

Physicochemical characterization and antioxidant activity of *Tamarindus indica* L. seed and shell flours

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ABSTRACT

Objective: To determine the physicochemical characteristics and antioxidant capacity of tamarind seed (*Tamarindus indica* L.) and peel flours.

Design/methodology/approach: To this end, tamarind pods were collected from San Antonio Sahcabchén, Calkiní, Campeche. They were subsequently pulped manually, and the peels and seeds were dried and ground to produce flour. To determine the physicochemical composition, several parameters were measured, including pH, titratable acidity (Aw), water solubility, moisture, protein, fat, ash, and fiber. In addition, antioxidant capacity was evaluated by determining total polyphenols and using DPPH and ABTS+ assays.

Results: There are differences ($P < 0.05$) between flour types for all the physicochemical characteristics evaluated. The seed flour showed higher values for aw (0.34), water solubility (12.40%), moisture (6.96%), protein (11.67%), fat (4.72%), and carbohydrates (52.65%). Meanwhile, the shell flour showed higher values for pH (5.66), titratable acidity (2.46%), ash (4.63%), and crude fiber (53.82%). In addition, greater ($P < 0.05$) antioxidant capacity was observed in the seed flour than in the tamarind shell flour.

Findings/conclusions: This information is useful in the search for alternatives for the use of these tamarind by-products, thus reducing their waste.

Keywords: Antioxidant activity, DPPH, total polyphenols.

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INTRODUCTION

Tamarind (*Tamarindus indica* L.) is a fruit tree native to tropical Africa. Currently, it is cultivated in regions of the world with a dry tropical climate. The most important producing countries are India, Thailand, Mexico, Indonesia, the Philippines, Brazil, Guatemala, Costa Rica, Nicaragua, among others (Anchundia-Salazar, 2021). In 2023, Mexico had a total tamarind production of 52,694.16 tons; the main producing states were Jalisco (43.4%), Colima (30.7%), Guerrero (14.4%), and Michoacán (7.27%) (SIAP, 2023). It is an economically valuable and multipurpose plant, since almost all of its parts can be used



(Limsangouan *et al.*, 2019). However, it is mostly known for its fruit, which is used for direct consumption or to produce various by-products, such as beverages, juices, jams, syrups, traditional sweets and culinary ingredients (Viveros-García *et al.*, 2012).

Tamarind contains 30% pulp, 40% seeds, and 30% peel relative to the total fruit weight (Rao *et al.*, 2015). The fruit is a rich source of protein (15-18%); it contains between 30 and 40% sugars and up to 11% organic acids, such as citric, acetic, ascorbic (vitamin C), and mainly tartaric, pectin, vitamins, and minerals (Montañez-Valdez *et al.*, 2023). Furthermore, the seeds, leaves, flowers, branches, bark, and roots are used as aphrodisiacs and for other medicinal purposes. Specifically, the seed has been reported to contain secondary compounds such as alkaloids, saponins, total phenolic compounds, and tannins, highlighting its antioxidant activity (Viveros-García *et al.*, 2012; Montañez-Valdez *et al.*, 2023). However, during peak tamarind production periods, much of the fruit remains in the orchards due to the low selling price or the product does not meet the quality standards required by the company, making its harvest economically unviable (Montañez-Valdez *et al.*, 2023). Traditionally, producers only sell the pulp and simply do not use the remaining seeds or peels (Viveros-García *et al.*, 2012). If these parts of the tamarind fruit are not managed properly, they end up as waste with no economic or other benefits.

