

## Obtaining a lipid extract from peach palm (*Bactris gasipaes* Kunth) epicarp. Quantification of carotenoid content and application as a food additive

### Obtención de un extracto lipídico a partir del epicarpio de chontaduro (*Bactris gasipaes* Kunth): Cuantificación del contenido de carotenoides y aplicación como aditivo alimentario

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#### ABSTRACT

The agro-industrial assessment of fruit by-products as food additives would allow compliance with Sustainable Development Goals. This research aimed at the homogenizer-assisted extraction of total carotenoids from peach palm (*Bactris gasipaes*) peel (epicarp) with sunflower oil. We also studied its application as a natural additive in white corn flour food. The response surface methodology and the rotational composite central design quantified the extraction process. The studied factors were extraction speed, temperature, time, and liquid-solid ratio. Total carotenoid content in the extract (336.06 µg/g dried epicarp) was optimized at 50°C, with 76 seconds, extraction speed of 19200 rpm, and liquid-solid ratio of 48.75 mL/g. The green extract obtained from homogenizer-assisted extraction constitutes a natural additive with agro-industrial potential for use in roasted corn cake, increasing carotenoid (30.60 µg/g of β-carotene), provitamin A (4.14 µg/g) and antioxidant activity (11.57 % DPPH).

#### Keywords

*Bactris gasipaes* • β-carotene • corn agroindustry • natural dye • homogenizer-assisted extraction

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## RESUMEN

El uso agroindustrial de los subproductos de las frutas, como aditivos alimentarios, podría ser una alternativa para el cumplimiento de los Objetivos de Desarrollo Sostenible. Por lo tanto, el objetivo de esta investigación fue la extracción asistida por homogeneización de carotenoides totales de la cáscara (epicarpio) de chontaduro (*Bactris gasipaes*) con aceite de girasol y su aplicación como aditivo natural en un producto alimenticio elaborado con harina de maíz blanco. Se aplicó la metodología de superficie de respuesta junto con el diseño central compuesto rotacional para cuantificar el proceso de extracción. Los factores de estudio fueron la velocidad de extracción, temperatura, tiempo y relación líquido-sólido. Los carotenoides totales en el extracto obtenido (336,06 µg/g de epicarpio seco) se optimizaron a una temperatura de 50°C, con un tiempo de 76 s, velocidad de extracción de 19200 rpm y relación líquido-sólido de 48.75 mL/g. El extracto verde obtenido de la extracción asistida por homogeneización es un aditivo natural con potencial agroindustrial para uso en alimentos como la arepa de maíz, debido al incremento en los valores de carotenoides (30,60 µg/g de  $\beta$ -caroteno), provitamina A (4,14 µg/g) y actividad antioxidante (11,57 % DPPH).

## Palabras clave

*Bactris gasipaes* •  $\beta$ -caroteno • agroindustria de maíz • colorante natural • extracción asistida por homogeneización

## INTRODUCTION

The peach palm (*Bactris gasipaes*) is cultivated in Nicaragua, Honduras, Costa Rica, Panama, Colombia, Venezuela, French Guiana, Brazil, Bolivia, Hawaii, Indonesia, Malaysia and Reunion Island (8). The common name of this fruit changes among countries. It is called pupunha (Brazil), pejobaye (Costa Rica and Nicaragua), pijuayo (Peru), person (Guyana), chontaduro (Colombia and Ecuador), and peach palm (English-speaking countries) (8). The fruit's pulp is consumed mainly cooked with salt, or processed into flour for bakery or animal feed (8, 13). Additionally, indigenous communities in Peru, Bolivia and Brazil obtain fermented beverages (8). Even though peels constitute an important source of carotenoids (330 µg/g) (12), and represent 10-12 % of total fruit weight, they are eliminated during consumption and processing (11, 15).

Carotenoids like  $\beta$ -carotene,  $\alpha$ -carotene,  $\beta$ -cryptoxanthin, zeaxanthin, and lycopene, provide fruits and vegetables with characteristic yellow, red, and orange colors (9). At a functional level, they have antioxidant activity, and some are a source of provitamin A, with potential applications for health and nutrition (14). Furthermore, carotenoids in processed foods highlight color and encourage consumption (5, 22). The agro-industrial use of peels as by-product would significantly contribute to the food, pharmaceutical and cosmetic industries (17) while reducing waste generation, avoiding economic losses (25, 27) and supporting the reduction of greenhouse gases (27).

