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Bioactive compounds preservation in functional flour production from *Cordia dodecandra* A. DC fruit: Impact of drying method and pretreatment

Preservación de compuestos bioactivos en la producción de una harina funcional del fruto *Cordia dodecandra* A. DC: Impacto del método de secado y pretratamiento

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Abstract

During the production of functional flours, bioactive compounds are affected by processing, and their bioactivity preservation is crucial for future applications. Using the ciricote fruit *Cordia dodecandra* A. DC as study model., convective dried (CD) peels, freeze dried (FD) peels, and pulps pretreated with ascorbic acid, favored the preservation of total phenolic content (TPC), total flavonoids (TF) and antioxidant activity (AA) over the non-pretreated ones. CD and FD processes did not present effects ($p < 0.05$) on TPC and AA of pulp and peel flours extracts. The pretreatment prevented the losses of the phenolic compounds rosmarinic acid and nepetoidin A and B, analyzed by UPLC-DAD-ESI-MS. The ciricote flours (pulp, peel, and seed) were rich in protein, low in fat, and preserved valuable Vitamin C content. The pretreated CD process represented a viable technology to obtain functional flours while preserving their compounds useful for both pharmaceutical and food applications.

Keywords: phenolics, convective drying, freeze drying, ascorbic acid, functional flours.

Resumen

Durante la producción de harinas funcionales, los compuestos bioactivos son afectados por el procesamiento, siendo crucial la preservación de la bioactividad para futuras aplicaciones. Empleando el fruto de ciricote *Cordia dodecandra* A. DC. como un modelo de estudio, las pieles secadas por secado convectivo (SC) y las pieles y pulpas pretratadas con ácido ascórbico y secadas por liofilización (L), favorecieron la preservación del contenido de fenoles totales (CFT), flavonoides totales (FT) y actividad antioxidante (AA) sobre las no pretratadas. Los procesos de SC y L no presentaron efectos significativamente diferentes ($p < 0.05$) sobre el CFT y AA de los extractos de harina de pulpa y piel. El pretratamiento previno las pérdidas de los compuestos fenólicos ácido rosmarínico, nepetoidin A y B, analizados por UPLC-DAD-ESI-MS. Las harinas de ciricote (pulpa, piel y semilla) fueron ricos en proteína, bajos en grasa y preservaron un contenido valioso de vitamina C. El proceso de SC con pretratamiento representó una tecnología viable para la obtención de harinas funcionales que preservan sus compuestos, útiles para aplicaciones farmacéutica o alimenticias.

Palabras clave: Compuestos fenólicos, secado convectivo, liofilizado, ácido ascórbico, harina.

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1 Introduction

The trees belonging to the genus Cordia are part of the Boraginaceae family, comprising over 300 species widely distributed in tropical regions, including East and West Africa, Central and South America in countries such as Mexico, Pakistan, Nigeria, Ghana, Sri Lanka, and India. Some of these species have been used in traditional medicine (Oza and Kulkarni, 2017). *Cordia dodecandra* A. DC., commonly known as "ciricote", which is a native tree from the Yucatán Peninsula, located in the southeastern region of Mexico, it has been reported as an unconventional native species with great properties (Janick and Paull, 2008; Zapata-Luna *et al*., 2023). The ciricote tree produces a fruit that is primarily processed in an artisanal manner on a small scale. Our previous research has demonstrated that the ciricote fruit contains valuable phenolic compounds with potential applications for both pharmaceuticals and food areas (Jiménez-Morales *et al*., 2022; Pacheco *et al*., 2020). The ciricote fruit, however, is a highly oxidizable product which is affected during its processing.

Continuous airflow, physical stress (compressing, cutting and bruising), and other conditions, can promote browning, oxidation or enzymatic reactions that can affect the appearance, flavor, and nutritive value, such as in other very perishable fruits (Derardja *et al*., 2019; Ramírez-Pulido *et al*., 2021; Onwude *et al*., 2022). Proceses like freezing, chilling, drying, and pasteurization, considered as conventional food preservation, are encouraged to avoid the chemical and microbial deterioration of foods (Amit *et al*., 2017). Dehydration and milling are generally combined to produce fruit and vegetable powders of great interest for the food industry. This market is growing due to the increasing use of these kinds of powders as food ingredients and sources of bioactive compounds. Furthermore, the combination of these current processes, reduces the volume of the material, minimizing the necessary requirements for the packaging, storage, and shipment of plant material for their use in the production of different dressings, bakery, confectionery, sweet, soups or ready-to-eat products, to improve the nutritional value of foodstuffs (Castañeda-Pérez *et al*., 2013; Patrón-Vázquez *et al*., 2019; Ramírez-Pulido *et al*., 2021).

Several commercially available drying methods have been used to process fruits and vegetables. Convective drying (CD) is considered a common method for achieving economic drying in food, as it reduces weight of the products and simplifies operations such as storage and transportation (Thuy *et al*., 2023). It is also reported as a good technology for processing food waste, like those coming from vegetables and fruits, allowing their integral recovery and valorization (Amit *et al*., 2017; Onwude *et al*., 2022; Tan *et al*., 2020). Freeze drying (FD) technology is an effective way to preserve nutrients in the final products, providing high quality and retention of their functional properties. An inconvenient aspect of this technology has been its high cost that have reduced its application in the industry (Castañeda-Pérez *et al*., 2013; Patrón-Vázquez *et al*., 2019; Ramírez-Pulido *et al*., 2021; Tan *et al*., 2020). On the other hand, even with convective drying treatments, structural or chemical changes occur during processing depending on operating conditions, that may affect the nutritional or organoleptic properties as well as the reduction or losses of bioactive metabolites, e.g. phenolic compounds, carotenoids or vitamins (Castañeda-Pérez *et al*., 2013; Patrón-Vázquez *et al*., 2019).