An interesting strategy for managing these residues is to convert them into useful by-products that can facilitate their utilization, such as flour. Studies have been reported describing the advantages and disadvantages of utilizing tamarind residues, such as seeds and shell, in different industries (Rao *et al.*, 2015; Mansingh *et al.*, 2021; Montañez-Valdez *et al.*, 2023; Rahmat *et al.*, 2023). A recent study explored the chemical composition and in situ degradability of the whole tamarind fruit, demonstrating its potential as a feed resource for ruminants (Montañez-Valdez *et al.*, 2023). Other authors showed that tamarind seeds could be used to enrich local cereals, such as corn, to produce complementary foods with high nutritional quality, locally available at low cost (Oluseyi & Temitayo, 2015). In this regard, Uthai & Chetyakamin (2020) evaluated the chemical composition, mineral and phenol content, antioxidant activity, and sensory acceptance of different noodle pasta formulations made from the partial replacement of wheat flour with tamarind seed flour. The authors concluded that replacing up to 10% of wheat flour with tamarind seed flour could be successfully used for pasta production, increasing its nutritional value without negatively affecting its acceptability. Despite this research, the use of tamarind seeds and peel flours remains underexplored, largely due to the scarcity of information on their nutritional profile. Therefore, it is necessary to generate information on their nutritional values so that they can be considered an alternative in the food industry. Therefore, the objective of this study was to determine the physicochemical characteristics and antioxidant capacity of tamarind (*Tamarindus indica* L.) seed and peel flours.

MATERIALS AND METHODS

Sample Collection and Processing

Tamarind (*Tamarindus indica* L.) fruit was collected in the community of San Antonio Sahcabchén, located northeast of the municipality of Calkiní, Campeche (19° 50' 16.91" N and 90° 31' 39.72" W), at an altitude of 10 masl. The region has a warm subhumid climate

with summer rainfall (A_w). The average annual temperature is 26.4 °C, and the total annual rainfall is 1,253.8 mm (INEGI, 2024).

The tamarind samples were transferred to the Chemical Biological Analysis Laboratory of the Calkiní Higher Technological Institute in the State of Campeche for processing. Initially, impurities and dust were removed from the tamarind pods by manual washing with distilled water. The pods were then dried in a convection oven (Isotemp Premium Oven, Fisher Scientific®) at 50 °C for 40 h. The average pod weight was 9.90 ± 4.45 g, with a length between 4 and 15 cm. The pulp, seed, and husk were separated manually. The dried husks were ground using an electric mill (GoldFruit® 300W Electric). The dried seeds were ground using a conventional grain mill (Estrella®, Mexico), followed by fine grinding using a commercial blender (Osterizer®, USA). The ground material, both husk and seed, was sieved through a #40 mesh sieve (W.S. Tyler®, USA), with a particle size of 0.45 mm, until the flours were obtained. The flours were stored in Ziplot® airtight bags in a cool, dry place to prevent moisture absorption until further use.

Physicochemical Analysis

The pH measurement was performed using a digital potentiometer (Science Med®, mod. SM-3BW) according to AOAC method 943.02 (Lane, 1995). 10 g of flour was weighed and placed in an Erlenmeyer flask, where 50 mL of distilled water at room temperature was added. The contents were subsequently mixed in an electric stirrer for 30 min. The contents were placed in a beaker, allowed to stand for 10 min, and the pH was measured (Natukunda *et al.*, 2016). Buffer solutions of pH 4.0 and 7.0 were used for potentiometer calibration. pH measurements were performed in triplicate.

Total titratable acidity was determined by titration according to the AOAC (1999). 1.5 g sample was weighed and placed in an Erlenmeyer flask, where 20 mL of distilled water was added. The mixture was then mixed on a hot plate at 40 °C for 60 min. The resulting mixture was filtered, and 10 mL of the filtered solution was taken, to which 3 drops of 1% phenolphthalein were added as an indicator. 0.1 N sodium hydroxide was used for titration until a faint pink color was observed or until the solution reached a pH of 8.5 (Araujo *et al.*, 2016). The results were expressed as % tartaric acid, using the molar mass of tartaric acid as the equivalent weight of acid (Natukunda *et al.*, 2016).

Water activity (a_w) was determined using the LabStart-aw kit (NOVASINA®, Lachen, Switzerland). To determine water solubility, 0.5 g of sample was weighed and mixed with 20 mL of distilled water. Soluble particles were separated by centrifugation (3,500 rpm) using a refrigerated centrifuge (Centrifuge 5702R, Eppendorf®) at 25 °C for 10 min. The supernatant was then transferred to a crucible and dried in a digital oven (Thermolyne®) at 105 °C for 5 h. Measurements were performed in triplicate.