The green extraction methodology can obtain molecules of interest in different plant matrices (1, 10, 16, 17, 18, 24). The interaction of emerging technologies with biodegradable solvents makes these extraction processes environmentally friendly while reducing health risks. Additionally, these processes become more efficient when fewer solvents, less extraction time, and less energy are used (16, 18). Despite extraction efficiency, green carotenoid extraction studies using homogenizer-assisted extraction (HAE) are still scarce (1, 3). HAE, also known as high-shear homogenization, is a mechanical method based on high-speed homogenization, generating a shear effect between analyte and solvent, causing cell wall rupture and releasing the active compound of interest (24).

Therefore, new research is needed on food enriched with bioactive compounds, such as carotenoid pigments (7). This research aimed to obtain a carotenoid-rich extract using the homogenizer-assisted extraction from peach palm epicarp with sunflower oil and study its application as a natural additive.

## MATERIALS AND METHODS

### Sample collection and preparation

Red peach palm fruits with commercial maturity were acquired in the local market of Palmira, Department of Valle del Cauca, Colombia. Whole, healthy fruits were washed with water and disinfected with sodium hypochlorite at 150 ppm. The fruits were conventionally cooked in water for 60 min at boiling temperature (kg fruit/2 L water). Then, peels (epicarp) were removed using a disinfected, manual, stainless steel fruit peeler. Epicarp flour was produced according to previous studies (11). The epicarp was dehydrated in a convection oven (Binder ED 53 UL, Germany) at  $60 \pm 2^\circ\text{C}$  until 10-11 % moisture. Dehydrated samples were crushed in an electric mill to particle size  $\leq 0.25$  mm. This flour was refrigerated in a sterile amber glass bottle at  $4^\circ\text{C}$  for later use.

### Homogenizer-assisted extraction of epicarp carotenoids

The homogenizer-assisted extraction (HAE) was carried out in an ultra-turrax (T 18 digital, IKA, Janke & Kunkel, Germany) using sunflower oil as extraction solvent. Treatments were processed according to the established extraction parameters shown in table 1.

**Table 1.** Central composite rotatable design with independent variables and coded levels.

**Tabla 1.** Diseño central compuesto rotacional con variables independientes y niveles codificados.

Independent variables	Coded levels				
	$-\alpha$ (-2)	-1	0	+1	$+\alpha$ (+2)
	Experimental levels				
Temperature ( $^\circ\text{C}$ )	30	40	50	60	70
Time (s)	60	70	80	90	100
Speed (rpm)	16000	18000	20000	22000	24000
Liquid-solid ratio (mL/g)	30	40	50	66	90

Total carotenoids ( $\mu\text{g/g}$  dried epicarp) were determined according to the spectrophotometric method (15), using a molar extinction coefficient of  $7.10 \times 10^4 \text{ M}^{-1}\text{cm}^{-1}$  and sunflower oil as blank (15). The HAE was optimized via the response surface methodology combined with the rotational composite central design (RCCD). Table 1 shows coded factors, central points, and extreme values. Preliminary experiments identified central points, confirming that the liquid-solid ratio, temperature, time, and extraction speed significantly affected extraction.

### Extract application in corn griddle cake

The optimized extract was used as a natural additive in corn griddle cake. Two treatments were elaborated: a control with precooked white corn flour (WCF), and another with white corn to which 50 mL of the lipid extract was added as a natural additive. In all cases, 100 g of flour were mixed with 2 g of salt and 145 g of water. Kneading time was 5 minutes, and standing time was 3 minutes. All samples were 4 cm diameter and 1 cm thick. They were cooked on a preheated plate at  $180^\circ\text{C}$  for 10 minutes (5 minutes on each side) obtaining the brownish and crunchy texture of traditional corn griddle cake.