The undesired effects from heat during the drying processing can be minimized by pretreating the product with the primary purpose to inactivating enzymes such as polyphenoloxidase (PPO), peroxidase (PDO) and phenolases. These pretreatments can also inhibit undesirable chemical reactions that may cause adverse changes in the product (Ramírez-Pulido *et al*., 2021). These pretreatments, however, can also change the tissue structures to make it soft, and favor the release of bioactive compounds, and impacting in the drying process responses (Ramírez-Pulido *et al*., 2021). Organic acids such as citric, ascorbic, oxalic, and cinnamic acid are widely used as pretreatments to inhibit PPO activity. Additionally, ascorbic acid (Vitamin C) is a major compound used in the food industry due to its effectiveness at replacing the use of sulfites (Derardja *et al*., 2019; Landi *et al*., 2013; Zhou *et al*., 2016). Considering the previous information, the objective of this study was to investigate the effect of the CD and FD drying methods and the pretreatment with ascorbic acid to preserve the presence of phenolic compounds, and antioxidant activity, during the obtention of flours from peel, pulp, and immature ciricote fruit seed. This study also evaluated the UPLC-ESI-PDA-MS phenolic profile, and the impact of the drying method on vitamin C content, amino acid content and the proximate composition of the ciricote flour.

2 Methods and materials

2.1 Fruits material and chemicals

The fruit of ciricote (approximately 4 kg), were collected at the spring in June 2021 and classified as immature stage according to Jiménez-Morales *et al*. (2022). The collection was obtained in Mérida City in the state of Yucatán, Mexico, at the coordinates MX 21º 01´52´´ N 89º 37´40´´ W, with a temperature

of 34 °C, humidity of 72 %, and rainfall of 99 mm. The fruits were bagged in tagged rafia sacks and immediately brought to the CIATEJ Southeast Unit. The preparation and storage were performed according to Jiménez-Morales *et al*. (2022). Briefly the fruits were separated from those that were deteriorated, washed and disinfected with an iodine solution (2 ppm), for 1 min. The peel was obtained using a potato peeler, the pulp was cut with a knife into uniform size segments and the seed was manually separated from the hard endocarp using a hammer. All parts of the fruit were individually and immediately processed as fresh or frozen at -20 ºC according to the subsequent pretreatment or drying process. The abbreviations of the parts of the fruits were named as: peel or exocarp (PE), pulp or mesocarp (PU), and seed (SE). The chemicals used in the experimentation were: Trolox ($C_{14}H_{18}O_4$), sodium nitrite ($NaNO_2$), trichloroalumane ($AlCl_3$), dipotassium;sulfonatooxy sulfate ($K_2S_2O_8$), the radicals DPPH ($C_{18}H_{13}N_5O_6$) and ABTS ($C_{18}H_{24}N_6O_6S_4$), acetonitrile chromatographic grade, formic acid; rosmarinic, trans-cinnamic, cinnamic, chlorogenic, gallic, ferulic and caffeic acids, quercetin, catechin, rutin, kaempferol, epicatechin and L-Ascorbic acid (USP) analytical standards, were purchased from SIGMA-Aldrich, St. Louis, MO, USA. The ascorbic acid food grade (Cosmos, CDMX, CX, MX), petroleum ether (Fermont, Monterrey, NL, MX) and hydrochloric acid (HCl), sodium hydroxide (NaOH), ethanol (C_2H_6O), sodium carbonate (Na_2CO_3), methanol (CH_4O) were purchased from Avantor JT Baker, Radnor, PA, USA. Distilled and ultrapure water were used in all experiments.

2.2 *Obtention of functional flours from ciricote fruit*

Prior to the drying process and after peelding and cutting, the parts of the fruit (peels, pulps or seeds) were immediately pretreated according to Anyasi *et al*. (2018) with adaptations. In a separate manner, the parts were immersed in a tray containing a 1 % ascorbic acid solution for 10 min, then they were drained with a strainer for 2 min. PU, PE and SE without pretreatment were used as controls. After the ascorbic acid solution was drained, samples and controls were immediately placed in polyethylene bags, frozen, and dried for 78 h by FD using a Free Zone equipment (Labcono Freezone 6, Kansas City, MO, US) (Jiménez-Morales *et al*., 2022) or were directly placed in steel trays and dried for 24 h by CD using a digital convection incubator (Manufacturers Feligneo, S.A. de C.V. Felisa®, JC, MX) at 50 °C, until the moisture content was below 10 % (Patrón-Vázquez *et al*., 2019). A factorial 2^2 design was performed to evaluate the effect of the drying process and pretreatment on the TPC, TF, and antioxidant activity in the extracts of PU, PE, and SE ciricote flours (separate). The categorical factors A: Drying method, were defined as Freezing-drying (FD) and Convective drying (CD), and factor B: Pretreatment, was defined as with pretreatment of ascorbic acid (WT) and without pretreatment of ascorbic acid (WOT) (Table S1, supplementary material). To discard any antioxidant activity due to ascorbic acid in the pretreated samples after the drying process, the residual content of ascorbic acid was obtained by subtracting the content of the samples without pretreatment from the content of the samples with pretreatment, using the chromatographic method of Vitamin C content. Three replicates were performed for all treatments.

2.2.1 *Obtention of the flours*

The ciricote fruit flours were obtained by milling and sieving (to achieve a particle size < 500 μm) the dried PU, PE, and SE, using a coffee mill (Hamilton Beach 80350R, Wareham, MA, US) and a sieve No.35 ATSEM-E11. The flours were then stored at -20 °C for less than one day prior to the analysis.

2.2.2 *Obtention of extracts from ciricote flours*

The phenolic extracts of flours from PE, PE and SE were processed according to Jiménez-Morales *et al*. (2022) and Herrera-Pool *et al*. (2021). Briefly 200 mg of flour (samples and controls) were immersed in 50 % ethanol /water (50 % EtOH) (10 mL). Then, they were processed by ultrasound extraction for 10 min, at 20 kHz, 130 W, and amplitude 80 %. The samples were then centrifuged, and the supernatants were collected by filtration. They were adjusted with 50 % EtOH to the initial volume and analyzed for antioxidant activity, phenolic profile, TPC and TF. Triplicate determinations were made.

