

## Optimization of the drying process of the chicozapote fruit (*Manilkara zapota* L.) on the techno-functional and nutraceutical properties

## Optimización del proceso de secado del fruto de chicozapote (*Manilkara zapota* L.) sobre las propiedades tecno-funcionales y nutraceuticas

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### ABSTRACT

Chicozapote is an endemic fruit in Mexico, which is also little known and consumed. This fruit has a high content of nutrients and bioactive compounds that could be used to improve the nutraceutical value of other foods as a source of such compounds. However, chicozapote fruit has a short shelf life. One of the most used conservation processes is drying, which offers less susceptibility to microbial degradation. Therefore, optimizing the drying process to produce functional flour with good techno-functional and nutraceutical properties was decided. This study aimed to optimize the drying process of processed chicozapote flour (OPCF) to preserve its techno-functional and nutraceutical properties using a rotatable central composite design of the response surface. Obtained data indicate that the best combination of drying process variables to obtain OPCF with better values for Oil Absorption Index, Water Absorption and Solubility Index, Antioxidant Activity, Total Anthocyanins, and Total Phenolics, were observed at a drying temperature and time of 74.1 °C and 12 h, respectively. Therefore, it is concluded that optimization of the drying process generated beneficial effects on the functional chicozapote flour.



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## RESUMEN

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El chicozapote es una fruta endémica de México, poco conocida y consumida. Esta fruta tiene un alto contenido de nutrientes y compuestos bioactivos que podrían utilizarse para mejorar el valor nutracéutico de otros alimentos al ser una fuente de obtención de compuestos. Sin embargo, el chicozapote tiene una vida útil muy corta. Uno de los procesos más utilizados de conservación es el secado al brindarnos menor susceptibilidad a la degradación microbiana. Por lo tanto, decidimos optimizar el proceso de secado para producir una harina funcional con buenas propiedades tecnofuncionales y nutracéuticas. El objetivo de este estudio fue optimizar el proceso de secado sobre las propiedades tecno-funcionales y nutracéuticas de una harina procesada de chicozapote (OPCF) utilizando un diseño compuesto central rotatable de superficie de respuesta. Los resultados obtenidos en este estudio indican que la mejor combinación de variables del proceso de secado para obtener la HPOC, con mejores valores en el Índice de Absorción de Aceite, el Índice de Absorción y Solubilidad en Agua, la Actividad Antioxidante, Antocianinas Totales y Fenólicos Totales, se observó a una temperatura de secado de 74.1 °C y un tiempo de 12 h, respectivamente. Por lo que concluimos que la optimización del proceso de secado generó efectos beneficiosos en la harina funcional de chicozapote.

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**PALABRAS CLAVE:** Actividad antioxidante; Compuestos fenólicos totales; Antocianinas totales; Alimentos funcionales; Optimización.

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## Introduction

The chicozapote (*Manilkara zapota* L.), is a tropical fruit considered a berry that fluctuates in shape from spherical to conical. Its mesocarp is fleshy, rich in starch and coloring pigments, with cells loaded with aromatic oils. The skin is rough and brown when green, becoming smooth as it ripens, while the soft, fleshy, juicy pulp is very sweet, releasing a mild and pleasant fragrance (Vargas y Vargas *et al.*, 2015). Worldwide, India is the leading production country with plantations of approximately 163,210 hectares with yields of 8.7 tons/ha, while in Mexico it is distributed in 13 states with plantations of 2,138 ha, with yields of 7.37 tons/ha (SIAP, 2024). In Mexico, this tree is known primarily for its nutritional use, while in other countries its main contribution is the latex extraction, used to produce chewing gum (García-Cuevas *et al.*, 2021). Traditionally, it has been used to treat coughs, diarrheal ulcers, hemorrhages, muscle spasms, pain, lung diseases, and neuronal disorders, among other medical conditions (Bashir, 2019). In the specific case of the chicozapote, it presents nutritional interest as it is a source of carotenoids, phenols, vitamins C and A, which are nutritional compounds with potential protective effects due to their antioxidant

activity. Additionally, it possesses a high nutrient content such as carbohydrates, proteins, fats, fiber, and minerals (Punia-Bangar *et al.*, 2022). As a fruit, it is consumed fresh when fully ripe, serving as a dessert fruit in many areas. After being cut from the plant, it continues its ripening process, exhibiting a climacteric pattern. Its shelf life at room temperature is very short, reaching its commercial maturity around 6-8 days after cutting. Within one or two days, it enters an advanced ripening stage, entering the senescence phase, making it highly perishable and challenging for conservation and commercialization (Rivas-Gastelum *et al.*, 2023).

Food is lost or wasted throughout the entire food chain, from initial agricultural production to final consumption in households. In general terms, the Food and Agriculture Organization (FAO) estimates that around one-third of the food produced for human consumption is worldwide lost or wasted, equivalent to 1050 million tons per year (FAO, 2013). A significant portion of this loss is due to the lack of suitable means of storage or processing. This underscores the importance of developing appropriate conservation processes that also preserve the nutritional value present in the food (Espinoza-Moreno *et al.*, 2021).

One of the most used processes in the industry is drying. This method is one of the oldest methods of food preservation, allowing for reduced susceptibility to microbial degradation. Additionally, it reduces the volume and weight of the raw material, resulting in a significant reduction in packaging, storage, and transportation costs (Serna-Cock *et al.*, 2015). Hence, this study aimed to optimize the drying of chicozapote fruit to obtain flour with good techno-functional and nutraceutical properties.

## **Material and methods**

### **Materials**

Chicozapote fruits from the El Saladito area, Elota, Sinaloa, were used (geographical coordinates, 23.8833 N, 106.85 W). The fruits were harvested when ripe and with no signs of biological or mechanical damage.