### Concentration of carotenoid and provitamin A in corn griddle cake

Carotenoids ( $\mu\text{g/g}$  of corn grilled cake) were determined by spectrophotometry (17). Absorbance of the organic phase was measured at 444, 450, and 451 nm and compared to hexane with a spectrophotometer (Genesys 20 UV-Vis, Thermo Electron Scientific Instruments LLC, Madison, WI, USA).

Carotenoid concentration ( $\mu\text{g/g}$  of sample) was calculated using extinction coefficients ( $E\% 1\text{ cm}$ ) in hexane: 2460, 2480, 2560, and 2800 for  $\beta$ -cryptoxanthin, zeaxanthin,  $\beta$ -carotene and  $\alpha$ -carotene, respectively. The provitamin A, expressed as retinol activity equivalents (RAE,  $\mu\text{g/g}$  of corn grilled cake), was calculated using a conversion factor of 12 for  $\beta$ -carotene and 24 for the other provitamins according to equation 1, as reported by the standard method (20).

$$\text{RAE} = \frac{\mu\text{g}(\beta - \text{carotene})}{12} + \frac{\mu\text{g}(\beta - \text{cryptoxanthin}) + \mu\text{g}(\alpha - \text{carotene})}{24} \quad (1)$$

#### Determination of antioxidant activity in corn griddle cake

Antioxidant activity AA (%) was determined as inhibition percentage of the radical DPPH (2,2-diphenyl-1-picrylhydrazyl) according to the colorimetric method (26).

#### Color parameters in corn griddle cake

Sample surface color was evaluated using the CIEL\*a\* b\* coordinates, measured with a CR-400 Colorimeter, Konica Minolta Tokyo, Japan, with 2° observer settings and D<sub>65</sub> deuterium lamp. The equipment was calibrated using a standard measurement plate: Y = 89.50, x = 0.3176, y = 0.3347. In addition, the Chroma (C\*), hue angle (h°), and total color difference, TCD, were calculated with equations 2-4:

$$C^* = (a^{*2} + b^{*2})^{1/2} \quad (2)$$

$$h^\circ = \tan^{-1} (b^*/a^*) \quad (3)$$

$$\text{TCD} = (\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2})^{1/2} \quad (4)$$

#### Experimental design and statistical analysis

The HAE was optimized with the response surface methodology combined with rotational central design of 29 experiments, where 16 were factorial points, 8 were axial points, and 5 were central points. The study factors were the liquid-solid ratio, temperature, time, and extraction speed. Factor effects and their interaction were evaluated with a second-order polynomial model to estimate the variables. Factor effects were identified with ANOVA ( $p < 0.05$ ), and model reliability was evaluated with the coefficient of determination,  $R^2$ , lack of Fit, and coefficient of variation. The statistical software Design Expert (Version 11, Stat-Easy, Godward, MN, USA) was used in optimization design. The t-Student test validated the optimization model, and evaluated the two grilled corn cake formulations. The statistical analysis was run in the Minitab version 18 statistical package for Windows.

## RESULTS AND DISCUSSION

#### Response surface optimization and contour plots

Table 2 (page 201), shows total carotenoids in each treatment evaluated during the HAE. Results ranged from 140.00 to 341.56  $\mu\text{g/g}$  dried epicarp. These values are lower than the 440-670  $\mu\text{g/g}$  obtained in peach palm epicarp (13).

The ANOVA model presented a  $p < 0.0001$ , monitoring HAE optimization of the response variable of interest (total carotenoids). Factors, interactions (temperature\*time, temperature\*ratio, time\*speed, and time\*ratio) and quadratic effect on the independent variables significantly affected carotenoid extraction (table 3, page 202). Lack of fit was not significant ( $p > 0.05$ ). The  $R^2 = 0.9994$ ,  $R^2 \text{ adj} = 0.9987$ ,  $R^2 \text{ pred} = 0.9966$  and  $\text{CV}\% = 0.844$  (table 3, page 202) indicated good regression fit according to the following equation:

$$Y = 340.115 - 9.136X_1 + 1.247X_2 - 2.866X_3 + 7.117X_4 + 1.539X_1 * X_2 - 0.629X_1 * X_3 + 10.182X_1 * X_4 + 1.325X_2 * X_3 + 15.919X_2 * X_4 - 0.802X_3 * X_4 - 45.374X_1^2 - 12.116X_2^2 - 15.498X_3^2 - 42.769X_4^2$$

**Table 2.** Central composite rotatable design with experimental total carotenoids.**Tabla 2.** Diseño central compuesto rotacional con resultados experimentales de carotenoides totales.