The TPC of the extracts and their antioxidant activity were determined using the Folin Ciocalteu and DPPH methods, as reported by Covarrubias-Cárdenas *et al*. (2018), and the antioxidant activity was also determined by the ABTS method as reported by Alonso-Carrillo *et al*. (2017). The TF was determined according to Al *et al*. (2009). For the TPC assay, 20 μL of extract was added to Folin reagent (250 μL) and allowed to react for 8 min. The mixture was then incubated for 30 min and read at 760 nm, after the addition of 7.5 % Na_2CO_3 (1250 μL) followed by water (480 μL) (Covarrubias-Cárdenas *et al*., 2018). For the TF assay, the sample (1 ml) or standard was mixed with 5 % $NaNO_2$ (0.300 mL) followed by the addition of 10 % $AlCl_3$ (0.300 mL) 6 min later, and 1 M NaOH (2 mL) 5 min after that. The mixture was then diluted 10 mL with water and measured at 510 nm. For the DPPH assay, 50 μL of flour extract or

standard was added to 3950 μL of DPPH, shaken, and incubated in darkness for 40 min, before being measured at 515 nm. For the ABTS assay, to 1000 μL of ABTS solution, 10 μL of extract or standard was added and mixed. After 6 min, the mixture was read at 734 nm. The TPC, TF and antioxidant activity (DPPH and ABTS) were calculated using calibration curves for: Gallic acid (GA) (50 to 800 ppm), rutin (RE) (5 to 800 ppm), and Trolox (50 to 400 ppm), and expressed as mg of GA, RE and μmol of Trolox equivalents (TE) per g of dry weight (dw) respectively.

2.2.3 *Chromatographic and mass spectrometry analysis of phenolic compounds*

The chromatographic analysis of phenolic compounds in ciricote flour extracts was performed using an Ultra Pressure Liquid Chromatograph (UPLC). The elution conditions during the chromatographic separation were based on Covarrubias-Cárdenas *et al*. (2018). The DAD was set at 350 nm for analyte detection and the curves of rutin, rosmarinic acid and caffeic acid (analytical standards) were used for quantification of phenolic compounds, reported as μmol g^{-1} dw (Jiménez-Morales *et al*., 2022). The mass spectrometry (MS) analysis was carried out according to Herrera-Pool *et al*. (2021) using a mass spectrometer (Waters XeVo TQ-S micro, MA, US). The analysis was conducted at collision energy from 10 to 150 eV in negative ion mode, with settings according to Jiménez-Morales *et al*. (2022).

2.2.4 *Content of vitamin C*

The vitamin C content in flours from the pulp, peel and seed of immature ciricote fruit was determined according to Cotruţ and Bdulescu (2016) with slight modifications. The UPLC system was used with a column as reported in the chromatographic analysis of phenolic compounds. Temperature of 30 °C, an isocratic flow of 0.45 mL min^{-1}, an injection volume of 2 μL, and an elution time of 5 min were used. An acetonitrile mobile phase [MeCN / Water (3:97 v/v), 0.09 % trifluoroacetic acid (TFA)] was also used. The PDA was set at 245 nm. The content of vitamin C was quantified in milligrams of vitamin C / per gram of dry weight (dw), using a calibration curve from 40 to 200 ppm of ascorbic acid standard United States Pharmacopeia (USP).

2.3 *Chemical composition of ciricote fruit flours*

2.3.1 *Proximate analysis*

The chemical composition analysis of the flours (obtained from PU, PE, and SE) was determined according to the Official Methods of Analysis AOAC (Horwitz and Latimer, 2005): 923.03 (ash), 925.09 (moisture), 962.09 (crude fiber), 978.04 (nitrogen), 920.39 (crude fat). The protein content was obtained by multiplying the nitrogen by the factor 6.25. The nitrogen free extract was obtained by mathematical calculation from the other components and expressed as the carbohydrate content.

2.3.2 *Soluble amino acids*

Flour samples (PU, PE or SE) (50 mg) were added and mixed with 10 mL of 0.05 M borate buffer adjusted to pH 8.05 and sonicated for 60 min in an ultrasonic cleaner (Branson 3510r-MT, Danbury, CT, US). The samples were then centrifuged at 4000 rpm for 15 min at 4 °C and filtered for injection. The Amino Acid Standard H, protein hydrolysate standard (Themo Scientific, Rockford, IL, USA) was used. Calibration curves from 12.5 to 500 pmol μL^{-1} of each amino acid standard, except for Cys (from 6.25 to 250 pmol μL^{-1}), were performed. Subsequently, standard solutions and samples were derivatized according to the "UPLC® Amino Acid Analysis Solution, System Guide" 71500129702/Revision B, using the AccQ · Tag^{TM} ultrachemistry kit (Waters, Milford, MA, USA). The chromatographic analysis was performed using the same UPLC System cited above in the chromatographic analysis of phenolic compounds, using a C_{18} column at 60 °C and according to the Guide " 71500129702/Revision B. The analytical response was registered at 260 nm. The results were expressed as μmol amino acid per gram or dried weight (μmol g^{-1}dw).

2.4 *Statistical analysis*

To analyze the factorial design a multifactorial analysis of variance (ANOVA) was performed to determine the effects of the factors ($p < 0.05$) using the Statgraphics software (Ver 16.1.03., Centurion XVI StatPoint Tech. Inc., Warrenton, VA, USA). The results were presented as the mean plus/minus the standard deviation (SD). They were followed by the Tukey test to compare the differences between treatments.

