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Research article

Phytochemical screening and DPPH radical scavenging activity of three morphotypes of *Mauritia flexuosa* L.f. from Peru, and thermal stability of a milk-based beverage enriched with carotenoids from these fruits

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ABSTRACT

Mauritia flexuosa L.f. is a palm tree which presents great morphological variability (morphotypes), represented mainly by the mesocarp color of its fruits. The objective of the study was to characterize the physicochemical and antioxidant properties of three morphotypes *of Mauritia flexuosa* L.f. ("Yellow", "Colour" and "Shambo") of greater economic importance in the Peruvian Amazon. "Shambo" showed a significantly high content of bioactive compounds (total phenolics, flavonoids and carotenoids) and DPPH radical scavenging activity compared to the "Yellow" and "Colour" morphotypes ($p \leq 0.05$). There was a significant correlation between DPPH radical scavenging activity and total phenolics, flavonoids and carotenoids ($p \leq 0.01$). Furthermore, milk-based beverages enriched with carotenoids of those morphotypes of *Mauritia flexuosa* L.f. have been shown to be a good source of bioactive compounds for use in the food industry. The milk-based beverages enriched with carotenoids of those morphotypes *of Mauritia flexuosa* L.f. showed higher lightness (L^*) and yellowness (b^*).

1. Introduction

Mauritia flexousa L.f., belonging to the Arecaceae family (Palmae), is one of the most abundant palm trees in the South American Amazon. In the Amazon basin, it has a wide distribution in Peru, Bolivia, Brazil, Colombia, Ecuador, Venezuela and Guyana (Pereira Freire et al., 2016). In Peru, this palm is known as aguaje and it is distributed in the basins of the Huallaga, Marañón and Ucayali rivers. In adulthood, this palm has a stem that reaches 35 m high and a diameter of 60 cm. This palm stands out due to the uses of various parts of the plant. The fruit of this palm is the most used part from which the pulp and oil are extracted, which have multiple beneficial properties for health due to their high content of bioactive compounds. These bioactive compounds are important in the prevention of oxidative stress and chronic diseases; they also act as antioxidant, anti-inflammatory and platelet antiaggregants. Other parts of this palm are also used. From the leaves, fibers are obtained for domestic use and crafts; the leaves are used directly on the roof of rustic houses while pulp for paper is obtained from the petiole (Del Castillo et al., 2006; Manzi and Coomes, 2009; Gilmore et al., 2013; van der Hoek et al., 2019).

In the Peruvian Amazon, specifically in the Loreto Region, there is approximately a consumption of 50 tons of the total fruit per day (only the mesocarp is consumed), which involves about 5,000 people in the value chain of this fruit (García and Pinto, 2002). The natural habitat of this palm is found in soils with permanent flooding or in seasons that have greater presence of water, also known as aguaje forests. In Peru, there are more than 5 million hectares of aguaje forests, distributed mainly in the areas of San Martín, Huánuco, Madre de Dios, Cusco, Loreto, Ucayali and Pasco (Del Castillo et al., 2006; Horn et al., 2012).

Mauritia flexuosa L f. is a palm tree of great social and economic relevance, and is considered as the native fruit tree of greatest demand

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due to its nutritional, nutraceutical and pharmacological properties in the Peruvian Amazon (Del Castillo et al., 2006). This palm presents great morphological variability (morphotypes), represented mainly by the mesocarp color of its fruits (Delgado et al., 2007; Vásquez-Ocmín et al., 2009). During this research, three morphotypes of *Mauritia flexuosa* L.f., according to the color of the mesocarp, were evaluated: "Yellow" (yellow mesocarp), "Colour" (red mesocarp on the outside and yellow on the inside) and "Shambo" (red mesocarp).

Previous studies have demonstrated that the pulp of *Mauritia flexuosa* L.f. is rich in bioactive compounds such as carotenoids, tocopherols, ascorbic acid, phenolic compounds and monounsaturated fatty acids mainly represented by oleic acid (Pereira Freire et al., 2016; Faustino Pereira et al., 2018). *Mauritia flexuosa* L. f. pulp is high in carotenoids due to its carotene-rich oil, 70% of which is β-carotene (Ribeiro, 2008). Some studies have reported that the incorporation of carotenoids in dairy foods can significantly increase their nutritional and chromatic properties, as well as slow their oxidation, prolonging the half-life of these products (Rizk et al., 2014; Izli et al., 2017; Senarathne and Wickramasinghe, 2019).

The objective of this study was to characterize the physicochemical and antioxidant properties of morphotypes of *Mauritia flexuosa* L.f. ("Yellow", "Colour" and "Shambo") and evaluate the use of their bioactive compounds in the food industry by analyzing the thermal stability of milk-based beverages enriched with carotenoids from these morphotypes of *Mauritia flexuosa* L.f.