Run	Temperature $X_1$	Time $X_2$	Speed $X_3$	Liquid-solid ratio $X_4$	Analytical results total carotenoids ( $\mu\text{g/g}$ dried epicarp)
1	1	1	1	1	251.03
2	0	0	0	2	184.00
3	1	-1	-1	1	218.67
4	-1	1	-1	1	247.84
5	-2	0	0	0	176.00
6	0	0	0	0	341.56
7	1	1	1	-1	182.84
8	-1	-1	1	1	212.00
9	0	0	-2	0	285.69
10	0	0	0	0	340.76
11	-1	-1	-1	1	219.49
12	0	0	0	0	339.52
13	2	0	0	0	140.00
14	-1	1	-1	-1	220.40
15	0	0	0	0	339.75
16	0	-2	0	0	287.49
17	1	-1	1	1	208.00
18	-1	1	1	-1	222.49
19	1	1	-1	1	252.00
20	1	1	-1	-1	186.08
21	-1	1	1	1	242.54
22	0	0	2	0	269.32
23	1	-1	1	-1	208.45
24	-1	-1	1	-1	251.96
25	-1	-1	-1	-1	254.25
26	0	0	0	0	338.99
27	0	0	0	-2	152.84
28	0	2	0	0	294.58
29	1	-1	-1	-1	216.62

**Table 3.** ANOVA for the fitted quadratic polynomial model estimated for total carotenoid content of peach palm epicarp.**Tabla 3.** Análisis de varianza del modelo polinomial cuadrático estimado para el contenido total de carotenoides a partir del epicarpio de chontaduro.

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	94301.14	14	6735.80	1584.26	< 0.0001	Significant
X <sub>1</sub> -Temperature	2003.51	1	2003.51	471.22	< 0.0001	
X <sub>2</sub> -Time	37.37	1	37.37	8.79	0.0102	
X <sub>3</sub> -Speed	197.14	1	197.14	46.37	< 0.0001	
X <sub>4</sub> -Ratio	1215.74	1	1215.74	285.94	< 0.0001	
X <sub>1</sub> *X <sub>2</sub>	37.93	1	37.93	8.92	0.0098	
X <sub>1</sub> *X <sub>3</sub>	6.34	1	6.34	1.49	0.2424	
X <sub>1</sub> *X <sub>4</sub>	1659.04	1	1659.04	390.21	< 0.0001	
X <sub>2</sub> *X <sub>3</sub>	28.11	1	28.11	6.61	0.0222	
X <sub>2</sub> *X <sub>4</sub>	4055.07	1	4055.07	953.75	< 0.0001	
X <sub>3</sub> *X <sub>4</sub>	10.29	1	10.29	2.42	0.1421	
X <sub>1</sub> <sup>2</sup>	53418.47	1	53418.47	12564.02	< 0.0001	
X <sub>2</sub> <sup>2</sup>	3808.82	1	3808.82	895.83	< 0.0001	
X <sub>3</sub> <sup>2</sup>	6232.16	1	6232.16	1465.80	< 0.0001	
X <sub>4</sub> <sup>2</sup>	47461.37	1	47461.37	11162.91	< 0.0001	
Residual	59.52	14	4.25			
Lack of Fit	55.25	10	5.52	5.17	0.0638	Not significant
Pure Error	4.28	4	1.07			
Cor Total	94360.66	28				