3 Results and discussion

3.1 *Effect of drying method and pretreatment on antioxidant activity, TPC and TF*

In Table 1 the results of the effect of pretreatment with ascorbic acid and the drying method (freeze drying (FD) or convective drying (CD)) on the total phenolic compounds, total flavonoids (TPC, TF), and

antioxidant activity of extracts of flours from peel, pulp, and seed (PE, PU and SE) are presented. The analysis of the experimental design showed that the drying method by CD or FD did not show a significant effect ($p > 0.05$) on the TPC and antioxidant activity (by DPPH and ABTS) of the extracts of flours. However, the application of the pretreatment with ascorbic acid (WT) significantly favored ($p < 0.05$) an increase in the TPC and antioxidant activity (by DPPH ad ABTS) in freeze-dried (FD) pulps and TPC, TF and antioxidant activity in peels dried by both methods (CD and FD) compared to those without pretreatment (WOT). This increase is attributed to the antioxidant properties on the phenolic compounds presented in the sample and not by thje acid ascorbic itself, considering that no residual of added ascorbic acid was detected in the dried pretreated samples (WT). With respect to the seed, the drying method and pretreatment affected the TPC and antioxidant activity ($p < 0.05$). The TPC was higher when the seed was dried by CD than FD. However, the antioxidant activity showed different responses according to the antioxidant activity methodology. This could be attributed to the principle of measurement of the assays and the properties of the bioactive compounds (such as chemical structure, polarity or antioxidant power) contained in the extract that react in a different way toward the synthetic radicals ABTS or DPPH considering that the extract is a mix of compounds that can act individually or in synergistic form. Since the ABTS method is capable of detecting both hydrophilic and lipophilic antioxidants, the DPPH assay is inclined to detect more lipophilic antioxidants (Cömert and Gökmen, 2018). Therefore, a high positive correlation was found between the main hydrophilic phenolic compounds such as caffeic or rosmarinic acids and the DPPH and ABTS responses (Pearson´s correlation coefficient, r = 0.977-0.999). However, a lower correlation was found between hydrophilic compounds such as rutin and more lipophilic compounds (such as nepetoidin A and B) and the DPPH (r = 0.517 - 0.810), and an even lower correlation with ABTS (r = 0.292 - 0.701), which could explain a lower response with the ABTS assay.

According to the main effects analysis, the TPC and antioxidant activity were not affected by the drying method (CD or FD), which indicates that the response variables are indistinct concerning the drying method but were affected by the pretreatment with ascorbic acid. This showed a protective effect on the phenolic compounds, mainly in FD pulps and FD and CD peels, all pretreated (WT), compared to those without pretreatment (WOT). The results of the drying method effect were different from those reported by Sęczyk *et al*. (2022) with Basil (*Ocimum basilicum* L.), where the antioxidant activity and TPC were higher in the FD material than CD, unlike those for ciricote. The difference with our results could be associated with the temperature and drying period used in that study (40 °C and 48 h), which favored oxidative enzymatic activity compared to our CD conditions (50°C and 24 h). However, our results agree with the reports for fruits such as ginger, pomegranate, mango and strawberry (Onwude *et al*., 2022).

Regarding the effect of pretreating ciricote fruit with ascorbic acid on the TPC, TF, and antioxidant activity (Table 1), our results are consistent with a study on mangoes, treated by CD or infrared drying, where the application of ascorbic acid prevented the degradation of TPC by delaying PPO activity (Yao *et al*., 2020). The positive results of pretreatment with ascorbic acid could be attributed to its natural properties. Ascorbic acid is a natural agent that can reverse the oxidation of polyphenols, temporarily preventing their oxidation (Jang and Moon, 2011; Landi *et al*., 2013) and consequently the reduction of antioxidant activity in the ciricote flour extracts. Ascorbic acid acts through different mechanisms, such as the reduction of oxidized compounds resulting from PPO or POD action, enzymes with high activity in fresh fruits or vegetables that act instantly during physical stress, e.g. cutting, when the cells are destroyed and the enzymes and substrate come into contact (Derardja *et al*., 2019; Jang and Moon, 2011). Ascorbic acid act through competitive inhibition for the catalytic site of the PPO, or also by reducing the medium pH, which affects the functionality of the enzyme.

Table 1. Effect of pretreatment with ascorbic acid and drying process on TPC, TF, and antioxidant activity in extracts of flours from pulp and peel of ciricote fruit.

		WT	WOT
TPC (mg GAE g^{-1} dw)			
Pulp	CD	49.02 ± 3.57^{a}	47.91 ± 0.97^{ba}
	FD	51.75 ± 0.95^{a}	41.58 ± 2.92^{b}
Peel	CD	58.50 ± 6.44^{a}	45.84 ± 1.11^{b}
	FD	62.32 ± 1.53^{a}	45.60 ± 3.67^{b}
Seed	CD	4.23 ± 0.24^{a}	4.43 ± 0.47^{a}
	FD	1.07 ± 0.28^{a}	1.16 ± 0.13^{a}

TF (mg RE g^{-1} dw)			
Pulp	CD	114.75 ± 11.47^{b}	122.68 ± 2.15^{ba}
	FD	135.66 ± 7.64	129.79 ± 3.29^{ba}
Peel	CD	156.37 ± 0.54^{a}	90.26 ± 5.40^{c}
	FD	153.35 ± 2.72^{a}	128.61 ± 12.06^{b}
Seed	CD	3.15 ± 1.13	NQ
	FD	NQ	NQ
DPPH (μM ET g^{-1} dw)			
Pulp	CD	427.20 ± 34.75^{a}	408.53 ± 15.25^{ba}
	FD	434.26 ± 5.73^{a}	366.14 ± 17.63^{b}
Peel	CD	479.23 ± 17.76^{a}	383.06 ± 12.38^{b}
	FD	511.42 ± 9.22^{a}	384.32 ± 22.11^{b}
Seed	CD	10.82 ± 0.79^{a}	1.93 ± 0.74^{b}
	FD	2.31 ± 0.23^{a}	1.89 ± 0.67^{a}
ABTS (μM ET g^{-1} dw)			
Pulp	CD	292.50 ± 15.12^{a}	276.89 ± 17.93^{a}
	FD	290.66 ± 6.40^{a}	214.51 ± 19.70^{b}
Peel	CD	336.47 ± 14.78^{a}	214.73 ± 4.77^{b}
	FD	376.92 ± 10.30^{a}	234.84 ± 24.73^{b}
Seed	CD	18.82 ± 1.25^{a}	9.66 ± 2.00^{b}
	FD	23.23 ± 0.41^{b}	31.80 ± 1.40^{a}

Different letters (a, b) for each group (pulp, peel, or seed) in same row, and for each response variable (TPC, TF, DPPH, ABTS) mean difference ($p < 0.05$). WT: With pretreatment of ascorbic acid; WOT: Without pretreatment of ascorbic acid. CD: Convective drying; FD: Freeze-drying.