### **Production of optimized processed chicozapote flour (OPCF)**

For the drying process, the chicozapote fruits were washed and disinfected. Subsequently, the peel was removed, and the fruits were sliced (0.4 cm) and placed on stainless steel trays. These trays were then placed inside a heating oven (ECOSHEL, 9023A) under conditions of drying temperature (DT) and drying time (Dt) according to the different combinations provided by the experimental design. Dehydrated fruits obtained from each treatment were ground using a coffee grinder, resulting in 13 corresponding dehydrated chicozapote flours. Subsequently, these flours were evaluated for the response variables: Oil Absorption Index (OAI), Water Absorption Index (WAI), Water Solubility Index (WSI), Antioxidant Activity (AoxA), Total Anthocyanin Content (TAC), and Total Phenolic Content (TPC).

## Oil Absorption Index, Water Absorption Index, and Water Solubility Index

OAI, WAI, and WSI were assessed according to Ruiz-Armenta *et al.* (2022). Briefly, samples of 0.5 g of flour were mixed with 3 mL of vegetable oil and were vortexed for 1 minute, left to stand for 30 minutes, and finally centrifuged (1,600xg/25°C/25 min). The volume of free oil was measured. The amount of absorbed oil was calculated by subtracting the volume of free oil from the initial volume of oil and expressed as mL of absorbed oil per gram of flour. The test was performed in triplicate. For WAI and WSI, samples of 1 g flour were mixed with 15 mL of distilled water at 25 °C and then shaken for 30 min. Then, the mixture was centrifuged (3000xg/25°C/10 min) and the supernatant and precipitate were recovered. The supernatant obtained after the WAI assay was decanted in a crystal vessel, evaporated in a stove (80°C/12 h), and then weighed. WAI and WSI were expressed as percentages of dry solids.

## Quantification of Total Anthocyanin Content

To determine the total anthocyanin content (TAC), a hydroalcoholic extract was prepared by mixing 1 g of the sample with 10 mL of 80 % cold ethanol (-20°C), as described by Abdel-Aal and Hucl (1999). The mixture was homogenized (500 rpm/30 min) and centrifuged (10,000xg/10 min). The supernatant obtained after centrifugation was used to measure absorbance at 535 nm and 700 nm. TAC was calculated and the results were expressed as milligrams of cyanidin-3-glucoside equivalents per gram of dry weight (mg C3GE/g dw) according to equation 1.

$$TAC = \left( \frac{A_{535} - A_{700}}{\epsilon} \right) * T_v * PW * \left( \frac{1}{SW} \right) \dots (1)$$

Where  $\epsilon$  = Molar absorption coefficient of cyanidin-3-glucoside (25,965 cm<sup>-1</sup> M<sup>-1</sup>), T<sub>v</sub> = Total volume (mL), MW = Molecular weight of cyanidin-3-glucoside (449.2 g/mol), SW = Sample weight (g).

## Extraction Quantification of Total Phenolic Content

For the determination of Total Phenolic Content (TPC), methanolic extracts were prepared following the method described by Quintero-Soto *et al.* (2022). 500 mg of sample was mixed with 20 mL of 80 % methanol and subjected to agitation (60 min/300 rpm). Subsequently, the samples were hydrolyzed (2 N HCl, 30 min/90°C) and centrifuged (10,000xg/30 min). The supernatant was washed with 40 mL of hexane, then mixed with water (20 mL) and ethyl acetate (40 mL). Phenolic compounds were recovered from ethyl acetate through evaporation. Dried samples were stored at -20°C until use.

The TPC was determined following the method described by Singleton *et al.* (1999). To 0.2 mL of the extract, 2200 µL of Folin-Ciocalteu reagent was added, and the mixture was maintained for 3 minutes in darkness. Subsequently, 60 µL of a 7 % (w/v) sodium carbonate solution was added, followed by incubation for 90 minutes. The absorbance of the samples

was measured at 765 nm. The results were expressed as milligrams of Gallic Acid equivalents/100 grams of dry weight (mg GAE/100 g, dw).

### **Antioxidant activity (AoxA)**

The *in vitro* antioxidant activity (AoxA) was determined using the ABTS assay (Quintero-Soto *et al.*, 2022). A 7.4 mmol/L ABTS radical solution was prepared by mixing ABTS with 2.6 mmol/L potassium persulfate and then incubated for 16 hours in the dark at room temperature (25°C). The ABTS radical solution was then diluted with 10 mmol/L phosphate buffer (pH 7.4) to achieve an absorbance of  $0.70 \pm 0.02$  at 734 nm. Subsequently, 3 mL of the solution was mixed with 0.75 mL of the sample and incubated at room temperature for 6 minutes before recording readings at 734 nm. The antioxidant activity (AoxA) was expressed as  $\mu\text{mol Trolox equivalents (TE)}/100 \text{ g}$  of sample (dw).

### **Experimental design**

A rotatable central composite experimental design (response surface methodology, RSM) with two factors (process variables) (drying temperature [X1 = DT, 40 °C to 80 °C]/ drying time [X2 = Dt, 7 h to 22 h]) and five variation levels (2 factorial [coded: -1, +1], 2 axials [coded: -1.414 (- $\alpha$ ), +1.414 (+ $\alpha$ )], and 1 central [coded: 0]) was used to obtain the process variables combination throwing 13 treatments (Table 1). The values for the levels of the independent variables were determined based on preliminary trials. The dependent variables in this study were the water Absorption Index (WAI), Water Solubility Index (WSI), Oil Absorption Index (OAI), Antioxidant Activity (AoxA), Total Anthocyanin Content (TAC), and Total Phenolic Content (TPC).