2. Materials and methods

2.1. Chemicals

The chemicals were purchased from Sigma-Aldrich (G5003, St. Louis, MO, USA). They were: Folin–Ciocalteu reagent, sodium carbonate, sodium nitrite, gallic acid, catechin, ammonium chloride, 1,1-diphenyl-2-picrylhydrazyl (DPPH), HCl, NaOH, ethanol, acetone, petroleum ether and hexane. The solvents were ACS reagent grade or higher.

2.2. Materials

The fruits of three morphotypes of *Mauritia flexuosa* L.f. ("Yellow", "Colour" and "Shambo") were purchased in the "Veinte de Enero" Community of the Marañon River, Iquitos, Peru (latitude: 4° 39′19.5″S, longitude: 73° 49′ 27.9″ W). The fruits were selected with respect to their sanity and ripening stage, and cleaned in water containing 25 ppm of sodium hypochlorite. Then, the pulp, peel and endocarp were separated. The pulp was stored at -70 °C until lyophilization. For the freeze-drying process, a stainless steel freeze dryer tray model Stellar (Millrock Technology, NY, USA) was used. Subsequently, the pulp was vacuum packed before grinding in a rotor mill (0.08 mm). The determinations of moisture (method 920.151), ash (method 940.26), lipid (method 930.09) and protein (method 920.152) were made according to the official methods of the Association of Official Agricultural Chemists (AOAC, 2012). Carbohydrate content was obtained by difference. The energy content (kcal) was calculated from the food content using the energy of the following factors: 4 kcal/g for protein, 4 kcal/g for carbohydrates and 9 kcal/g for fat (FAO, 2003).

2.3. Extraction

For the analysis of bioactive compounds, an extract was prepared from 1 g of pulp lyophilized powder from each of the morphotypes of *Mauritia flexuosa* L.f., which were mixed with 10 mL of 80% ethanol. Then, the samples were sonicated for 30 min at 30 °C. After sonication, the samples were centrifuged at 4,000×*g* (Universal 320R, Hettich GmbH & Co. KG, Tuttlingen, Germany) for 20 min at 20 °C.

2.4. Total phenolics

The content of total phenolics was measured using a modified Folin-Ciocalteau method (Ramos-Escudero et al., 2012; Castro-López et al., 2016). In summary, an aliquot of 100 μL of the translucent extract was reacted with 750 μL of 0.2 N Folin-Ciocalteau reagent. The reaction was allowed to stand for 5 min. After this time, 750 μL of 7.5% sodium carbonate solution was added and the mixture was incubated in a water bath at 40 °C for 30 min. The absorbance reading was recorded at 725 nm and the results were expressed in μg of gallic acid equivalents per g of sample (μg GAE/g).

2.5. Total flavonoids

The aluminum chloride colorimetric method was used for the determination of the total flavonoid content (Alvites-Misajel et al., 2019). An aliquot of 100 μL of extract was mixed with 75 μL $NaNO_2$ (5%) solution and the mixture was incubated for 5 min; then 150 μL of $AlCl_3.6H_2O$ (10%) solution was added and the mixture was allowed to stand for 5 min. Finally, 500 μL NaOH (1 M) was added to the reaction and the mixture was incubated for 15 min at room temperature. The absorbance was read at 510 nm in an Orion AquaMate 8100 Uv-Visible spectrophotometer (Thermo Scientific, Waltham, MA, USA). Total flavonoid content was expressed as μg of catechin equivalents per g of sample (μg CE/g).

2.6. Total carotenoids

The content of total carotenoids was determined according to (Biehler et al., 2010; de Carvalho et al., 2012) with some minor modifications. In summary, 0.5 g of each lyophilized sample was reconstituted in 10 mL of petroleum ether and sonicated for 30 min. Subsequently, the samples were centrifuged at 4,000×*g* (Universal 320R, Hettich GmbH & Co. KG, Tuttlingen, Germany) at 20 °C for 20 min. Visible spectra (390–750 nm, 2 nm interval) were collected using a 1-mL quartz cuvette (101-QS, Hellma GmbH, Mullheim, Germany) in an Orion AquaMate 8100 Uv-Visible spectrophotometer (Thermo Scientific, Waltham, MA, USA). The total carotenoid content was calculated using Eq. (1) below:

$$\text{Carotenoids content } (\mu g/g) = \left(A \times V_o \times 10^4\right) / \left(A^{1\%}{}_{1cm} \times W(g)\right) \quad (1)$$

where A = Absorbance; V_o = Total extract volume (mL); W = sample weight; $A^{1\%}{}_{1cm}$ = 2592 (β-carotene extinction coefficient in petroleum ether).

2.7. Free radical scavenging activity of DPPH radical

In vitro antioxidant activity was evaluated by the DPPH radical scavenging assay according to (Alvites-Misajel et al., 2019) with some modifications. Briefly, 50 μL of extract was mixed with 950 μL of DPPH (65 μmol/L in ethanol) and shaken vigorously for 10 min and thereafter the mixture was placed in a 10 mm semi-micro cuvette. The absorbance at 515 nm was measured using an Orion AquaMate 8100 UV-visible spectrophotometer (Thermo Scientific, Waltham, MA, USA). DPPH radical scavenging activity was expressed as Trolox equivalents (μmol TE/g).