The enzymes PPO or POD are responsible for browning reactions, degradative changes, oxidation, and loss of phenolic compounds. However, these effects can also result from a reduction-oxidation imbalance due to overproduction of reactive oxygen species (ROS) and loss of antioxidant capacity, or lipid peroxidation of the membranes (Zhang *et al*., 2015). These phenomena occur during process such as cutting, drying, freezing, or in the presence of oxygen (Jang and Moon, 2011; Queiroz *et al*., 2011; Landi *et al*., 2013; Tan *et al*., 2020a). During the freezing process, the food is partially frozen, cell rupture occurs, and a series of degradative changes can oxidize the phenolic compounds, limiting the quality of the plant material. Tropical fruits and vegetables are the most sensitive to cold damage (Amit *et al*., 2017). Therefore, the protective effect of the pretreatment (WT) on TPC and/or TF and antioxidant activity in FD pulps and peels compared to those without treatment (WOT) could be attributed to its action during the freezing process.

Both convective drying (CD) and freeze drying (FD) methods promote the loss of soluble phenolic compounds, primarily through oxidative reactions (Yao *et al*., 2020). However, in the ciricote pulps, the effect of the pretreatment was observed only in FD pulps, which suggests a protection of phenolics by the ascorbic acid during the damage that can occur in the freezing process, unlike in CD, where freezing is not required. Sęczyk *et al*. (2022) mention that plant material processed by CD could generate stable plant material and be less enzymatically active due to induction of biochemical degradation enzymes at temperatures up to 40°C, compared to FD, where higher enzymatic activity is maintained. The reason why TPC, TF, and antioxidant activity were not significantly different in CD pulps with and without pretreatment is likely due to the temperature used in our study of 50 °C, which could generate less enzymatic activity without damaging to phenolic substances.

The drying process by heat such as the CD, could cause the loss of bioactive compounds and their antioxidant capacity, especially when the product starts reach its moisture in equilibrium when the degradation of bioactive compounds is faster due to the temperature as was observed in a study on the drying of red pepper at 60 or 70 °C. This could explain why in CD peels of ciricote the pretreatment with ascorbic acid (WT) had an effect with an increase in TPC, TF, and antioxidant activity compared to without pretreatment (WOT). This suggests that CD peels reached their moisture equilibrium first compared to CD pulps, making their bioactive compounds more prone to degradation than the pulps. CD peels exhibited less moisture content than CD pulps (8.54 ± 0.07 and 9.87 ± 0.93 %, respectively) at the same final drying process time.

The TPC, TF and antioxidant activity in FD peel WT were also favored. However, in the case of the seed (SE) the contents of TPC and antioxidant activity (by DPPH and ABTS) were lower than those for

PU and PE. The above could be attributed to the low presence of phenolic compounds in the ciricote flour extract, and no relevant findings were observed in these treatments. Therefore, in CD and FD peel, the tissue of the part of the fruit (peel) could also explain by itself the susceptibility to oxidation or loss of phenolic compounds concerning pulps and consequently, the positive effect of the pretreatment with ascorbic acid on the response variables. Freeze-drying is a prolonged dehydration process and is expensive compared to convective drying, which requires less time. This time difference is a decisive factor in selecting a drying method such as CD or FD. SęSęczyk *et al*. (2022) reported that changes in food matrix properties seem to be responsible for the results of applying different drying methods, such as FD or CD. Therefore, the combination of convective drying (CD) and pretreatment with ascorbic acid (WT) was appropriate for the peel of ciricote, as the TPC, TF, and antioxidant activity were higher than CD, WOT, and were not statistically different ($p < 0.05$) from those of FD, WT peels. However, convective drying with WT or WOT would be suitable for the pulp of ciricote fruit, as TPC and antioxidant activity were not different from FD pulps with WT or WOT ($p > 0.05$). This is due to the fact that freeze-drying is a very slow dehydration process and is expensive compared to convective drying, which requires less time (Onwude *et al*., 2022; Tan *et al*., 2020).

3.2 Chromatographic and mass spectrometry analysis of phenolic compounds

The phenolic compounds analyzed by UPLC-DAD-ESI-MS in the extracts of flours (PU, PE) obtained by CD, WT, or WOT (selected as the best treatments) are shown in Table 2, and the typical chromatograms of PU, PE, and SE are shown in the Fig. 1. In table 2, compound [1] was identified as caffeic acid, compound [2] as rutin and compound [3] as rosmarinic acid (Jiménez-Morales *et al*., 2022). The compound [4] (Rt: 23,05 min; λ_{max}: 249, 337 nm) and compound [5] (Rt: 23,17 min; λ_{max}: 249, 337 nm) were tentatively identified as the isomers nepetoidin A and nepetoindin B, corresponding to two caffeic acid esters (Z,E)-[2-(3-(3,5-dihydroxyphenyl)ethenyl] 3-(3,4-dihydroxyphenyl)-2-propenoate and (Z,E)-[2-(3,4-dihydroxyphenyl)-ethenyl] 3-(3,4-dihydroxyphenyl)-2-propenoate (Aničć *et al*., 2021; Grayer *et al*., 2003; Nakanishi *et al*., 1990). In Table 2, it was observed that the content of caffeic acid did not vary significantly in CD pulps and peels whether WT or WOT. In our previous investigation, it was determined that rutin is found mainly in the peel (Jiménez-Morales *et al*., 2022), which is why it was not quantified in the pulp. Its content in CD or FD peel whether WT or WOT did not vary ($p > 0.05$). The rosmarinic acid in CD