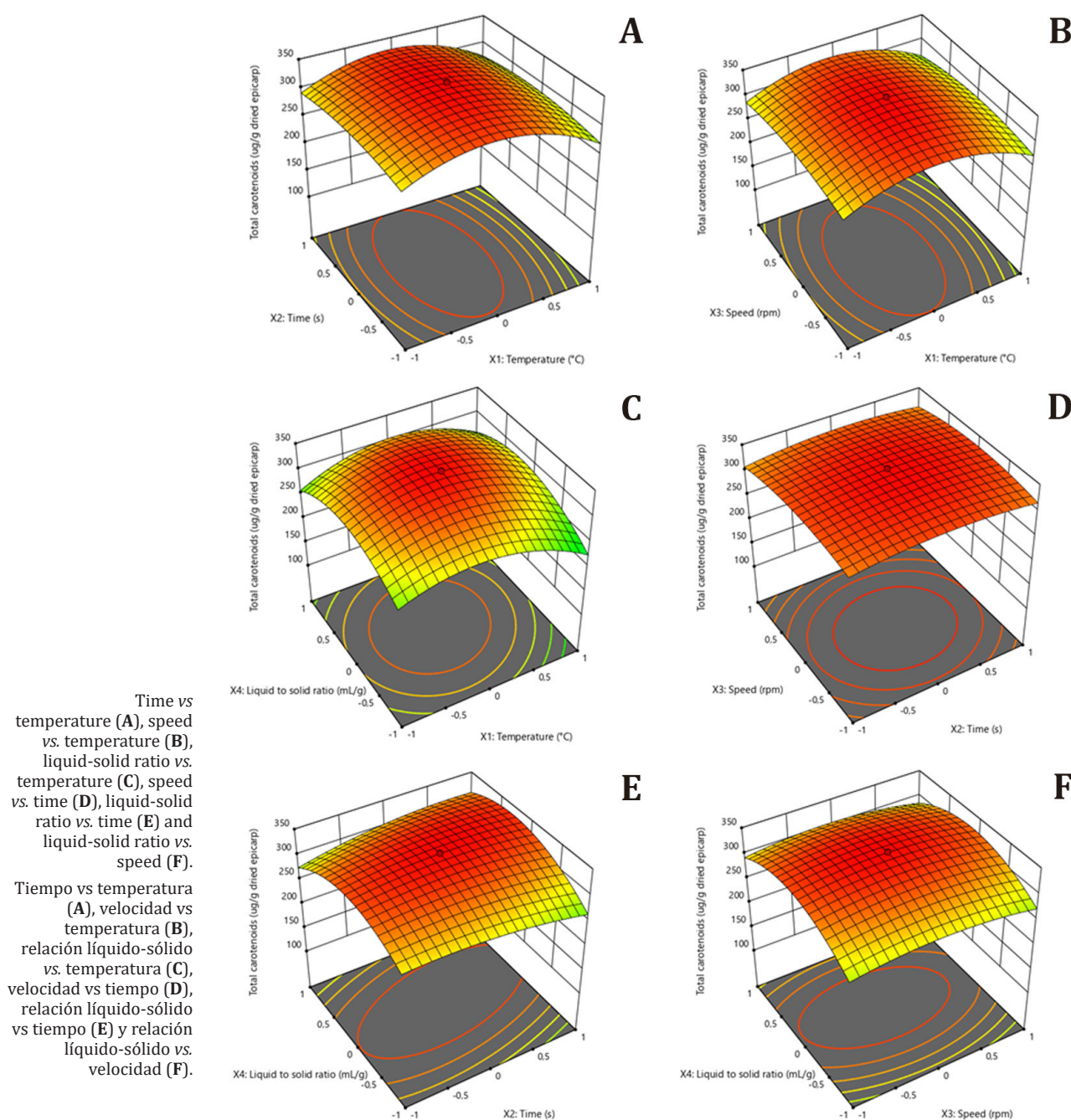
$$R^2 = 0.9994, R^2 \text{ adj} = 0.9987, R^2 \text{ pred} = 0.9966, \text{ and } CV\% = 0.844$$

Figure 1 A-F (page 203), shows response surfaces of interaction and quadratic effects in carotenoid extraction.

Figures 1A and 1B (page 203), show response surfaces generated by significant effects of time and temperature, and speed and temperature. Augmented extractions were observed with increasing time, speed, and temperature, while at over 50°C, extraction was reduced. This was previously observed on HAE in oligosaccharides from banana pulp (2, 18). However, these authors did not observe a significant impact on extraction speed of phenolic compounds and chlorophylls during this process (2, 16).