2.8. Lyophilized powder and carotenoids-enriched milk-based beverages color measurement

Color measurements of pulp lyophilized powder and carotenoids-enriched milk-based beverages were acquired using an image analysis technique. Image acquisition was obtained using a digital camera (Canon, Power Shot SX60 HS, full HD 65X optical zoom, Tokyo, Japan) under controlled and defined illumination conditions using OSRAM 17W high power led lamp, luminous flux: 1836 lm, luminous efficacy: 108 lm/

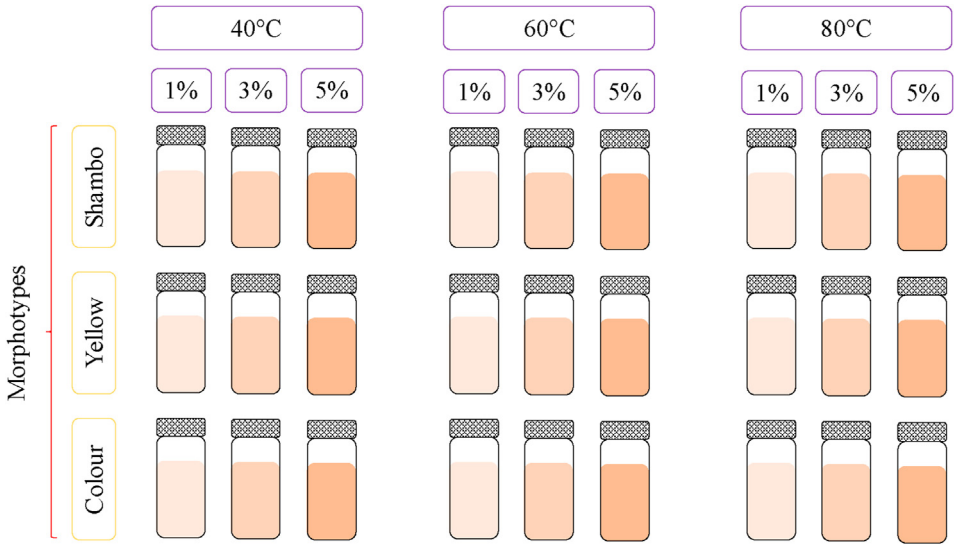

Figure 1. Treatment combinations of carotenoids-enriched milk-based beverages. The colors in the vials are referential.

W and a color temperature of 6500 K according to the manufacturer. RGB signals were obtained using the color histogram tool of Image J programme (Abderrahim et al., 2015). Color squares were generated by converting R, G and B values to lightness (L^*), redness (a^*) and yellowness (b^*) values using Nix Pro Color Sensor (Nix Sensor Ltd.). Illuminant and reference angle for input values were D65 and 10°, respectively. From the CIELAB color space, the psychophysical parameters for chroma (C^*_{ab}) and hue (h_{ab}) were calculated using the Eq. (2) and Eq. (3) below:

$$C^*_{ab} = \left[(a^*)^2 + (b^*)^2\right]^{0.5} \quad (2)$$

$$h_{ab} = \arctan\,(b^*/a^*) \quad (3)$$

Chroma (C^*_{ab}) is an attribute of color used to indicate the degree of difference of the color from a gray color of the same lightness. Hue (h_{ab}) is the attribute of the visual sensation according to which colors are usually defined as reddish, yellowish, greenish, and bluish and is used to define the difference of a color with reference to a gray color with the same lightness.

2.9. Thermal stability

The thermal stability of the carotenoids was carried out on a dairy matrix (partially skimmed) enriched with pulp lyophilized powder from three morphotypes of *Mauritia flexuosa* L.f. ("Yellow", "Colour" and "Shambo") at different proportions (1, 3 and 5%) and heat treated at 40, 60 and 80 °C (Figure 1). The heat treatment was carried out on a water bath (Memmert GmbH + Co. KG, Schwabach, Germany) for 20 min for each temperature. The total soluble solids in the carotenoids-enriched milk-based beverages were measured using an OPTi digital handheld refractometer (Bellingham + Stanley Ltd., Kent, UK) at 20 °C and expressed in °Brix. pH was analyzed with a pH-meter (Multi-parameter portable meter, MultiLine® Multi 3630 IDS, Weilheim, Germany). Total carotenoid and phenolic content was measured following the procedure described by (de Carvalho et al., 2012; Raikos et al., 2019). Three mL of a solution composed of acetone was added to 1 g of carotenoids-enriched milk-based beverage samples: hexane-ethanol in a ratio of 1:2:1, respectively. The extraction of the non-polar and polar fraction was carried out for 1 h, stirring at room temperature. Samples were then centrifuged at 3,500×*g* (Universal 320R, Hettich GmbH & Co. KG, Tuttlingen, Germany), 20 °C for 20 min. The non-polar fraction was used for the determination of total carotenoids, while the polar fraction was used to determine the content of total phenolics.