Proximate Analysis

The proximate composition of tamarind shell and seed flours was determined using standard methods (AOAC, 2019). Moisture content was determined by oven drying at 105 °C to constant weight (AOAC 925.10). Ash content was determined by ashing in a muffle furnace (BL Barnslead/Thermolyne, Thermo Fisher Scientific MX®) at 550 °C

(AOAC 923.03). Crude protein content was calculated using the Kjeldahl method with a nitrogen-to-protein conversion factor of 6.25 (AOAC 984.13). Crude fat was extracted using the Soxhlet method using petroleum ether as a solvent (AOAC 920.39). Crude fiber was determined using the Weende method, which involves digesting the sample in acidic and alkaline solutions (AOAC 978.10). Carbohydrate content was determined by difference (Geethalaxmi *et al.*, 2024). Carbohydrate (%) = $100 - (\% \text{moisture} + \% \text{fat} + \% \text{protein} + \% \text{crude fiber})$. All analyses were performed in triplicate, and the results were expressed as dry weight.

Total Polyphenol Quantification

Total polyphenols were quantified using the Folin-Ciocalteu method (Blainski *et al.*, 2013). First, 50 μL of the sample was taken and placed in 15 mL Falcon tubes, followed by the addition of 3 mL of distilled water and 250 μL of Folin-Ciocalteu reagent. The contents were homogenized and left to stand for 8 min in complete darkness. After this time, 750 μL of 20% sodium carbonate (NaCO_3) and 950 μL of distilled water were added. The solution was then homogenized and kept in complete darkness at room temperature for 2 h, and the absorbance was measured at a wavelength of 765 nm using a PerkinElmer[®] UV-Vis spectrophotometer. An external calibration was performed using gallic acid as a polyphenol reference.

Determination of Antioxidant Capacity by DPPH

Antioxidant capacity was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method, following the methodology described by Brand-Williams *et al.* (1995), with some modifications. 3800 μL of the DPPH solution (0.1 mM) was added to 15 mL Falcon tubes, followed by 200 μL of the extract, and the solution was homogenized by vortexing. The extracts were allowed to stand for 30 min to react with the radical. Subsequently, the absorbance at 515 nm was measured using a Perkin Elmer[®] UV-Vis spectrophotometer, and the % DPPH inhibition was calculated for each sample. The calibration curve was prepared using Trolox at different concentrations as a reference standard.

Determination of Antioxidant Capacity by ABTS+

Antioxidant capacity was determined using the ABTS following the methodology of Nenadis *et al.* (2004), with some modifications. 2970 μL of the ABTS solution was added to a 15 mL Falcon tube, followed by 30 μL of antioxidant extract, and the solution was homogenized by vortexing. The sample was allowed to stand for 6 min, and the absorbances were read on a spectrophotometer at 784 nm to calculate the % ABTS inhibition for each sample. The calibration curve was prepared using Trolox at different concentrations as an antioxidant reference.

Statistical Analysis

Data processing consisted of exploratory analysis, descriptive statistics, and one-way analysis of variance (ANOVA) with a significance level of 0.05%. Means between

flour types were compared using Tukey test ($P \leq 0.05$). Results were expressed as mean \pm standard deviation. All analyses were performed using IBM® SPSS® version 21.0 (Armonk, NY, USA).