Carotenoids increase during HAE due to a higher mass transfer coefficient between carotenoid pigments and sunflower oil. This generates a mechanical breakdown of the biological matrix through shearing during HAE, reducing viscosity and accelerating diffusion and breakdown of protein-carotenoid bonds in the plant matrix (16, 21). Carotenoid reduction with increasing temperature during HAE may be associated with isomerization and oxidative degradation (21).





**Figure 1.** 3D surface plots of the effect of temperature, time, speed and liquid-solid ratio in the homogenizer-assisted extraction (HAE) of total carotenoids from *Bactris gasipaes* epicarp.

**Figura 1.** Gráficos de superficie 3D del efecto de la temperatura, el tiempo, la velocidad y la relación líquido-sólido en la extracción asistida por homogeneizador (HAE) de carotenoides totales del epicarpio de *Bactris gasipaes*.

Figure 1C (page 203), shows the response surface generated by temperature and liquid-solid ratio effects on the total carotenoid extraction. A quadratic effect was evidenced, at the beginning by a significant increase in extraction after an increase in liquid-solid ratio and temperature. Later, extraction levels were reduced when the liquid-solid ratio surpassed 50 mL/g, and temperature exceeded 50°C. Liquid-solid ratio effects in HAE of bioactive compounds in plant by-products were reported by Eyiz *et al.* (2020) in red grape pomace. The results presented here are consistent with the principles of mass transfer exposed by Wong *et al.* (2015), who stated that the concentration gradient between liquid and solid constitutes the driving force, which is greater for a higher liquid-solid ratio. On the other hand, extraction reduction of total carotenoids for ratios above 50 mL/g could prolong solvent diffusion distance into the matrix (23, 29).

Figure 1D (page 203), shows the response surface generated by speed and time effects on total extraction. Factors interaction positively affected extraction. Increased concentrations may have resulted from shearing and mechanical damage, transferring pigments to the solvent (16). Figures 1E and 1F (page 203), validate liquid-solid ratios, time, and speed effects in extracting total carotenoids from peach palm epicarp. A quadratic and interaction effect was observed on the response variable. The optimal HAE point of total carotenoids was 336.06 µg/g dried epicarp, with 50°C, 76 s time, 19200 rpm and a liquid-solid ratio of 48.75 mL/g. When experimentally validating the process factors established in HAE optimization, a carotenoid content of  $334.97 \pm 1.06$  µg/g dried epicarp was obtained, not significantly different ( $p > 0.05$ ,  $n = 4$ ) from the theoretical one. Therefore, experimental values were adjusted to the quadratic model. When comparing optimized values with the maceration method (sunflower oil for 24 h), HAE exceeds the concentration of the conventional method (113.94 µg/g dried epicarp) by 2.95 times. Extraction efficiency of bioactive compounds with HAE in plant matrices was previously described (16). These authors achieved high extraction rates with shearing, rupturing the plant matrix in a few seconds, increasing mass transfer coefficient (16). In addition, this method uses agitation, accelerating extraction and increasing mass transfer from the plant matrix to the solvent with diffusion and osmotic processes.

#### Application of the optimized extract in corn griddle cake

All response variables were significantly affected ( $p < 0.05$ , table 4, page 205). The enriched corn griddle cake (ECC) presented statistically higher carotenoids, provitamin A, and antioxidant activity than white corn flour (WCF). These differences are mainly due to the incorporation of the lipid extract in the ECC formulation. Other studies have used peach palm lipid extract in food matrices as bakery products, emulsions, and Frankfurt sausages (5, 17, 19). For example, de Souza Mesquita *et al.* (2020) reported increasing carotenoid pigments and provitamin A in mayonnaise made with this lipid extract. Bioactive compounds can influence antioxidant capacity in food matrices, and the addition of carotenoid pigments in the samples may increase antioxidant capacity, as stated in guava pulp after homogenization treatment (4).

Color attributes evaluated in crumb and crust showed  $L^*$  and  $h^\circ$  were statistically reduced in ECC, and  $C^*$  significantly increased compared to WCF. The TCD had a greater difference between ECC and WCF (table 4, page 205). These results are explained by the higher concentration of carotenoid pigments in ECC.