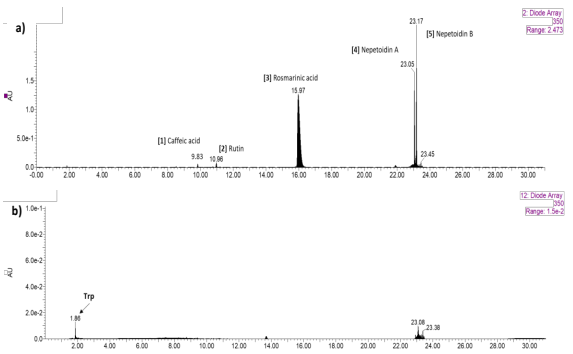

Figure 1. Chromatogram of phenolic components in flours extracts from a) Pulp and Peel, b) Seed of ciricote flour (*Cordia dodecandra* A. DC) obtained at 350 nm.

pulp WT was higher than WOT ($p < 0.05$), and in the CD peel, it did not vary WT compared to WOT. The contents of the isomers nepetoidin A and B were higher in the CD pulp and peel WT than in those WOT. Regarding the ciricote seed flour extract, the UPLC-PDA-ESI-MS profile revealed no significant presence of phenolic compounds (Fig. 1). Instead, the major component tentatively identified was the essential amino acid tryptophan (Rt: 8.39 min; λ_{max}: 217, 278 nm) (Lesniak *et al*., 2013).

In this work two new compounds, tentatively identified as nepetoidin A and B were found in the ciricote fruit compared to our previous work with *Cordia dodecandra* A. DC. This result was attributed to the batch being harvested at a different date and under different climatic conditions, which can lead to variations in the availability and concentration of beneficial phytochemicals present in the fruits (Anyasi *et al*., 2018). The rest of the factors such as location, maturity and extraction conditions were maintained. The compounds nepetoidin A and B, have been found in species of the subfamily *Nepetoideae*, considered a specialized group of the *Lavandula* family and recognized for their relevant antioxidant properties, even more significant than caffeic or rosmarinic acid (Grayer *et al*., 2003). It has been reported that these compounds are sometimes not detected in vegetal material because they do not resist drying (Héral *et al*., 2021). However, the relevance of this work was the finding that the pretreatment with ascorbic acid during the drying process significantly ($p < 0.05$) favored the content of these compounds in the ciricote fruit, more than other phenolic compounds present in the fruit (Table 2). The pretreatment with ascorbic acid prevented the loss or oxidation, mainly of rosmarinic acid in the pulp, and nepetoidin isomers in the pulp and peel, which suggested that these compounds are more susceptible to degradation or oxidation during CD. This behavior was consistent with those reported for banana varieties pretreated with 1.5 % ascorbic acid and dried by CD, whose content of some phenolic

Table 2. Phenolic compound by UPLC-PDA-ESI-MS.in flour extract from pulp and peel of ciricote.

CN	tr	PDA UV (λ_{max})	[M-H]$^-$	(m/z)	Identification		Quantification (μmol g^{-1} dw) Convective drying (CD)	
							WT	WOT
1	9.83	217 323	179	135	Caffeic acid	PU	3.92 ± 0.48^b	4.21 ± 0.20^{ab}
						PE	3.87 ± 0.20^a	4.88 ± 0.27^a
2	10.96	209	609	300	Rutin	PU	NQ	NQ
		255		301			2.77 ± 0.16^a	2.99 ± 0.25^a
		353		271		PE		
				254				
				243				
3	15.97	219	359	197	Rosmarinic	PU	57.22 ± 2.09^a	44.36 ± 0.22^b
		318		179	acid	PE	48.90 ± 2.03^a	42.87 ± 4.55^a
				161				
				132				
				135				
4	23.05	249	313	161	Nepetoidin A*	PU	3.93 ± 0.02^a	1.67 ± 0.06^b
		337		133		PE	13.27 ± 1.27^a	2.45 ± 0.21^b
5	23.17	249	313	161	Nepetoidin B*	PU	33.02 ± 2.14^a	23.85 ± 0.16^b
		337		133		PE	71.86 ± 3.29^a	49.58 ± 3.25^b

CN: compound number; tr: retention time; *TI: tentative identification; NQ: not quantifiable. PU: Pulp, PE: Peel; [M-H]$^-$: ion molecular in negative mode, m/z: mass/charge. Different letters (a, b) mean difference ($p < 0.05$) in same row.

compounds or some that had not been detected in the variety of fruit were slightly increased after the pretreatment was applied (Anyasi *et al*., 2018). Our results also agreed with those reported in a study with apple juice, where the loss of content of phenolic compounds (flavonoids and phenolic acids) was lower when incubated with ascorbic acid pretreatment (Li *et al*., 2015).

3.3 Analysis of content of vitamin C

The content of vitamin C in flours from the PU, PE, and SE of ciricote is presented in Figure 2. The drying method had a slight influence on the contents of vitamin C in PU and SE. The content of vitamin C content in CD pulp and seed flours was slightly higher than in FD pulp and seed flours ($p < 0.05$) but the CD and FD peel flours did not show a significant difference. These results differed from a study with tomatoes dried by CD at 60 °C reported by Gümüşay *et al*. (2015), where the content of vitamin C decreased compared to FD. The difference could be explained by the effect of the high temperature used in that study compared to our study (50 °C). Ascorbic acid is sensitive to high temperatures, which can decrease its content (Castañeda-Pérez *et al*., 2013; Gümüşay *et al*., 2015).