2.10. Statistical analysis

Data were presented as mean ± standard deviation (SD) and compared by one-way analysis of variance (ANOVA) followed by Tukey's post hoc test ($p \leq 0.05$) using SPSS for Windows version 24.0 (SPSS, Inc., Chicago, IL, USA). Statistical correlation among different variables was performed using the Pearson coefficient (r) and the results were statistically significant when $p \leq 0.05$.

3. Results and discussion

3.1. Chemical composition of pulp lyophilized powder of morphotypes of *Mauritia flexuosa* L.f.

The chemical composition of the pulp lyophilized powder of morphotypes of *Mauritia flexuosa* L.f. are shown in Table 1. The morphotype "Shambo" showed significantly higher levels of lipids and energetic value than "Yellow" and "Colour" morphotypes ($p \leq 0.01$). Carbohydrate levels

Table 1. Chemical composition of pulp lyophilized powder of morphotypes of *Mauritia flexuosa* L. f.

	"Yellow"	"Colour"	"Shambo"
Moisture (%)	1.36 ± 0.04^a	1.08 ± 0.01^b	1.03 ± 0.04^b
Ash (%)	2.53 ± 0.01^c	2.88 ± 0.04^a	2.65 ± 0.03^b
Lipids (%)	46.83 ± 0.04^c	50.47 ± 0.04^b	55.81 ± 0.06^a
Protein (%)	4.05 ± 0.06^c	5.21 ± 0.09^a	4.88 ± 0.01^b
Carbohydrates (%)	45.24 ± 0.05^a	40.38 ± 0.16^b	35.64 ± 0.04^c
Energetic value (kcal/100 g)	618.59 ± 0.44^c	636.53 ± 0.06^b	685.85 ± 1.81^a

Values (mean ± SD) in the same row with different letters (a–c) are significantly different (One-way ANOVA with Tukey's multiple comparison test, $p \leq 0.05$).

Table 2. Total content of phenolics, flavonoids and carotenoids, and DPPH radical scavenging activity of pulp lyophilized powder of morphotypes of *Mauritia flexuosa* L. f.

	"Yellow"	"Colour"	"Shambo"
Total phenolics (μg GAE/g)	2220.58 ± 23.06^{c}	2751.55 ± 16.51^{b}	3423.94 ± 24.93^{a}
Total flavonoid (μg CE/g)	117.41 ± 4.15^{c}	137.90 ± 1.75^{b}	165.34 ± 4.11^{a}
Total carotenoids (μg β-carotene/g)	135.68 ± 2.28^{c}	260.34 ± 5.95^{b}	713.38 ± 12.03^{a}
DPPH radical scavenging activity (μmol TE/g)	6262.53 ± 356.64^{b}	6255.23 ± 213.55^{b}	7573.44 ± 273.15^{a}

Values (mean ± SD) in the same row with different letters (a–c) are significantly different (One-way ANOVA with Tukey's multiple comparison test, $p \leq 0.05$). Gallic acid equivalents (GAE), Catechin equivalent (CE) and Trolox equivalents (TE).

were significantly higher in the "Yellow" morphotype compared to "Colour" and "Shambo" morphotypes ($p \leq 0.01$). In addition, the "Colour" morphotype presented significantly higher levels of protein than "Yellow" and "Shambo" morphotypes ($p \leq 0.01$). There was a significant correlation between the energetic value and the lipid content ($r = 0.999$, $p \leq 0.01$).

The fruits of *Mauritia flexuosa* L.f. present a high demand for its nutraceutical, pharmaceutical and nutritional properties. Previous results show that lipids and carbohydrates are the main components present in the pulp of this fruit (Carneiro and Carneiro, 2011; Darnet et al., 2011; Manhães and Sabaa-Srur, 2011). In our study, the proximal composition was evaluated in the pulp-lyophilized powder of the three morphotypes of *Mauritia flexuosa* L.f.; we also found high levels of carbohydrates and lipids. The carbohydrate content ranged from 35.61 to 45.27%, while the lipid content ranged from 46.80 to 55.85%, the latter being the highest levels in the "Shambo" morphotype. Our results are in accordance with previous studies on dehydrated pulp of *Mauritia flexuosa* L. f., where the carbohydrate and lipid content was 31.24 and 51.67%, respectively (Carneiro and Carneiro, 2011).