These pigments absorb part of the visible spectrum, favoring the yellow color in ECC. Meanwhile, low carotenoid concentration in WCF resulted in greater reflection of the visible spectrum, generating a white color in the samples. Suo *et al.* (2023) confirm changes in the white color of French fries to reddish tones when fried in corn oil enriched with carotenoid.



**Table 4.** Carotenoids, provitamin A, antioxidant activity and color attributes in two corn griddle cake formulations.**Tabla 4.** Carotenoides, provitamina A, actividad antioxidante y atributos de color en dos formulaciones de arepas de maíz.

Parameter	Formulation type	
	White corn flour (WCF)	Enriched corn griddle cake (ECC)
$\beta$ -Carotene <sup>1</sup>	0.80 $\pm$ 0.01 <sup>b</sup>	30.60 $\pm$ 0.02 <sup>a</sup>
$\alpha$ -Carotene <sup>1</sup>	0.40 $\pm$ 0.01 <sup>b</sup>	28.50 $\pm$ 0.03 <sup>a</sup>
$\beta$ -Cryptoxanthin <sup>1</sup>	4.30 $\pm$ 0.02 <sup>b</sup>	9.80 $\pm$ 0.03 <sup>a</sup>
Zeaxanthin <sup>1</sup>	9.20 $\pm$ 0.05 <sup>b</sup>	30.30 $\pm$ 0.06 <sup>a</sup>
Provitamin A <sup>2</sup>	0.26 $\pm$ 0.01 <sup>b</sup>	4.14 $\pm$ 0.03 <sup>a</sup>
DPPH (%)	4.61 $\pm$ 0.57 <sup>b</sup>	11.57 $\pm$ 1.64 <sup>a</sup>
$L^*$ <sub>crumb</sub>	81.93 $\pm$ 0.24 <sup>a</sup>	72.05 $\pm$ 0.06 <sup>b</sup>
$C^*$ <sub>crumb</sub>	14.40 $\pm$ 0.35 <sup>b</sup>	43.09 $\pm$ 0.60 <sup>a</sup>
$h$ <sub>crumb</sub>	93.50 $\pm$ 1.75 <sup>a</sup>	87.81 $\pm$ 0.04 <sup>b</sup>
TCD <sub>crumb</sub>		30.45 $\pm$ 2.76
$L^*$ <sub>crust</sub>	80.65 $\pm$ 0.48 <sup>a</sup>	72.29 $\pm$ 0.02 <sup>b</sup>
$C^*$ <sub>crust</sub>	14.27 $\pm$ 0.21 <sup>b</sup>	45.97 $\pm$ 0.57 <sup>a</sup>
$h$ <sub>crust</sub>	95.48 $\pm$ 1.34 <sup>a</sup>	86.16 $\pm$ 0.05 <sup>b</sup>
TCD <sub>crust</sub>		33.05 $\pm$ 1.45

<sup>1</sup>  $\mu$ g of compound/g of corn griddle cake, averages on the same column followed by different letters vary significantly from each other ( $p < 0.01$ ) according to t-Student test.

<sup>1</sup>  $\mu$ g de compuesto/g de arepa de maíz, <sup>2</sup> RAE  $\mu$ g/g de arepa de maíz, los valores promedios en la misma columna seguidos de letras diferentes varían significativamente entre sí ( $p < 0,01$ ) según la prueba t-Student.

## CONCLUSION

HAE was adequate for carotenoid extraction in peach palm epicarp. Maximum extraction of total carotenoids was reached when processing the samples at 50°C, 76 s, 19200 rpm, and liquid-solid ratio of 48.75 mL/g. In addition, the HAE method presented the best extraction performance for total carotenoids compared to extraction with maceration. The green extract obtained from homogenizer-assisted extraction is a natural additive with agro-industrial potential for use in roasted corn cake, increasing carotenoids (30.60  $\mu$ g/g of  $\beta$ -carotene), provitamin A (4.14  $\mu$ g/g) and antioxidant activity (11.57 % DPPH).

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