The content of vitamin C was higher in the pulp and peel than the in seed ($p < 0.05$). The content of vitamin C in ciricote flours was even higher than the content in some red fruits such as fresh blueberry (42.45 mg / 100 g dw), dried blueberry (13.30 mg / 100 g dw), and strawberry (42.45 mg / 100 g dw), which are considered good sources of this compound, as well

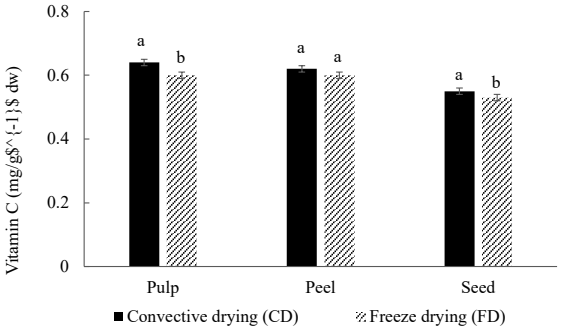

Figure 2 Content of vitamin C in flours from pulp, peel, and seed of ciricote fruit. Different letter for each part of fruit, means significant difference ($p < 0.05$). ■ Convective drying (CD). ■ Freeze Drying (FD).

as other fruits such as plum, nectarine or peach (from 3 to 14 mg / 100 g dw) (Cardoso *et al*., 2011; Gil *et al*., 2002; Zia and Alibas, 2021). The results were similar to some lettuce varieties, which had vitamin C content ranging from 52. 27 to 60.35 mg / 100 g dw (Medina-Lozano *et al*., 2021). The presence of vitamin C in ciricote flours after the drying process makes them a valuable source of this bioactive compound. Vitamin C is considered a quality index and an indicator of the nutritional value of final products, as it is sensitive to light, temperature and oxygen conditions (Zia and Alibas, 2021).

The above suggests that ciricote fruit flours retain essential nutrients as such as vitamin C, even after drying conditions, which could be associated with its genetic composition, drying conditions and food type (Onwude *et al*., 2022). Therefore, ciricote flour has become, a valuable raw material for the development

of functional food. For example, the human need for vitamin C is 10-75 mg daily (for adults, adolescents or in a condition of pregnancy or lactation) (Secretaría de Economía/Secretaría de Salud (2010)). Therefore 100 g or less of ciricote flour could be helpful in developing products to meet that intake.

3.4 Chemical composition of flours of ciricote

3.4.1 Proximate analysis

In Table 3 is presented the proximate composition of flours from the peel, pulp, and the seed of ciricote fruit. The protein content in the seed was significantly higher than the pulp and peel. The protein content in pulp and peel was higher than those reported for African tropical fruits such as the desert date (*Balanites aegyptiaca*), the fruit of the Eben tree (*Dacryodes edulis*) and the fruit of the Pacific (*Boerhavia diffusa*) with a protein content from 4.6 to 8.3 (Latham, 2002). The protein content was also close to the values for cereals (ranging from 6 to 12 %). The highest protein content exhibited by the ciricote seed was similar to the contents for cotton seeds (*Gossypium* spp.), desert date tree fruit seed (*Balanites aegyptiaca*), melon seed (*Cucumis melo*), and African baobab fruit seed (*Adansonia digitata*), which range from 20 to 30% protein (Latham, 2002). Even higher than the protein content of pistachios, almonds, and peanuts which have values ranging from 21.43 to 24.35 % (Bonku and Yu, 2020). Flours from the PE, PU and SE of the ciricote fruit represented a good source of protein. The pulp and the peel were low in fat; however, the seed was richer in this nutrient. The fat values for SE were within those reported for walnuts and almonds, with contents around 53.57 to 65.21 % (Bonku and Yu, 2020).

Table 3. Proximal composition and amino acid profile of flours from pulp (PU), peel (PE), and seed (SE) of ciricote fruit.

	PU	PE	SE
	Proximal composition (%)		
Protein	11.27 ± 0.06^{b} 1	2.65 ± 0.18^{b}	29.45 ± 0.95^{a}
Crude fat	2.04 ± 0.09^{b}	2.89 ± 0.04^{b}	53.75 ± 0.75^{a}
Ash	8.34 ± 0.13^{a}	7.47 ± 0.31^{a}	4.50 ± 0.57^{b}
Crude fiber	12.45 ± 0.23^{b}	14.26 ± 0.14^{b}	10.15 ± 0.40^{a}
FNE	65.89 ± 0.06	62.72 ± 0.22	2.78 ± 0.14
	Aminoacid profile (μmol g^{-1} dw)		
NH_3	1.16 ± 1.05^{a}	2.44 ± 0.00^{a}	0.42 ± 0.32^{a}
Asparagine (Asn)	ND	ND	1.19 ± 0.49
Glutamine (Gln)	ND	ND	ND
Histidine (His)	ND	ND	ND
Glycine (Gly)	ND	ND	*
Serine (Ser)	1.46 ± 0.01^{b}	1.24 ± 0.00^{c}	1.64 ± 0.05^{a}
Arginine (Arg)	ND	ND	4.79 ± 0.13*
Glutamic Acid (Glu)	0.42 ± 0.01^{c}	0.49 ± 0.01^{b}	3.25 ± 0.02^{a}
Aspartic Acid (Asp)	0.87 ± 0.01^{b}	0.59 ± 0.20^{b}	3.17 ± 0.13^{a}
Threonine (Thr)	0.65 ± 0.01^{b}	0.55 ± 0.04^{b}	1.36 ± 0.11^{a}
Alanine (Ala)	1.54 ± 0.00^{a}	1.42 ± 0.00^{a}	1.67 ± 0.07^{b}
Gaba	1.96 ± 0.01^{a}	1.68 ± 0.01^{b}	1.35 ± 0.06^{c}
Proline (Pro)	0.69 ± 0.11^{b}	0.56 ± 0.03^{b}	1.47 ± 0.21^{a}
Cysteine (Cys)	14.58 ± 0.18^{a}	14.06 ± 0.18^{a}	11.19 ± 0.12^{b}
Lysine (Lys)	ND	ND	2.29 ± 0.25
Tyrosine (Tyr)	ND	ND	1.24 ± 0.28
Methionine (Met)	0.73 ± 0.32^{a}	0.71 ± 0.13^{a}	0.90 ± 0.22^{a}
Valine (Val)	0.82 ± 0.09^{b}	0.65 ± 0.08^{b}	2.11 ± 0.38^{a}
Isoleucine (Ile)	1.85 ± 0.00^{b}	1.09 ± 0.00^{c}	2.12 ± 0.00^{a}
Leucine (Leu)	ND	ND	2.05 ± 0.94
Phenylalanine (Phe)	1.73 ± 0.00^{a}	0.98 ± 0.00^{b}	1.87 ± 0.11^{a}
Tryptophan (Trp)	ND	ND	1.16 ± 0.10

PU: Pulp, PE: Peel, SE: Seed, FNE: Free nitrogen extract Different superscript letter in the same row means significant difference ($p < 0.05$). * (Arginine + Glycine)

The ash content was higher in the PE and PU than in the SE and the crude fiber was higher in the SE, since its main component was fat. The ciricote fruit can be considered a food low in fat, with high protein content that could be used as a whole fruit or as functional ingredients for the development of products (Latham, 2002; Onwude *et al*., 2022; Secretaría de Economía / Secretaría de Salud, 2010).