### **Optimization**

The drying process was optimized to find maximum values of WAI, WSI, AoxA, TPC, and TCA, and minimum values of OAI using the numerical method. The stepwise regression procedure was applied, and non-significant terms ( $p > 0.1$ ) were deleted from a second-order polynomial. Subsequently, a new polynomial is calculated until a predictive model is obtained for each response variable. The statistical software Design Expert 7.0.0 was used for the RSM analyses.

### **Obtaining and experimental evaluation of the optimized processed chicozapote flour (OPCF)**

Once the best temperature and time conditions were determined, the selected model was experimentally validated by drying the fruit under the predicted optimal conditions: a temperature of 74.1 °C and a drying time of 12 hours. After obtaining the dehydrated fruits, they were ground in a coffee grinder, resulting in an optimized processed chicozapote flour (OPCF). Finally, OPCF was packed in polyethylene bags and stored at room temperature. After obtaining OPCF, it was

characterized based on its techno-functional and nutraceutical properties. (WAI, WSI, OAI, AoxA, TPC, and TAC).

**Table 1. Experimental design (13 treatments) and experimental results of water absorption index (WAI), water solubility index (WSI), oil absorption index (OAI), total phenolic compounds (TPC), Total anthocyanin content (TAC), and antioxidant activity (AoxA) from optimized processed chicozapote flour (OPCF).**

Treatments	Process variables <sup>1</sup>		Response variables <sup>2</sup>					
	DT (°C)	Dt (h)	WAI	WSI	OAI	TPC	TAC	AoxA
1	45.86	9.20	0.33	35.14	1	197.33	67.54	26.588
2	45.86	19.80	0.84	44.49	0.8	400.10	132.89	58.601
3	74.14	9.20	1.97	50.84	1	510.40	138.84	60.602
4	74.14	19.80	2.76	54.76	0.7	276.77	100.88	42.584
5	60.00	<b>7.00</b>	0.96	40.74	1.1	303.18	105.151	45.501
6	60.00	<b>22.00</b>	2.18	50.67	1.1	396.15	124.79	47.699
7	<b>40.00</b>	14.5	0.67	38.56	1	399.27	113.58	40.934
8	<b>80.00</b>	14.5	2.56	52.02	0.5	499.93	121.86	49.402
9	60.00	14.5	1.94	51.31	1.2	542.76	123.53	54.618
10	60.00	14.5	1.92	50.82	1	432.87	127.77	43.884
11	60.00	14.5	1.58	50.63	1	448.04	143.98	50.317
12	60.00	14.5	1.92	51.23	1	453.66	125.80	48.500
13	60.00	14.5	1.65	48.37	1	440.50	126.40	54.067

<sup>1</sup>DT = Drying temperature; Dt = Drying time; <sup>2</sup>WAI = Water absorption index (g of gel/g sample); WSI = Water solubility index (%); OAI = Oil absorption index (g of gel/g oil); TPC = Total phenol compounds (mg of gallic acid equivalents [GAE]/100g sample); AoxA = Antioxidant activity (mmol of Trolox equivalents [TE]/100g sample).

## Statistical analysis

The techno-functional and nutraceutical properties data was subjected to a one-way analysis of variance (ANOVA), and mean comparisons were conducted using the least significant difference method (LSD, Least Significant Difference) with a confidence level of 95 %, using the STATGRAPHICS plus Software version 5.1 (Statistical Graphics Corporation<sup>TM</sup>, Rockville, Maryland, EUA).

## Results and discussion

### Response surface methodology (RSM) analysis

#### Modeling of response variables

The optimized flours obtained from the drying process exhibited different techno-functional and nutraceutical properties. The maximum and minimum values obtained for each response are shown in Table 1. Additionally, Table 2 shows the analysis of variance for the responses WAI, WSI, OAI, AoxA, TAC, and TPC. It is observed that the data exhibited a significant regression model, with  $R^2$  values  $\geq 0.71$ , coefficient of variation  $CV < 13.88$ , F-value  $p < 0.0084$ , and no lack-of-fit.

#### Water Absorption Index

Table 2 displays the statistical models for each evaluated response variable, noting that time ( $p < 0.001$ ) and temperature ( $p < 0.0001$ ) in their linear expressions were the factors that affected water absorption in OPCF. The mathematical model for this response variable showed an  $R^2 = 0.921$ ,  $CV = 13.88\%$ , and F-value  $p < 0.0001$ , with a significant lack-of-fit ( $p = 0.2200$ ). The following equation 2 shows the mathematical model used for the WAI.

$$WAI = 2.71 + 0.055 * T + 0.071 * t \dots (2)$$

Figure 1A describes the WAI behavior in response to the effect of temperature and drying time. The highest water absorption occurred in samples dried at high temperatures and extended drying times, attributed to greater cellular damage in the samples causing a greater number of water molecules to bind to the polar chain residues (Meda & Ratti, 2005). Techeira *et al.* (2014), when characterizing flours from various cassava varieties, found that employing higher temperature conditions leads to an increase in WAI. They remark that this behavior is due to the presence of weaker intragranular forces, which allow the passage of water molecules into the starch granules, causing an increase in size. Cornejo and Rosell (2015) have specified that a high protein level also increases the water interaction. An increase in WAI is associated with the leaching and solubility of amylose and the loss of the crystalline starch structure. This behavior is similar to what was found in this study, where subjecting the flour to high-temperature conditions and extended drying times can affect the starch structure, resulting in similar behaviors. García-Pacheco *et al.* (2019) reported that the variation in WAI among different flours may be due to the relationship between variable protein concentrations, the degree of interaction with water, along with original characteristics they possess.