RESULTS AND DISCUSSION

Physicochemical composition

One-way ANOVA showed significant differences ($P < 0.05$) between flour types for all physicochemical characteristics evaluated (Table 1). Both pH and titratable acidity were higher in shell flour compared to tamarind seed flour. pH is a chemical parameter that measures the acidity or alkalinity of the solution and is used to evaluate titratable acidity, which indicates the concentration of organic acids present in the flour. Therefore, these variables are important indicators in evaluating the conservation status of a food product. In addition, they influence flavor, consistency, texture, and aroma, which are fundamental attributes in determining the degree of acceptability by consumers (Bernal-Morales *et al.*, 2024). pH values below 4.5 can prevent the growth of microorganisms (Santos *et al.*, 2014). The pH value found for tamarind seed flour was close to 4.5; therefore, it could be considered a slightly acidic flour that offers antimicrobial characteristics. Similar results were reported by Silva *et al.* (2022), who observed pH values of 2.9 and 5.8 in flours from the shell and seed of tamarind grown in Brazil, respectively.

On the other hand, it was observed that tamarind seed flour presented higher ($P < 0.05$) aw and water solubility compared to shell flour. Specifically, a water solubility of 12.40 and 7.02% was observed for seed and shell flour, respectively. According to Osei-Tutu *et al.* (2024), this functional characteristic indicates the % of soluble solids, being essential in food systems such as pastry, since flours with high solubility can produce a less cohesive dough. These authors also mention that the variation in the % water solubility in vegetable flours is due to starch concentration, the amylose/amylopectin ratio and proteins, as well as the degree of interaction with water.

Proximate analysis provides essential information for characterizing tamarind seed and shell flour. It was observed that the seed flour had higher ($P < 0.05$) moisture content

Table 1. Physicochemical characteristics of seed and shell flours of *Tamarindus indica* L.

Item	Seed flour	Shell flour	P-value
pH	3.69 \pm 0.02 ^b	5.66 \pm 0.18 ^a	0.001
Titrable acidity (%)	0.13 \pm 0.03 ^b	2.46 \pm 0.04 ^a	0.001
a _w	0.34 \pm 0.01 ^a	0.22 \pm 0.02 ^b	0.001
Water solubility (%)	12.40 \pm 1.14 ^a	7.02 \pm 0.19 ^b	0.023
Moisture (%)	6.96 \pm 0.31 ^a	3.27 \pm 0.32 ^b	0.001
Protein (%)	11.67 \pm 1.09 ^a	3.77 \pm 1.07 ^b	0.001
Fat (%)	4.72 \pm 0.62 ^a	1.26 \pm 0.08 ^b	0.001
Ash (%)	4.25 \pm 0.04 ^b	4.63 \pm 0.02 ^a	0.001
Crude fiber (%)	23.37 \pm 3.53 ^b	53.82 \pm 0.73 ^a	0.001
Carbohydrates (%)	52.65 \pm 6.05 ^a	33.21 \pm 1.14 ^b	0.005

^{ab} Different letters in the same row indicate a statistically significant difference ($P < 0.05$).

(6.96%), protein (11.67%), and fat (4.72%), but lower ash (4.25%) and fiber (23.37%) contents than shell flour. Regarding the moisture content, this is of great relevance because moisture contents greater than 13-14% favor damage caused by the presence of fungi and other microorganisms during prolonged storage, consequently affecting the quality of flours in the manufacture of food products (Rodríguez-Pérez *et al.*, 2023). The moisture content obtained in tamarind flours is less than 13%, which indicates its storage stability.

On the other hand, high protein percentages can provide good nutritional value due to the presence of essential amino acids (Bernal-Morales *et al.*, 2024). Legume seeds are known to contain 10-30% protein, which is confirmed by our findings. Thus, tamarind seed flour can be considered a useful functional ingredient in the production of soups, pastas, and baked goods, due to its protein content. However, Geethalaxmi *et al.* (2024) recently observed that roasting tamarind seed significantly reduced the protein content of the flour, but improved the ash, carbohydrate, fat, and flavonoid contents.

In this regard, ash content is a parameter related to the amount of minerals present in foods, which is essential for determining their nutritional value. Low ash percentages are favorable because they can provide a greater amount of minerals for food. On the contrary, high ash percentages are particularly undesirable because they darken the flours. The ash concentration in flours for food use is expected to be less than 2% (Rodríguez-Pérez *et al.*, 2023). Although the results showed that the flours evaluated in the present study had an ash content greater than 2%, they are consistent with those observed by Silva *et al.* (2022), who reported ash values of 5.0 and 2.3 g.100 g⁻¹ in tamarind shell and seed flours, respectively.