3.4.2 Soluble amino acids analysis

The results of the soluble amino acid profile of the flours from PE, PU and SE of ciricote are shown in Table 3. Essential amino acids such as Phe, Ile and non-essential amino acids such as Cys, Ala were identified as the majority in PU and PE flours. The seed was richer in essential and non-essential amino acids such as Asp, Glu and Arg, and amino acids not found in PU and PE such as Leu, Tyr, Lys, Arg, Asn were present. The amino acids are not only basic components of proteins but are also important in the fruits due to their flavor, as they can be tasteless, bitter or sweet (Iordnescu *et al*., 2015). The relevant presence of bitter amino acids such as Phe, tasteless amino acids such as Ile and less sweet amino acids such as Pro or Met, could be associated with the characteristic flavor of ciricote fruit (CONABIO, 2020), considered a factor that impacts its abandonment and consumption (Martínez-Castilla, 2013). The results agreed with species such as *Cordia evolutior* fruit where Phe and Ile were among the primary amino acids (Arunachalam and Parimelazhagan, 2014). The PE and PU of ciricote fruit presented high contents of Cys, similar to such as fruits as banana, Nita tree fruit (*Parkia* spp.) and the Asian persimmon kaki fruit (*Dispyros kaki*), which are rich in this amino acid (FAO, 1981). The consumption of edible and non-edible parts (PU, PE, and SE) could be useful as raw material or functional ingredients in food development due to the relevant properties of amino acids such as antioxidant (Cys), growth factor (Lys) and essential amino acid for children (Arg) (Bourdon *et al*., 2005; Elias *et al*., 2008).

Conclusions

Convective drying proved to be a viable method for the drying of ciricote fruit and obtention of functional flours with preservation of essential phenolic compounds and antioxidant activity. The factorial design study did not show an effect of the drying method on the response variables total flavonoids (TF) or total phenolic content (TPC) and antioxidant activity. This is noteworthy considering that freeze-drying is a prolonged and expensive dehydration process compared to a conventional drying process. Combining the convective drying process with the pretreatment of ascorbic acid resulted in benefits for extracting flours from the peel and pulp of ciricote as the phenolic compounds and the bioactivity was higher than those without the pretreatment. The main phenolic compounds preserved of loss or oxidation with the pretreatment of ascorbic acid in PE and PU flours included the rosmarinic acid, nepetoidin A, and B. The ciricote flour (PU, PE and SE) also preserved an excellent vitamin C content after the dying processes by CD or FD. The flours (PU, PE, and SE) represented a good source of protein, low in fat, and relevant essential and non-essential amino acids such as Cys, Ile, Arg, Lys, Glu, Asp beneficial for adults or children. The present work demonstrated that it was possible to obtain, in a viable way, vegetable flours materials with preservation of its compounds and bioactivity using a native subtilized product such as the ciricote fruit with many promising applications.

Authorship and acknowledgements

Karina Jiménez-Morales: Investigation; methodology; acquisition of data; analysis and interpretation; writing-original draft. Emanuel Herrera-Pool: Methodology; acquisition of data; analysis and interpretation. Teresa Ayora-Talavera: Writing-review and editing; funding acquisition; approval of the final version. Juan Carlos Cuevas-Bernardino: Conceptualization; writing-review, approval of the final version. Ulises García-Cruz: Writing-review, approval of the final version. Soledad Pech-Cohuo: Writing-review, approval of the final version final. Douglas David Crockett: English writing-review. Neith Pacheco: Conceptualization; analysis and interpretation, writing- original draft, supervision, validation, funding acquisition. Authors also acknowledge to CONACYT for the scholarship 703763 for Karina Jiménez-Morales.

Nomenclature

CD	Convective drying
DAD	Diode array detector
dw	Dry weight
ESI	Electrospray ionization
EtOH	Ethanol
FD	Freeze drying
GAE	Gallic acid equivalents
MS	Mass spectrometry
NA	Not available
NC	Number of components
NQ	Not quantifiable
PDA	Photodiode array
PE	Peel
PU	Pulp

RE	Rutin equivalents
SE	Seed
TE	Trolox equivalents
TF	Total flavonoid
TI	Tentative identification
TPC	Total phenolic content
tr	Time retention
UAE	Ultrasound-Assisted Extraction
UPLC	Ultra-high performance liquid chromatography
WOT	Without pretreatment of ascorbic acid
WT	With pretreatment of ascorbic acid

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Appendix

Experimental design for the evaluation of the effect of drying method and ascorbic acid pretreatment on the TPC, TF and antioxidant activity of ciricote fruit flours (for pulp, peel, or seed) extracts.

Treatment	Factor A: Drying process	Factor B: Pretreatment	
			Response variables
1	CD	WT	Y1: Total phenolic content (mg GAE g^{-1} dw)
2	CD	WOT	Y2: Total flavonoids content (mg RE g^{-1} dw)
3	FD	WT	Y3 Antioxidant activity by DPPH inhibition (μEq Trolox g^{-1} dw)
4	FD	WOT	Y4: Antioxidant activity by ABTS inhibition (μEq Trolox g^{-1} dw)

CD: Convective drying; FD: Freeze-drying; WT: With pretreatment of ascorbic acid; WOT: Without pretreatment of ascorbic acid.