3.2. Total phenolics, flavonoids and carotenoids, and DPPH radical scavenging activity

Total phenolic, flavonoid and carotenoid content, as well as the DPPH radical scavenging activity were evaluated in the pulp-lyophilized powder of morphotypes of *Mauritia flexuosa* L.f. The content of bioactive compounds and the DPPH radical scavenging activity varied greatly among the morphotypes *of Mauritia flexuosa* L.f. As shown in Table 2, the levels of total phenolics, flavonoids and carotenoids were significantly higher in the "Shambo" morphotype compared to "Colour" and "Yellow" morphotypes ($p \leq 0.01$). Antioxidant activity, measured by the DPPH method, varied significantly ($p \leq 0.05$) from 6010.34 to 7766.59 μmol Trolox equivalent/g. In addition, the "Shambo" morphotype presented significantly higher DPPH radical scavenging activity than "Yellow" and "Colour" morphotypes ($p \leq 0.05$). When the correlation among the different parameters was evaluated, a strong correlation was observed between DPPH radical scavenging activity with total phenolic ($r = 0.848$, $p \leq 0.05$), flavonoid ($r = 0.813$, $p \leq 0.05$) and carotenoid ($r = 0.923$, $p \leq 0.01$) content.

Other studies also show the presence of bioactive compounds such as total phenolics, flavonoids, carotenoids, as well as *in vitro* antioxidant capacity in fruits of *Mauritia flexuosa* L.f. In the study conducted by Dos Santos et al. (2015), which included five species of palm trees from the Brazilian Amazon, in *Mauritia flexuosa* L.f, the levels of total phenolics, flavonoids and carotenoids (118 mg GAE/100 g, 28 mg EQE/100 g and 4.7 mg ßCTE/100 g; respectively), were found below that reported in the present study. Another study reports that the levels of bioactive compounds and *in vitro* antioxidant capacity in *Mauritia flexuosa* L.f. varied between two Brazilian regions (Cerrado and Amazon Region). A higher content of total phenolics and *in vitro* antioxidant capacity evaluated by the ABTS, DPPH, FRAP and ORAC methods was observed in the Cerrado Region, while higher levels of total carotenoids were observed in the Amazon Region. However, contrary to our study, a negative correlation was observed between *in vitro* antioxidant capacity and total carotenoid content, while a significant positive correlation was observed between *in vitro* antioxidant capacity and the total phenolic content (Cândido et al., 2015). These differences could be due to the fact that in the study by Cândido et al. (2015), the fruits of *Mauritia flexuosa* L.f. they were obtained from different biomes (Cerrado and Amazon region), which influenced their content of bioactive compounds. In our study, the samples were obtained from the same biome (Amazon region). Nonato et al. (2018) also carried out a chemical analysis and evaluation of *in vitro* antioxidant capacity using different fractions (chloroform, ethyl acetate and ethanol) obtained from the pulp of *Mauritia flexuosa* L.f. using a Soxhlet extractor. A higher content of phenolic and flavonoid compounds was observed in the ethyl acetate and ethanolic fractions (26.84 and 22.69 μg GAE/g, and 11.82 and 12.47 μg EQE/g; respectively); however, these values were much lower than the ones reported in our research because in the previous study only the lipid fraction of the samples was evaluated. Likewise, Resende et al. (2019) evaluated the content of phenolics and natural antioxidants in different products of the peel, mesocarp and endocarp of the fruits of *Mauritia flexuosa* L.f. In defatted pulp obtained by solvent extraction, the total phenolic content was 740 mg GAE/100 g, while carotenoids were not detected in the sample evaluated. These findings differ from our results because in our study the extraction of bioactive compounds was performed from the lyophilized whole pulp.

3.3. Lyophilized powder color measurement

The color values for the pulp lyophilized powder of *Mauritia flexuosa* L.f. morphotypes ("Yellow", "Colour" and "Shambo") are shown in

Table 3. L^* a^* b^* C^*_{ab} h_{ab} values of pulp lyophilized powder of morphotypes of *Mauritia flexuosa* L.f.

Color parameters	"Yellow"	"Colour"	"Shambo"
L^*	80.94 ± 3.60^{a}	80.86 ± 2.80^{a}	76.96 ± 3.75^{b}
a^*	3.70 ± 1.44^{b}	-0.68 ± 3.09^{c}	9.35 ± 3.46^{a}
b^*	47.70 ± 4.40^{c}	69.04 ± 3.17^{b}	76.14 ± 2.29^{a}
C^*_{ab}	47.85 ± 4.08^{c}	69.11 ± 3.18^{b}	76.79 ± 1.82^{a}
h_{ab}	85.62 ± 1.37^{b}	88.03 ± 1.26^{a}	82.94 ± 2.73^{c}

Values (mean ± SD) in the same row with different letters (a–c) are significantly different (One-way ANOVA with Tukey's multiple comparison test, $p \leq 0.05$). Lightness (L^*), redness (a^*), yellowness (b^*), chroma (C^*_{ab}) and hue (h_{ab}).

Figure 2. Color of pulp lyophilized powder of morphotypes of *Mauritia flexuosa* L. f.

Table 4. Color parameters of carotenoids-enriched milk-based beverages.