It has been demonstrated that WAI primarily depends on proteins and some of their parameters, such as size, shape, steric factors, and hydrophilic-hydrophobic balance of amino acids in molecules, as well as lipids and carbohydrates (Guerra-Baños *et al.*, 2020). It is also noted that for low temperatures and drying times, the response showed minimum values. This can be explained by the higher moisture content in the samples which prevents the added amount

of water from being absorbed in large quantities. Additionally, the low applied conditions did not affect the protein structure, reducing absorption. A low WAI may indicate that the starch is of low quality, as it tends to produce thin and unstable pastes. However, a very high WAI can negatively impact the mechanical properties of the dough (von Atzingen & Machado Pinto e Silva, 2005). The reference range for the WAI is between 0.82 and 15.52 g gel/g sample (Anderson, 1982).

**Table 2. Results of the regression analysis of second-order polynomial models for each of the response variables studied.**

Parameters	Regression parameter coefficients					
	WAI	WSI	OAI	TPC	TAC	AoxA
Interception	-2.71	-58.21	-1.225	-1905.38	-416.44	11.19E5
Lineal						
DT	0.055***	2.093***	0.089***	36.24**	NS	2683.13**
Dt	0.071***	4.046***	-0.012**	NS	31.08**	NS
Quadratic						
DT x DT	NS	-0.012***	-0.0008***	NS	-0.0373**	NS
Dt x Dt	NS	-0.0770***	NS	-2.498*	-0.3143**	NS
Interaction						
Dt x Dt	NS	NS	NS	-1.457***	-0.3443***	-166.77***
P-value for model	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
R <sup>2</sup>	0.92	0.97	0.71	0.85	0.90	0.85
R <sup>2</sup> adj	0.90	0.94	0.60	0.74	0.83	0.79
R <sup>2</sup> pred	0.86	0.86	0.17	0.32	0.64	0.72
P-value for lack-of-fit	0.2200	0.3200	0.2300	0.3400	0.5300	0.6500
CV (%)	13.88	2.84	12.05	12.19	6.68	8.39

NS = Not significant ( $p > 0.1$ ); \*Significant at  $P < 0.1$ ; \*\*Significant at  $P < 0.05$ ; \*\*\*Significant at  $P < 0.01$ . DT = Drying temperature; Dt = Drying time.

### Water Solubility Index

Time variable ( $p < 0.001$ ) and temperature ( $p < 0.0001$ ) in the linear expression were the factors with the most significant effect on the WSI response in the evaluated chicozapote flour. However, it is noticeable that the factors in the quadratic expressions and the interaction also showed significant effects on the response. The obtained model had an  $R^2 = 0.9700$ ,  $CV = 2.84\%$ , and F-test  $p$ -value  $< 0.0001$ , without lack-of-fit with a  $p$ -value of 0.32.

Equation 3 represents the mathematical model used for WSI, in which a quadratic model is observed.

$$WSI = -58.207 + 2.093 * T + 4.046 * t - 0.019 * T * t - 0.0118 * T^2 - 0.076 * t^2 \dots (3)$$

In figure 1B, the WSI behavior in response to temperature and drying time is observed. It can be seen that as both drying times and temperatures increase, WSI also increases. García-Jimenez and Vázquez (2016) indicate that WSI is a directly proportional parameter to the amount of water-dissolved solids, which, in turn, is related to the degree of flour drying. This is a critical factor since a long drying time breaks the starch chains and generates short chains that retain a greater amount of water molecules. Njintang and Mbofung (2015) described that, while drying at high temperatures is more efficient, the process negatively impacts the product quality in terms of color, functionality, and flour performance during reconstitution. Techeira *et al.* (2014) obtained a similar behavior in cassava flour. The highest solubility values were obtained in the highest temperature range as they exhibit a weaker intragranular structure, which breaks more easily under these conditions, allowing the exudation of more molecular components into the dispersion.