The results regarding fat content showed significant differences among the flours evaluated, which has important implications for the nutritional profile and functional quality of the products made from them. Tamarind seed flour had the highest fat content (4.72%), while shell flour had the lowest (1.26%). The variability in fat content among tamarind flours can be explained by the differential concentration of lipids, which can be beneficial, including essential unsaturated fatty acids, which characterize legume seeds (Moo-Huchin *et al.*, 2025). Similar results were reported by Silva *et al.* (2022), who reported mean values of 0.6 and 4.3 g.100 g⁻¹ in shell and seed of tamarind grown in Brazil. For their part, Yusuf *et al.* (2007) observed that the lipid value in raw seeds of *T. indica* was 6.94%, while in whole seeds it was 11.43%.

Crude fiber is composed mainly of cellulose (60-80%) and lignin (4-6%); additionally, by minerals (Madhu *et al.*, 2017). In the present study, the crude fiber content ranged between 23.37% and 53.82% for tamarind seed flour and shell, respectively. The shell flour presented a high crude fiber content ($P < 0.05$) compared to the seed flour, this is because the tamarind shell is mainly made up of cellulose, hemicellulose and lignin (Cruz-Montesinos *et al.*, 2024). Likewise, the high crude fiber values in the seed flour could be related to the lack of separation of the coat and endosperm. Fiber has a positive impact on human health, reducing blood cholesterol levels, the risk of cardiovascular diseases and helping to prevent colon cancer (Sheikh *et al.*, 2019). In this sense, both flours evaluated could be a potential source of crude fiber for food formulation. The carbohydrate content of the flours ranged from 33.21 to 52.65%. Flour made from tamarind seeds had a higher carbohydrate content

($P < 0.05$) compared to the data obtained for shell flour. Similar results were reported by Kumar & Bhattacharya (2008) where they indicated that a considerable amount of carbohydrates (50-57%) are found in tamarind seeds. In accordance with other studies, carbohydrate contents ranging from 42.76 to 71.11% have been reported for tamarind seed flour, noting that factors such as temperature and roasting time significantly influence the final carbohydrate content (Geethalaxmi *et al.*, 2024).

Determination of antioxidant capacity

Figure 1(A) shows the concentration of total polyphenols in the seed and shell flours of *Tamarindus indica* L. These results demonstrate that, of both flours analyzed, the flour from the seed had the highest concentration of these compounds (127.8 ± 9.5 mg gallic acid/g of sample). Meanwhile, the flour from the shell had a concentration of 82.4 ± 9.1 mg gallic acid/g of sample. This behavior is consistent with the findings reported by Sudjaro *et al.* (2005), who indicated that the seeds of *Tamarindus indica* L. have a significantly higher concentration of total polyphenols (6.54 g kg^{-1}) compared to the pericarp (2.82 g kg^{-1}). The results indicate that the seed has a high potential as a raw material for obtaining bioactive compounds. This statement is supported by the study carried out by Aengwanich & Suttajit (2013), who reported that tamarind seeds constitute a significant source of polyphenols. These compounds are of great importance for the agri-food sector focused on the production of functional foods, because they have been shown to have relevant biological effects, such as the reduction of oxidative damage and the inhibition of lipid peroxidation, both *in vitro* and *in vivo* studies (Aengwanich & Suttajit 2009).