Morphotypes	Code	T (°C)	°Brix	pH	Ratios	L^*	a^*	b^*
"Yellow"	B1	40	10.5	7.77	1%	92.48 ± 0.83	0.33 ± 0.15	15.72 ± 0.05
	B2		10.4	7.75	3%	90.79 ± 0.96	0.98 ± 0.44	17.16 ± 0.96
	B3		10.5	7.79	5%	87.70 ± 1.79	1.15 ± 0.28	20.22 ± 0.83
"Yellow"	B4	60	11.5	6.49	1%	88.78 ± 3.02	0.45 ± 0.25	15.12 ± 0.75
	B5		11.4	6.51	3%	89.66 ± 1.66	0.75 ± 0.25	16.98 ± 0.65
	B6		11.6	6.50	5%	88.64 ± 2.26	1.81 ± 0.53	19.54 ± 0.13
"Yellow"	B7	80	11.4	6.33	1%	92.22 ± 1.44	0.35 ± 0.08	15.08 ± 0.99
	B8		11.4	6.32	3%	90.34 ± 1.11	0.64 ± 0.29	15.71 ± 0.28
	B9		11.5	6.30	5%	88.47 ± 1.79	1.48 ± 0.37	19.77 ± 0.27
"Colour"	B10	40	10.8	6.68	1%	90.85 ± 2.90	-0.68 ± 0.38	24.83 ± 1.72
	B11		10.6	6.65	3%	88.31 ± 0.38	1.32 ± 0.03	31.26 ± 0.74
	B12		10.7	6.64	5%	85.98 ± 1.50	2.50 ± 0.46	31.29 ± 1.19
"Colour"	B13	60	11.3	6.36	1%	90.56 ± 1.23	-0.13 ± 0.28	20.35 ± 1.57
	B14		11.2	6.35	3%	89.43 ± 0.49	0.71 ± 0.50	32.71 ± 2.78
	B15		11.4	6.33	5%	89.72 ± 1.16	1.72 ± 0.42	33.01 ± 1.63
"Colour"	B16	80	11.8	6.08	1%	91.15 ± 1.83	-0.41 ± 0.29	21.55 ± 1.21
	B17		11.7	6.10	3%	89.58 ± 1.27	0.59 ± 0.23	27.60 ± 0.86
	B18		11.7	6.09	5%	88.09 ± 0.98	1.19 ± 0.36	30.74 ± 0.98
"Shambo"	B19	40	10.5	6.70	1%	90.47 ± 0.87	0.84 ± 0.05	33.14 ± 0.95
	B20		10.5	6.68	3%	86.97 ± 0.67	3.15 ± 0.06	45.55 ± 0.66
	B21		10.6	6.65	5%	83.65 ± 1.20	3.43 ± 0.17	51.10 ± 412
"Shambo"	B22	60	11.0	6.30	1%	92.01 ± 0.33	0.46 ± 0.12	32.98 ± 0.27
	B23		11.2	6.31	3%	86.64 ± 1.50	2.65 ± 0.32	41.68 ± 0.74
	B24		11.3	6.33	5%	87.41 ± 0.59	4.70 ± 0.07	39.82 ± 0.95
"Shambo"	B25	80	11.7	6.05	1%	88.00 ± 0.97	0.77 ± 0.13	29.82 ± 1.33
	B26		11.5	6.03	3%	86.03 ± 0.84	1.89 ± 0.27	46.52 ± 4.04
	B27		11.6	6.05	5%	85.07 ± 2.26	2.72 ± 0.11	47.68 ± 0.96

$L^*a^*b^*$ values were calculated from RGB values obtained from 12 images. Data are expressed as mean ± SD.

Table 3 and Figure 2. The color parameters (L^*, a^* and b^*) showed significant differences ($p \leq 0.05$). In general, higher L^* and b^* values and lower a^* values were obtained for the pulp lyophilized powder of morphotypes of *Mauritia flexuosa* L.f. Morphotypes show a positive b^* value which signifies yellow color, whereas for "Colour" morphotype, the a^* value was negative. For the "Shambo" morphotype, the a^* and b^* values were higher than the other morphotypes, while the value of L^* was lower than "Yellow" and "Colour". When the morphotypes were considered, it was observed that the C^*_{ab} values varied between 47.70 to 76.14 CIELab units. On the other hand, it is interesting to note that the C^*_{ab} values were similar to those corresponding to the values of b^*. The variations in the mean hue values among the different morphotypes were numerically less ranged from 82.94 to 88.03 CIELab units, this angular color coordinate varied little in numeric terms for qualitatively similar colors. The color of morphotypes ("Yellow", "Colour" and "Shambo") is an extremely important criterion to consumer perception. Many fruit extracts rich in

