg 100 g^{-1}), and C1M (R2) (119.4mg 100 g^{-1}). These results showed a better preservation than the results recorded by control (S1T, C2T, C1T, S2T).

Regarding microencapsulation, Tecoanapa had a better response to the spray drying process (4.08 mg AG 100 g^{-1}) than Sudan (3.60 mg AG 100 g^{-1}). Meanwhile, the best response to the spray drying process was recorded by S2M (R2) with 4.86 mg of gallic acid 100 g^{-1}, followed by C2M (R2), S1M (R1), and C1M (R1) with 4.15, 3.67, and 3.27 mg of gallic acid 100 g^{-1}, respectively. Sudan recorded the highest antioxidant activity (49.88%). For its part, Tecoanapa reached 49.13%. S2M (R2) obtained the highest antioxidant

activity (60.00%), followed by C2M (R2), S1M (R1), and C1M (R1) with 59.20, 43.83, and 43.20%, respectively. These percentages account for their antioxidant potential.

Descriptive analysis of the phenol variables

A significant effect ($p \leq 0.01$) was recorded for *Hibiscus sabdariffa* L. regarding the resin, variety, and treatment variables and their response to anthocyanins, phenols, and DPPH preservation and the distribution of particle size. S2E obtained more anthocyanins (555.4 mg 100 g^{-1}) and phenols (5.983 mg of gallic acid 100 g^{-1}), as well as a better antioxidant activity (64.77%). The similarity regarding antioxidant activity for both cultivars is determined and established by the total phenolic fraction and not just by the anthocyanin concentration. The best solvent to extract anthocyanins was boiling water (H_2O) for 25 minutes.

CONCLUSIONS

The Sudan and Tecoapana varieties of *Hibiscus sabdariffa* L. share chemical-taxonomic characteristics; nevertheless, they are quantitatively different. Sudan has a higher anthocyanin content (1,319.8 mg 100 g^{-1}) than Tecoanapa (557.2 mg 100 g^{-1}). Maltodextrin was the best microencapsulation process for the extracted and encapsulated extract of Sudan. It recorded the highest percentage of preservation of phenols and anthocyanins in the microencapsulation (85% ± 5%).

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