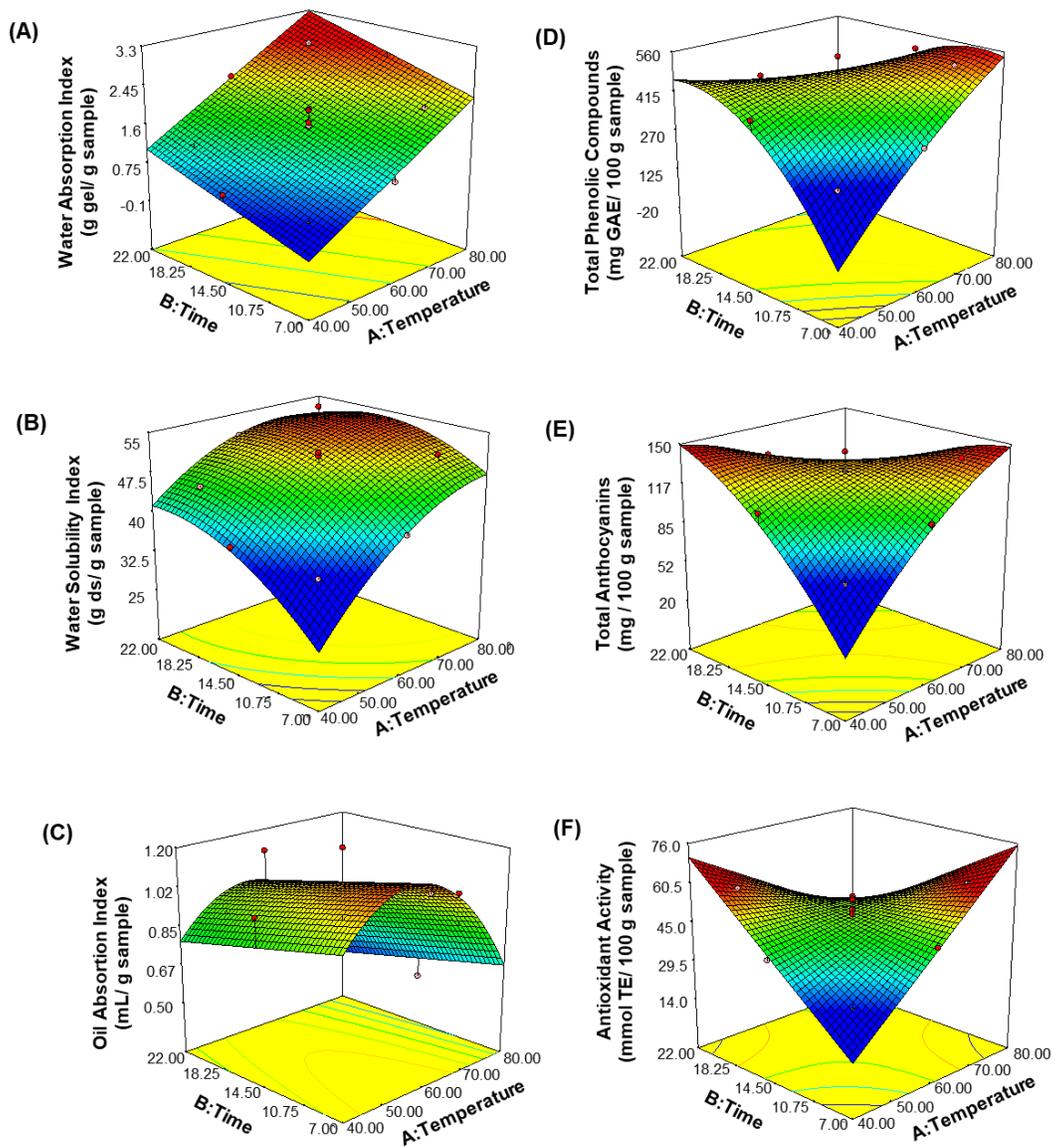
### Oil Absorption Index

In Figure 1C, the behavior of OAI of the OPCF concerning temperature and drying times is illustrated. The factors showing the most significant effect on the response were temperature and time in its linear expression. The model for this response variable showed an  $R^2 = 0.7115$ ,  $CV = 12.05\%$ , and F-test p-value = 0.0084, without lack-of-fit with a p-value of 0.23. Equation 4 represents the mathematical model used for the water solubility index, in which a quadratic model is observed.

$$OAI = -1.225 + 0.088 * T - 0.018 * t - 7.989 \times 10^{-4} * T^2 \dots (4)$$

It is observed that as the temperature values increase, the response is better, increasing the OAI until reaching a maximum. Punia-Bangar *et al.* (2022) mention that the fruit has large amounts of proteins, so this response is explained by the findings reported by Miquilena *et al.* (2016), who mentions that OAI is the ability of flour proteins to physically bind to fat through capillary attraction. This occurs under moderate conditions within a certain range of temperatures and drying times for flours, where the protein structure seems to undergo small structural alterations that favor the physical fat retention. This could be a result of the physical entrapment of fats by proteins through the formation of micelles, leading to an increase in the response, similar to the results of the present study. However, when the values of temperature and drying time conditions continue to increase, the proteins begin to lose structure, subsequently leading to a decrease in the response.

The OAI is attributed to the physical entrapment of fat within proteins, where non-covalent bonds such as electrostatic forces, hydrophobic interactions, and hydrogen bonds play a key role in lipid-protein interaction (Lawal, 2004). This property is crucial for formulating bakery products, meat products and substitutes, soups, and frying foods, as it is related to the flavor retention capacity and the smoothness of the end product (El-Adawy & Taha, 2001). Additionally, OAI reduces the development of oxidative rancidity, thereby increasing stability over storage (Shate, 2002).



**Figure 1. Contour plots showing the effect of the drying process variables (temperature and time) on the response variables (A) Water Absorption Index, (B) Water Solubility Index, (C) Oil Absorption Index, (D) Total Phenolic Compounds, (E) Total Anthocyanins, and (F) Antioxidant Activity of processed chicozapote flour.**

## Total Phenolic Content

In this study, it was found that temperature ( $p = 0.049$ ) was the factor showing the most significant effect on the TPC in its linear expression. Additionally, the temperature and time factors in its quadratic expression also showed a significant effect on the response. The model for this response variable exhibited an  $R^2 = 0.8500$ ,  $CV = 12.19\%$ , and F-test  $p$ -value  $< 0.0084$ . Equation 5 represents the mathematical model used for determining the total phenolic content:

$$TPC = -1905.38 + 36.24 * T - 1.46 * T * t - 0.10 * T^2 - 2.49 * t^2 \dots (5)$$

Figure 1D shows the behavior of TPC under the influence of varying temperatures and drying time. It can be observed that under high temperatures and short drying times, the TPC is favored. Gómez-Maqueo *et al.* (2020) reported that the content of phenolic compounds and carotenoids in tomatoes remains stable during processing at high temperatures. Furthermore, they also reported that the thermal process can increase the content of phenolic compounds by releasing phenolic acids bound to cell constituents. Similarly, Kim and Chin (2016) observed a similar behavior in the drying process in the range of 60 and 70 °C, attributing this effect to Maillard reactions occurring during toasting. Yao *et al.* (2020) reported an increase in TPC with increasing temperature due to the destruction of covalent bonds and cellular constituents, releasing antioxidants, such as phenolic acids, flavones, phenols, and other compounds, which are bound to macromolecules produced by heat treatment. Thus, it can be concluded that the effect of heat treatment on the content of phenolic compounds depends on the species and variety of the raw material used. These variations also depend on the chosen method for extracting phenolic compounds as well as the temperature and processing time.

