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Proceeding Paper

Effect of the Type of Thermal Treatment on the Nutritional and Nutraceutical Characteristics of Pacaya Inflorescences (*Chamaedorea tepejilote* Liebm) †

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Abstract: *Chamaedorea tepejilote* Liebm is a palm native to the south of Mexico and Central America. In Mexico, the male inflorescences are roasted, fried, boiled, or accompanied by other ingredients to decrease their bitter aftertaste, and they can be consumed by the inhabitants. However, it has been observed that raw inflorescences have hypoglycemic, antitussive, and antimicrobial potentials, but the thermal treatment effect in these activities has not been studied; for this reason, this study evaluated the impact of three thermal treatments (hydrothermal (HP), steaming at elevated pressure (SEP), and microwave (MW)) on the nutritional and nutraceutical characteristics of Pacaya inflorescences; inflorescences without thermal treatment (WTT) were considered as the control. In the nutritional characterization, only the protein content was the fraction that increased significantly ($p < 0.05$) when thermal treatment was applied. On the other hand, all thermal treatments significantly modified ($p < 0.05$) the chlorophyll "a" content (HP reduced 0.59-fold; SEP and MW increased 0.07-0.25-fold), and chlorophyll "b" decreased. A significant ($p < 0.05$) carotenoids content increase in all thermally treated samples (between 0.80-fold and 8.73-fold) and total phenolic compounds (between 7.75-fold and 8.16-fold) compared to the WTT samples was observed. Microwave cooking was the only thermal treatment that significantly ($p < 0.05$) increased (0.97-fold) the antioxidant activity in the DPPH radical. HP (14.11%) and SEP (18.20%) significantly ($p < 0.05$) reduced the dipeptidyl peptidase-IV enzyme inhibition when compared to WTT (24.42%). These changes have been associated with the partial loss, destruction, or denaturalization of cell walls' proteins, lipids, or cellulose, causing the liberating or creation of compounds with nutritional and nutraceutical activity.

Keywords: Pacaya; thermal treatment; antioxidant activity; nutraceutical characteristics

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1. Introduction

Modifications in the functional characteristics of raw materials affect their application in food processing, quality, acceptance, and how they are used as ingredients in formulations of other foods. Thermal treatments on foods could significantly impact nutritional, nutraceutical, and functional properties, such as solubility, water, and oil absorption, gelation, the ability to create emulsions, and others; the main food components that modify these properties are polymers such as proteins or carbohydrates because thermal treatment may transform the composition, structure, conformation, and interaction with other food components such as lipids and polyphenols. In particular, thermal treatments may change carbohydrates' composition, structure, and functionality, mainly on polysaccharides such as pectins, mucilages, cellulose, hemicelluloses, and starch [1]; these modifications can be observed primarily in foods such as flowers, which are becoming increasingly popular because they represent a new source of nutraceutical foods that have proteins, carbohydrates, lipids, and carotenoids, among others. Recent research has reported that edible flowers may have antioxidant, anti-inflammatory, antibacterial, antifungal, and antiviral activity [2–4]. The *Chamaedorea tepejilote* Liebm palm grows in the south of Mexico, in Central America, and in northwest Colombia [5]. In some Mexico regions (Veracruz, Oaxaca, and Chiapas), their male inflorescence is consumed by indigenous communities; however, due to their bitter aftertaste, inflorescences are treated with different thermal treatments, being for instance, roasted, fried, boiled, or mixed with other ingredients such as eggs and tomato sauce [6]; in addition, the incorporation of Pacaya powder has been reported in frequently consumed foods such as Mexican tostadas, Mexican chorizo, and breakfast cereal [5,7]. Some authors have evaluated the in vivo hypoglycemic potential of raw inflorescences in normoglycemic rats [8] and the antitussive and antimicrobial activities related to their nutraceutical characteristics [9]. However, the Pacaya is an underutilized plant that has not been thoroughly studied; for this reason, this work aimed to identify some nutritional and nutraceutical modifications on the inflorescences of Pacaya through the effect of thermal treatment.

2. Materials and Methods

2.1. Vegetal Material

Male inflorescences from Tapachula, Chiapas, Mexico, were collected in February 2022. Opaque yellow inflorescences without mechanical or microbiological damage were selected; later, they were cut into cubes of approximately 1 cm and stored under vacuum in PVC bags in batches of 300 g. Batches were refrigerated for no more than 24 h before processing.

2.2. Thermal Treatments

Batches (300 g) in vacuum bags were thermally treated with established conditions previously reported by Hernández–Castillo et al. [10]: hydrothermal processing (90 °C in a water bath for 15 min), steaming at elevated pressure (121 °C and ~ 124 Pa for 15 min in a pressure cooker) and microwave cooking (1500 W and operating frequency of 2450 MHz for 15 min in a microwave oven). At the end of each thermal treatment, the samples were cooled, frozen, lyophilized, ground, sieved in mesh No. 40, and stored in airtight glass jars. Inflorescences without thermal treatment were considered a control.