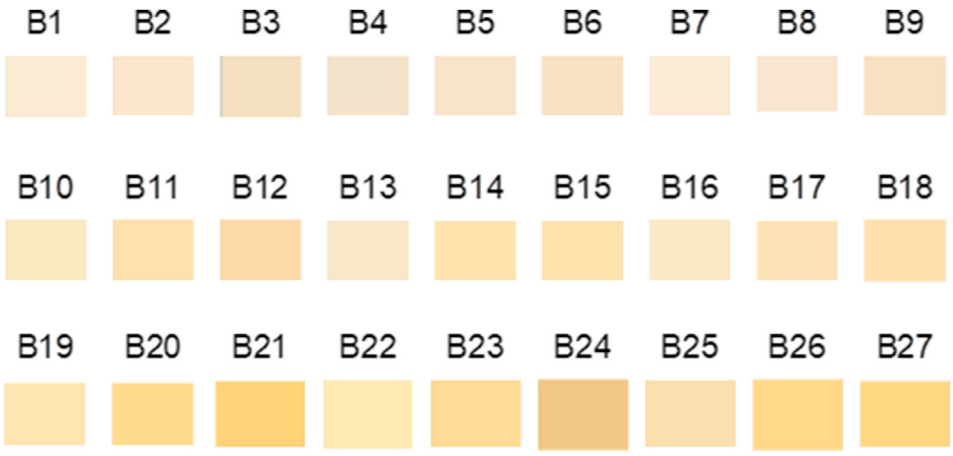

Figure 3. Color of carotenoids-enriched milk-based beverages (pictures taken with Nix sensor color). The codes correspond to Table 4.

carotenoids have been used to enrich dairy products and improve nutritional, sensory, chromatic and antioxidant properties (Rizk et al., 2014; Izli et al., 2017; Patel et al., 2019). Lyophilized powder of *Mauritia flexuosa* L.f., contains natural pigments that can contribute directly through their yellowing properties due to its chromatic characteristics.

3.4. Thermal stability

The color coordinates of carotenoids-enriched milk-based beverages are shown in Table 4. According to the data shown in Table 3, it was expected that "Yellow" morphotype-enriched milk-based beverages showed lower chromatic parameters with respect to the "Colour" and "Shambo" morphotypes. In general, lightness (L^*) values varied between 87.70 to 92.48, 85.98 to 91.15, and 78.41 to 92.01 in "Yellow", "Colour'' and "Shambo" morphotypes; respectively. On the other hand, a^* values ranged from 0.33 to 1.81 CIElab units, -0.68 to 2.50 CIElab units and 0.46 to 4.70 CIElab units in "Yellow", "Colour'' and "Shambo" morphotypes; respectively. Finally, b^* values showed lower values ("Yellow" morphotype), and wider in "Colour" and "Shambo" morphotypes.

The results of the CIElab color coordinates of the carotenoids-enriched milk-based beverages analyzed as a function of heat treatment (40, 60 and 80 °C) are summarized in Table 4 and Figure 3. As it

Table 5. Total carotenoid and phenolic content, and UV-vis absorption spectrum of carotenoids-enriched milk-based beverages.

Morphotypes	T (°C)	Ratios	Total carotenoids (μg/g)	Total phenolics (μg/g)
"Yellow"	40	1%	0.18 ± 0.06	210.92 ± 4.11
		3%	0.35 ± 0.05	303.46 ± 4.56
		5%	0.54 ± 0.06	362.16 ± 0.99
"Yellow"	60	1%	0.14 ± 0.02	234.18 ± 4.85
		3%	0.23 ± 0.04	239.53 ± 4.13
		5%	0.45 ± 0.04	264.78 ± 4.76
"Yellow"	80	1%	0.14 ± 0.04	222.61 ± 3.90
		3%	0.24 ± 0.01	184.68 ± 3.92
		5%	0.36 ± 0.02	163.28 ± 4.52
"Colour"	40	1%	0.44 ± 0.03	233.56 ± 2.83
		3%	1.57 ± 0.05	352.34 ± 4.42
		5%	2.06 ± 0.07	418.76 ± 3.99
"Colour"	60	1%	0.39 ± 0.03	210.30 ± 4.40
		3%	1.37 ± 0.05	240.77 ± 3.22
		5%	1.77 ± 0.07	262.54 ± 4.76
"Colour"	80	1%	0.32 ± 0.03	207.69 ± 3.93
		3%	1.25 ± 0.05	188.91 ± 3.39
		5%	1.40 ± 0.06	204.33 ± 2.82
"Shambo"	40	1%	1.70 ± 0.13	222.24 ± 3.56
		3%	5.24 ± 0.23	275.97 ± 4.48
		5%	7.24 ± 0.22	355.70 ± 1.41
"Shambo"	60	1%	2.23 ± 0.05	285.41 ± 1.41
		3%	5.54 ± 0.09	254.95 ± 1.14
		5%	7.25 ± 0.09	288.28 ± 0.75
"Shambo"	80	1%	2.06 ± 0.05	288.41 ± 0.94
		3%	4.84 ± 0.11	182.44 ± 4.16
		5%	5.93 ± 0.10	207.44 ± 3.76

Data are expressed as mean ± SD. Superposition of experimental UV-vis absorption spectrum of carotenoids-enriched milk-based beverages. Ratios of 1%, 3% and 5% of different morphotypes of *Mauritia flexuosa* L. f.