## Total Anthocyanin Content

In Figure 1E, it can be observed that the factors with a significant effect on anthocyanins were the time variable in its linear term ( $p = 0.0446$ ) and in its quadratic term ( $p = 0.0223$ ), as well as the temperature variable ( $p = 0.043$ ) in its quadratic term and the interaction between these factors with  $p = 0.0003$ . The mathematical model for this response variable showed values of  $R^2 = 0.9040$ ,  $CV = 6.68\%$ , and F-test  $p$ -value  $< 0.0019$ , with a significant adjustment probability ( $p = 0.5315$ ). Equation 6 presents the mathematical model used for the TAC determination.

$$TAC = -416.44 + 9.92 * T + 31.08 * t - 0.34 * T * t - 0.037 * T^2 - 0.314 * t^2 \dots 6$$

It can be observed that the TAC increased as temperature increased. Menchaca-Armenta *et al.* (2020) reported higher levels of anthocyanin at intermediate-high temperatures (85 to 105 °C), similar to the observations in this study. Yemmireddy *et al.* (2013) propose that the effect of temperature on anthocyanins is a hydrolysis of the glycosidic bond, followed by a conversion from aglycone to chalcone (colorless pigments). It should be noted that temperatures exceeding 100 °C result in much greater color degradation than temperatures below 90 °C, since temperature can induce an increase in the content of negative transcription factors, thus binding to promoters

and inhibiting the expression of anthocyanin genes Zou *et al.* (2021). The stability of anthocyanins in foods is significantly affected by temperature.

In general, the structural characteristics that lead to an increase in pH stability also contribute to thermal stability. Highly hydrolyzed anthocyanins are less stable than methylated, glucosylated, or acetylated forms (Habibi *et al.*, 2020a). The thermal stability of anthocyanins varies with their structure, pH, oxygen presence, and interactions with other components in the system (Habibi *et al.*, 2020b). Wang and Xu (2007) specify that anthocyanins are unstable when exposed to high temperatures.

### Antioxidant Activity (AoxA)

In Figure 1F, it is observed that the factors that had a significant effect on AoxA were the temperature variable and drying time variable in its linear terms, as well as the interaction between both factors with  $p = 0.002$ . The mathematical model for this response variable showed values of  $R^2 = 0.8417$ ,  $CV = 8.39 \%$ , and F-test  $p$ -value = 0.0006, with a significant adjustment probability ( $p=0.648$ ). Equation 7 represents the mathematical model used for the AoxA by the ABTS method of dried chicozapote fruit:

$$AoxA = -1.189E05 + 2683.13 * T + 10409.53 * t - 166.77 * T * t \dots (7)$$

The parameters that showed the greatest effect on AoxA were drying time and temperature in their linear expression. It can be observed that at high temperatures and low drying times, the response increases. Alvis-Celis (2020) explains that the increase in AoxA could result from high temperatures allowing the formation of new compounds with higher antioxidant activity, such as those formed in the Maillard reaction. Giovanelli *et al.* (2001) reported that heat treatment increases the level of free flavonols, which have significant antioxidant activity. Krzykowski *et al.* (2020) state that heating is the main cause of the loss of antioxidants, but it can also induce the formation of compounds such as melanoidins through the Maillard reaction, and these compounds have antioxidant effects. These compounds are generated at high temperatures, such as 100 °C, and short drying time.

Zapata Bustamante *et al.* (2014) reported a similar behavior as in the present study when increasing the temperature during the roasting of cocoa beans. They mentioned that this occurs due to the generation of intermediate products and melanoidins caused by the Maillard reaction, as the radical-scavenging activity increases due to the presence of melanoidins. However, it is possible to observe that as the temperature continues to increase, there is a diminishing trend, which may be attributed to the formation of other compounds with lower antioxidant activity during the Maillard reaction at higher temperatures. Piga *et al.* (2003) subjected "Presidente" plum cultivars to drying using two temperatures (60 and 85 °C) and observed that the AoxA doubled at 85°C. Similar results were found in the response to this project. They explain that the increase in AoxA could be the result of two factors: a) partial oxidation of phenolic compounds increases the antioxidant power present in the sample (temporarily), b) high temperature allows the formation of new compounds with greater antioxidant capacity, such as those formed in the Maillard reaction.

## Optimization

The optimization process was carried out to determine the best temperature and drying time conditions, aiming to obtain dehydrated flour with good techno-functional and nutraceutical properties. A numerical method was employed for this procedure, and different criteria were established for each of the response variables. High values are expected for the responses of total phenolic content, total anthocyanin content, antioxidant activity, water absorption index, and water solubility index. In contrast, low values are desired for the oil absorption index. These variables were chosen for optimization as the development of a food product with good characteristics and health benefits depends on its functional and nutraceutical properties.

In Figure 2, the bar graphs of individual desirability for the response variables and the overall or combined desirability of the process are shown. The maximum desirability has a value of 1, and the minimum desirability has a value of 0. It can be observed that the individual desirability for both temperature and drying time was 1.0, as they were assigned a range as their target, indicating that they will always have a desirability of 1.

The AoxA showed a desirability of 0.881, indicating that it achieved approximately 88.1 % of the difference from the upper limit to the lower limit of AoxA. The TAC showed a desirability of 0.887, exhibiting that it covered approximately 88.7 % of the difference from the upper limit to the lower limit. TPC presented a desirability of 0.918, indicating that it reached 91.8 % of the difference from the upper limit to the lower limit of TPC. In the case of OAI, it showed a desirability of 0.541, indicating that it covered approximately 54.1 % of the difference from the upper limit to the lower limit of OAI. The WSI showed a desirability of 0.872, indicating that it reached approximately 87.2 % of the difference from the upper limit to the lower limit of WSI. The WAI presented a desirability of 0.784, indicating that it reached approximately 78.4 % of the difference from the upper limit to the lower limit of WAI.