2.3. Nutritional Characterization

The proximal analysis was carried out with methods proposed by the AOAC [11]: moisture (925.09), protein (N $\times$ 6.25, 955.04), lipids (920.39), and ash (923.03). The nitrogen-free compound's content was determined by difference. The results are expressed as a percentage for each component on a wet basis of lyophilized samples.

2.4. Nutraceutical Characterization

2.4.1. Total Phenolic Compounds (TPC)

The phenolic compounds were extracted using methanol 80% v/v for 30 min under stirring; extracts were later centrifugated at 5000 rpm for 5 min. The supernatants were stored at -20 °C and protected from light until used. The Folin–Ciocalteu method quantified total phenolic compounds [12] using gallic acid as the standard. Total phenolic compounds are expressed as micrograms equivalents of gallic acid per gram of sample (μg EGA/g).

2.4.2. Chlorophyll a and b Content and Total Carotenoids

To extract these components, 400 mg of Pacaya powder was dispersed in 20 mL of acetone 80% v/v and kept under constant stirring (150 rpm) for two hours, protected from light at 4 °C. Subsequently, the dispersion was centrifuged at 3000 rpm for 5 min. The content of carotenoids and chlorophyll was estimated spectrophotometrically according to Equations (1)–(3) proposed by Lichtethaler & Wellburn [13]:

$$C_a\ (\mu g/g) = 12.21\ [\text{Absorbance}_{663\ nm}] - 2.81\ [\text{Absorbance}_{646\ nm}] \quad (1)$$

$$C_b\ (\mu g/g) = 20.13\ [\text{Absorbance}_{646\ nm}] - 5.03\ [\text{Absorbance}_{663\ nm}] \quad (2)$$

$$C_{carotenoids}\ (\mu g/g) = \frac{1000\ [\text{Absorbance}_{470\ nm}] - 3.27\ [C_a] - 104\ [C_b]}{229} \quad (3)$$

2.4.3. Antioxidant Activity Assays

The DPPH* assay [14] was used with methanol 80% *v*/*v* as a dissolvent. The results were expressed as % radical inhibition.

2.4.4. Dipeptidyl Peptidase IV (DPP-IV) Inhibition Assay

According to Lin et al. [15], the DPP-IV inhibitory activity was tested using porcine DPP-IV enzyme and Gly-Pro-p-nitroanilide as substrate. The reaction was incubated at 37 °C for one hour, and its absorbance was measured at 405 nm. Commercial sitagliptin (0.1 mM as positive control) and samples (1 mg/mL) were diluted in distilled water and centrifugated at 5000 rpm for 5 min. The results were expressed as % DPP-IV inhibition.

2.5. Experimental Design and Statistical Analysis

The experiments were set up on a single-factor, completely randomized design with three replicates per level of treatment. The "thermal treatment" factor was evaluated at four levels: hydrothermal, steam pressure, microwave, and without thermal treatment (as control). All data are presented as the mean ± standard deviation. A one-way analysis of variance (ANOVA) was performed, followed by a post-hoc Tukey–Kramer analysis to identify differences between treatments at a p-value < 0.05. The statistical package Origin Pro, Version 2021 (OriginLab Corporation, Northampton, MA, USA) was used.

3. Results and Discussion

3.1. Nutritional Characterization

Table 1 shows the proximal analysis of the Pacaya powder samples treated with different thermal treatments. The Pacaya powder obtained after the microwave cooking showed a significant ($p < 0.05$) decrease in moisture content compared to the other treatments. The protein content in all thermal treatments increased significantly ($p < 0.05$), while no significant differences ($p > 0.05$) in ash content were detected between all thermal treatments. Similar findings were already reported by Sun et al. [16], who associated increased protein with the partial loss or destruction of other components, for example, lipids or fiber. Microwave cooking may cause the partial loss or destruction of volatile and water-soluble fatty acids, which decreases the lipids content [16].

Table 1. Proximal analysis of Pacaya inflorescences with different thermal treatments.

Treatment	Moisture (%)	Protein (%)	Lipids (%)	Ash (%)	NFC * (%)
Without thermal treatment	8.67 ± 1.15 [a]	18.40 ± 0.79 [c]	9.00 ± 0.16 [a]	12.35 ± 0.39 [a]	51.58 ± 0.47 [a]
Hydrothermal processing	7.58 ± 0.38 [a]	26.82 ± 0.98 [ab]	9.83 ± 0.29 [a]	10.59 ± 0.13 [a]	45.18 ± 0.43 [b]
Steaming at elevated pressure	7.33 ± 0.58 [a]	27.31 ± 0.83 [a]	9.29 ± 0.21 [a]	11.15 ± 1.57 [a]	44.92 ± 2.74 [b]
Microwave cooking	3.33 ± 0.58 [b]	24.81 ± 0.81 [b]	6.33 ± 1.05 [b]	12.12 ± 0.86 [a]	53.41 ± 1.51 [a]

Different letters in each column indicate significant differences at $p < 0.05$. * Nitrogen's free compounds (NFC = 100 − moisture − protein − lipids − ash).