On the other hand, the results corresponding to the antioxidant capacity determined by the DPPH radical assay are presented in Figure 1(B). It can be observed that the seed flour was the one that presented the highest antioxidant activity (128.98 ± 3.2 mg of Trolox/g

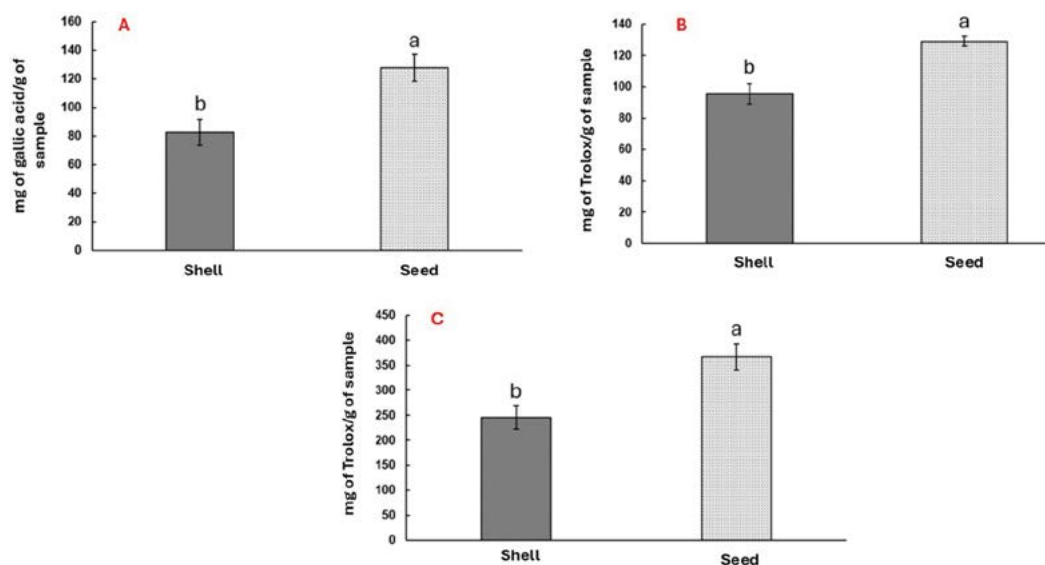


Figure 1. Quantification of antioxidant capacity in seed and shell flours of *Tamarindus indica* L. A) total polyphenols, B) DPPH, and C) ABTS+.

of sample). While the shell flour presented an antioxidant activity of 95.45 ± 6.6 mg of Trolox/g of sample. The study carried out by Farooq *et al.* (2022) agrees that the antioxidant activity, determined by the DPPH technique, was significantly higher in the tamarind seed ($74.1 \pm 0.8\%$) compared to the pulp ($72.2 \pm 0.4\%$).

While the antioxidant capacity determined with the ABTS+ technique (Figure 1(C)) showed the same behavior, the *Tamarindus indica* L. seed flour presented a higher antioxidant activity (366.7 ± 25.9 mg of Trolox/g of sample) compared to the shell flour (245.2 ± 23.4 mg of Trolox/g of sample). This corroborates that the seed flour has a higher concentration of compounds with antioxidant activity compared to the shell flour. Farooq *et al.* (2022) also evaluated the antioxidant activity of the tamarind seed using the ABTS technique, comparing their results with those obtained in the pulp. Their findings coincide in that the seed presented significantly higher values of antioxidant capacity ($79.2 \pm 0.4\%$) compared to the pulp ($75.7 \pm 0.2\%$), which suggests a higher concentration of bioactive compounds responsible for this activity.

These results are due to the fact that *Tamarindus indica* L. seeds are a rich source of polyphenols, flavonoids, and other secondary metabolites with recognized antioxidant capacity, particularly due to their effectiveness in neutralizing free radicals. This phytochemical composition could explain the high levels of antioxidant activity observed using the DPPH and ABTS methods. Furthermore, the dense structure and lower water content of the seed matrix, compared to the pulp, could favor the accumulation and stability of bioactive compounds.

CONCLUSIONS

Tamarind seed flour stood out in parameters such as moisture, protein, fat, and carbohydrates. It also exhibited greater antioxidant activity compared to tamarind shell flour. This information is useful for industries interested in utilizing tamarind products, allowing them to reduce waste and adding value to the fruit. Future studies on the determination and quantification of antinutritional compounds are needed to understand its true nutritional value.

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