Global desirability (GD) or combined is presented by the following equation 8:

$$GD = (0.881 * 0.887 * 0.918 * 0.541 * 0.784 * 0.872)^{\frac{1}{6}} = DG = 0.802 \dots (8)$$

With the obtained results from numerical optimization, the best process conditions were determined to be DT of 74 °C and Dt of 12 hours. Under these optimal conditions, the following predicted values were obtained for each of the mathematical models: OAI= 0.82 mL of oil absorbed/g of flour, WAI= 2.24 g gel / g (d.b.), WAI=52.27 g gel / g (d.b.), AoxA = 56.57 mmol TE/ 100 g (d.b.), TAC= 134.36 mg/100 g (d.b.), TPC= 514.58 mg GAE/100 g (d.b.).

For the validation of the employed models, chicozapote fruit was dehydrated under the optimal conditions found in the process. After obtaining the dehydrated fruit, flour was produced and evaluated to verify the predicted values.

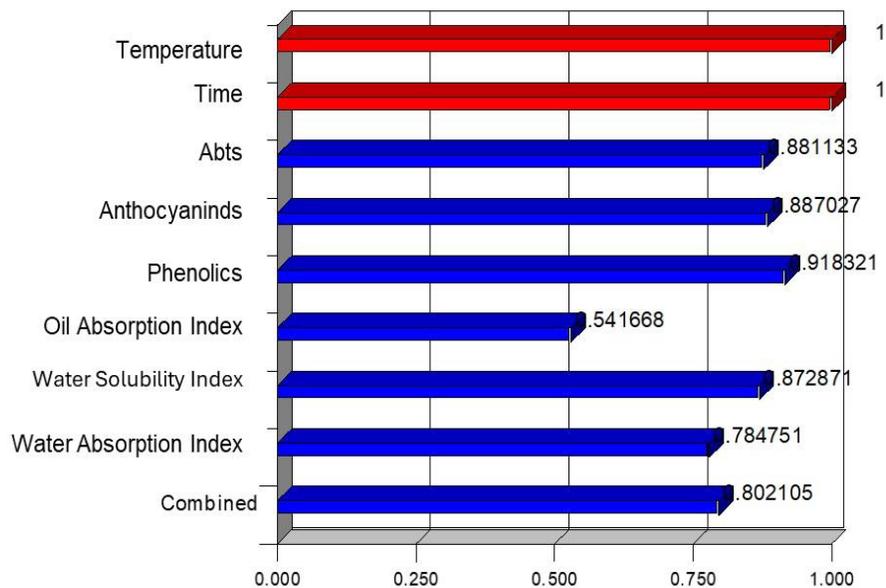
From the characterization of the OPCF the following average values and standard

deviations were obtained: OAI=  $0.7 \pm 0.25$  mL of oil absorbed / g of flour, WAI=  $2.259 \pm 0.38$  g gel / g (d.b.), WSI =  $49.90 \pm 0.30$  g dry solids / g original solids, AoxA = 64.42 mmol TE/ 100 g (d.b.), TAC= 131.07 mg/100 g (d.b.), TPC = 587.77 mg GAE/100 g (d.b.). When the experimental values were compared against the values predicted by the mathematical models, no significant difference was observed between them ( $p < 0.05$ ). Therefore, the employed model was demonstrated experimentally to have a good fit for finding the optimal temperature and drying time conditions for obtaining dehydrated fruit with good techno-functional and nutraceutical properties.

## Characterization of optimized processed chicozapote flour

### Techno-functional properties

To extend the shelf life of the fruit, its techno-functional properties were modified through the optimization of the drying process, obtaining a functional and nutraceutical flour to promote its use as an ingredient in the development of new foods as a way of valorizing the crop. The techno-functional properties evaluated were WAI, WSI, and OAI. The fresh fruit showed low values of WAI and OAI ( $0.001 \pm 0.00$  g gel/g and  $0.000 \pm 0.00$  mL of oil absorbed/g, respectively); which was expected due to its high water content (88.02 %). On the other hand, for the OPCF, WAI values of  $2.259 \pm 0.38$  g water/g were observed. This property can be influenced by several factors; one is the chemical composition of the flour. Martínez-Martí *et al.* (2023) investigated the water absorption index of clementine peel dried by hot air, reporting values of 6.6 g water/g. The lower WAI values observed in this study could be due to the different environmental conditions where the products were developed, as well as the chemical composition of each material. The OAI ( $0.7 \pm 0.25$  mL of oil absorbed/g) obtained for the OPCF in this study is lower than that reported for functional corn flour with cowpea obtained by extrusion (Sotelo-Díaz *et al.*, 2023). A lower OAI would ensure that if the product is fried or comes into contact with oil, it will absorb less oil. This makes OPCF a good option for making fried foods. The WSI determined for fresh and processed samples was  $20.12 \pm 0.75$  g dry solids/100 g original solids and  $49.90 \pm 0.30$  g dry solids/100 g original solids, respectively. The observed WSI values for OPCF suggest that it can be used for functional beverage production.