3.2. Nutraceutical Characterization

The chlorophyll "a" and "b", total carotenoids, total phenolic compounds, and other nutraceutical characteristics after different thermal treatments are shown in Table 2. The hydrothermal thermal treatment showed a significant ($p < 0.05$) decrease in the chlorophyll "a" content compared with the other treatments; nonetheless, there was an increase in steaming under the elevated pressure treatment and microwave cooking treatment compared to without thermal treatment. Regarding the chlorophyll "b" content, all thermal treatments significantly ($p < 0.05$) reduced it. Mazzeo et al. [17] observed a similar behavior of these phytochemicals by type of thermal treatment in green beans, asparagus, and zucchini. They ascribe these results to their conversion to pheophytins or possibly to the thermal degradation of chlorophylls.

Table 2. Effect of thermal treatment on chlorophyll a and b, total carotenoids, total phenolic compounds, and other nutraceutical characteristics of Pacaya inflorescences.

Treatment	Chlorophyll a	Chlorophyll b	Carotenoids	TPC	DPPH	DPP-IV
	μg/g			μg GAE/g	% Inhibition	
Without thermal treatment	27.71 ± 2.62 [a]	35.07 ± 3.95 [a]	4.28 ± 0.80 [d]	0.36 ± 0.23 [c]	5.60 ± 0.14 [b]	21.42 ± 1.04 [a]
Hydrothermal processing	16.26 ± 2.52 [b]	15.44 ± 3.42 [b]	8.11 ± 0.68 [c]	3.15 ± 0.06 [b]	5.03 ± 0.29 [bc]	14.11 ± 0.33 [c]
Steaming at elevated pressure	34.64 ± 4.77 [a]	21.15 ± 2.97 [b]	28.11 ± 1.96 [b]	3.96 ± 0.06 [a]	4.55 ± 0.05 [c]	18.20 ± 0.22 [b]
Microwave cooking	29.83 ± 0.81 [a]	17.12 ± 1.31 [b]	41.66 ± 0.44 [a]	3.30 ± 0.32 [b]	11.06 ± 0.56 [a]	20.76 ± 0.13 [a]

Different letters in each column indicate significant differences at $p < 0.05$. Abbreviations: total phenolic compounds (TPC), gallic acid equivalents (GAE), and dipeptidyl peptidase-IV (DPP-IV).

All thermal treatments significantly ($p < 0.05$) increased the total carotenoid content, which could be attributed to the carotenoids on some occasions being associated with proteins and cellulose or being immersed in lipid droplets, and the thermal treatment released a higher carotenoid content than in the samples without thermal treatment. Therefore, an appropriate thermal treatment (with adequate cooking conditions) could denature proteins and break down the cellulose structure, releasing carotenoids by softening cell walls [18,19]. The other point is that some pigment complexes with protein may be created during thermal treatments and liberated during extraction, altering the carotenoids' quantification. Therefore, it is essential to determine the carotenoid profile to know crucial changes in these phytochemicals' content.

Additionally, thermal treatments significantly ($p < 0.05$) increased the total phenolic compounds approximately 8-fold to 11-fold. These changes are associated with a substantial weakening of cell walls by heat that facilities polyphenols' release; another point is that the effect of the concentration in the food matrix after partial or total moisture evaporation increases the polyphenols' concentration. Similarly, de novo compounds' production has been reported, such as Maillard reaction products reacting with Folin–Ciocalteu reagent [20].

Microwave cooking was the only thermal treatment that significantly ($p < 0.05$) increased the antioxidant activity (0.97-fold) by the DPPH method in the Pacaya after thermal treatments. Hydrothermal processing and steaming at an elevated pressure did not cause substantial changes. Jiménez–Monreal et al. [21] suggested four possibilities for the increase in antioxidant activity in some cooking methods: (1) the release of high amounts of antioxidants due to thermal destruction of cell walls and subcellular compartments; (2) the production of more robust radical-scavenging antioxidants by thermal–chemical reaction; (3) the elimination of the oxidation capacity of antioxidants by thermal inactivation of oxidative enzymes; and (4) the formation of new compounds with antioxidant activity as a result of the Maillard reaction.

It was observed that hydrothermal processing and steaming at an elevated pressure significantly ($p < 0.05$) reduced the DPP-IV enzyme inhibition. As a positive control, Sitagliptin (0.1 mM) showed a 95% inhibition. The hypoglycemic activity of *Chamaedorea tepejilote* inflorescences has been studied. However, the mechanism of action and thermal treatment's effect is not reported. Riquett Robles et al. found that the administration of 300 mg/kg of aqueous extract to normoglycemic mice reduced blood glucose by 29.77% compared to the control group [8]. The characterized and studied plant dipeptidyl peptidase-IV inhibitors are phenolic compounds and protein hydrolysates (bioactive peptides) [22].

4. Conclusions

Tepejilote inflorescences are an excellent source of nutrients, mainly proteins and lipids, as well as nutraceutical compounds, such as carotenoids and chlorophyll (to mention a few), which have shown antioxidant activity and inhibit the enzyme DPP-IV (important in glucose metabolism).

However, thermal treatments modified all the evaluated characteristics, possibly due to denaturation, the partial or total loss of cell wall components such as proteins, cellulose, and hemicellulose, causing the release or creation of compounds with nutraceutical activity.

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