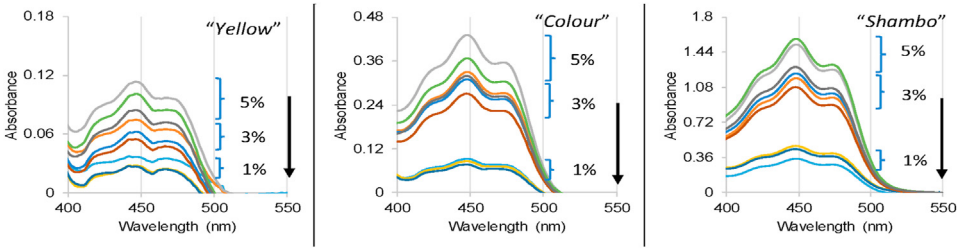

Figure 4. UV-vis absorption spectrum of carotenoids-enriched milk-based beverages.

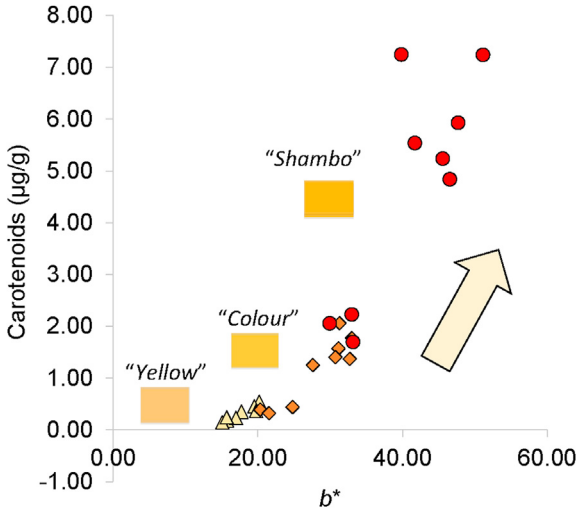

Figure 5. Correlation between chromatic parameter *b** and carotenoid content during thermal stability. Red circle: "Shambo" morphotype, orange square: "Colour" morphotype and yellow triangle: "Yellow" morphotype.

was also observed in terms of the relevant color parameters of the CIElab space, thermal treatment affects the chromatic parameters; in this sense, lightness (*L**) values show slight variations, the morphotypes with a higher value of *b** show greater changes when the temperature increases. These results agree with Izli et al. (2017) who studied the influence of different drying techniques that can affect color, increasing yellowness (*b**), and decreasing *L** values in drying parameters of mango. With respect to the content of total phenolics and carotenoids, it is clearly observed that there is a decrease due to heat treatment (Table 5). The UV-vis absorption spectra for the "Yellow", "Colour" and "Shambo" morphotypes showed a decrease in absorbance units (Figure 4), due to a decrease in carotenoid content according to the percentage and morphotype. Furthermore, the UV-vis absorption spectra are correlated to the chromatic parameter *b** and the carotenoid content, these last two variables showed an $r^2 = 0.8361$. On the other hand, even though the thermal treatment influences the decrease of the carotenoid content, the "Shambo" morphotype presented better yellowing (Figure 5).

In the case of total phenolics, the presence of certain flavonoids can have a positive effect when the temperature increases. When the "Yellow" morphotype is considered for a proportion of 1%, the total phenolic content is as follows: at 40 °C (210.92 µg GAE/g), at 60 °C (234.18 µg GAE/g) and finally, at 80 °C (222.61 µg GAE/g); however, in the "Colour" and "Shambo" morphotypes a decrease is observed when the temperature increases. The type of flavonoid must be considered in this type of study, which evaluates the effect of heat treatment on the phenolics content. Some reports show increases in the phenolics content up to 120 °C/30 min, while this content decreases at 150 °C/30 min (Sharma et al., 2015).

4. Conclusion

Our results show that there is a variability of bioactive compounds and DPPH radical scavenging activity among the morphotypes of *Mauritia flexuosa* L.f. evaluated in this study, associating the color of the fruit mesocarp with its content of bioactive compounds. Within this group, carotenoids represent a natural source to enrich dairy foods and enhance their sensory properties in order to achieve greater acceptance of these products. A few limitations of this article shall be highlighted; first, further studies are required to evaluate the chemical composition of phenolic and flavonoid compounds in the morphotypes of *Mauritia flexuosa* L.f. from the Peruvian Amazon. Secondly, the antioxidant capacity of these *Mauritia flexuosa* L.f. fruits should be characterized using different *in vitro* methods.

Declarations

Author contribution statement

Ivan Best, Fernando Ramos-Escudero: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Sandra Casimiro-Gonzales, Alan Portugal, Luis Olivera-Montenegro, Luis Aguilar: Performed the experiments; Analyzed and interpreted the data.

Ana María Muñoz: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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