**Figure 2. Individual and global (combined) desirability of the process variables (temperature and time) and responses (water absorption index, water solubility index, oil absorption index, total phenolic compounds, total anthocyanins, and antioxidant activity) were analyzed during optimization.**

### Nutraceutical properties

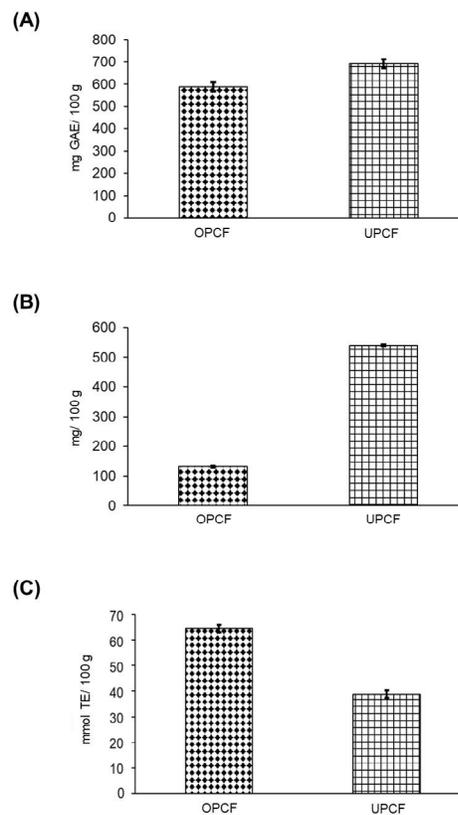
In Figure 3A, the TPC of the OPCF, prepared with the best drying process conditions (DT= 74.1 °C / Dt = 12 h), is shown. The TPC of the optimized flour was significantly lower ( $p < 0.05$ ) than the unprocessed chicozapote flour (UPCF) [587.77 vs 691.28 mg GAE/100 g (d.b.); retention= 85.03 %]. Similar behaviors have been reported in microalgae (retention = 50 %) and mango (retention= 47 %) by researchers such as Subbiah *et al.* (2023) and Medeiros *et al.* (2022), who attribute such decrease to the degradation of phenolic compounds due to prolonged exposure to high temperature. However, despite the observed TPC decrement of the optimized flour, the values in this study are similar to those reported by Baky *et al.* (2022) for a fruit from the same family as chicozapote (*Manilkara hexandra* = 650 mg GAE/100 g) extracted with a hydroalcoholic solution and without processing.

The TAC of OPCF was significantly lower ( $p < 0.05$ ) than the UPCF [131.67 vs 539.07 mg/100 g (d.b.); retention = 24.43 %] (Figure 3B). The low retention values of TAC are due to the drastic effect of the drying process; temperatures above 60 °C may cause the denaturation of anthocyanins (Patras *et al.*, 2010). The values observed in this study for UPCF are similar to

those obtained by Li *et al.* (2023) for fresh red radish (608.91 mg/100 g), but higher than those of red radish dried with hot air at 60 °C (52.97 mg/100 g) (retention = 9 %). This suggests that the anthocyanin type in chicozapote is more resistant to temperature, or the matrix in which they are found partially protects them from degradation.

The AoxA of the UPCF and OPCF was determined using the ABTS colorimetric technique (Figure 3C). The values obtained for the analyzed samples were 38.86 y 64.42 mmol ET/100 g (d.s.) for UPCF and OPCF, respectively [increase = 65.75 %]. Despite the notable decrease in the content of phytochemicals to which various authors have attributed AoxA (Li *et al.*, 2023; Patras *et al.*, 2010; Quintero-Soto *et al.*, 2018; Xu & Chang, 2007), this study observed a 1.6-fold increase in this activity. These values are higher than those reported by Jiménez-Ortega *et al.* (2024) for 10 antioxidant supplements. This indicates that OPCF could be a good option to be used as a dietary supplement.

This behavior could be explained by the fact that during the drying process, temperatures are applied that trigger a large number of reactions in the matrix undergoing processing. One of the reactions with a significant impact is the Maillard reaction, where free amino acids present in the food react with available reducing sugars, forming compounds that give certain brown tones to the fruit (Félix-Medina *et al.*, 2021). These compounds are known as melanoidins, which have been reported to generate AoxA (Quintero-Soto *et al.*, 2022). These compounds, together with phytochemicals, could be acting synergistically to increase the antioxidant activity of OPCF. However, further studies are required to confirm this effect.



**Figure 3. Content of phenolic compounds (A), anthocyanins (B), and antioxidant activity (C) of an optimized processed chichozapote flour (OPCF) and unprocessed chichozapote flour (UPCF).**

## Conclusions

The predictive mathematical models obtained for the response variables analyzed in this study were adequate, as they showed values of  $R^2 \geq 0.71$ ,  $F$  p-value  $\leq 0.001$ ,  $CV \leq 13\%$ , and did not show a lack-of-fit. The optimal conditions found (DT: 74°C and DT: 12 h) were useful for producing a dehydrated chichozapote flour with good techno-functional properties (water absorption and solubility capacity) and nutraceutical properties (total phenolic compounds, total anthocyanins, and antioxidant activity). The retention percentages of total phenolic compounds (85.03 %) and the increase in antioxidant activity (65.75 %) after the optimized drying process of chichozapote suggest that the optimal conditions obtained are suitable for carrying out the dehydration process of the fruit, thereby extending its shelf life while largely preserving its nutraceutical capacities.

## Authors' contribution

Work conceptualization: MFQS, MEVO, and GDRH. Methodology development: EMCM, CNBM, IDP, and JVFM, Software management: MFQS and MEVO. Experimental validation: FSL and ODAL. Analysis of results: EMCM, MFQS, and MEVO. Manuscript writing and preparation: EMCM. Drafting, revising, and editing: MEVO and MFQS. Project management: MEVO.

All authors of this manuscript have read and accepted the submitted version.

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## Declaration of interest

The authors declare that there are no conflicts of interest.

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