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# Exhaustive Extraction of Bioactive Compounds and Antioxidants from Ataulfo Mango Seed

# Extracción Exhaustiva de Compuestos Bioactivos y Antioxidantes de la Semilla de Mango Ataulfo

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**Abstract**
Mango (*Mangifera indica* L.) is an important fruit worldwide, with high production that allows the production products such as juices and jams. Mango processing generates wastes, such as peels and seeds, which have been explored for their phytochemical profile and bioactivity. However, mango seed extraction processes generate waste sediment that has not been analyzed to determine whether polyphenols or antioxidants are present. Therefore, the objective of this study was to perform exhaustive extraction of mango seeds and evaluate their polyphenolic content (PC) and antioxidant activity (AA). The results showed a strong relationship between the content of the extracted material, PC, and AA. Extraction 1 showed a PC of 24.79 ± 1.41 mg GAE/g (hydrolysable) and 12.38 ± 1.70 mg CE/g (condensed) and AA was DPPH: 334.65 ± 18.71 mg TE/g, ABTS: 576.50 ± 16.91 mg TE/g and FRAP: 325.20 ± 14.09 mg TE/g, these results were significant concerning extractions 2, 3, 4, and 5. However, the second extraction was significant in hydrolysable tannins, ABTS, and FRAP against extractions 3, 4, and 5. Extractions 4 and 5 showed the lowest results, with no significant differences between them in all assays. Therefore, it is possible to make at least two extractions of mango seed and obtain significant values of polyphenols and antioxidants in a second extraction, which allows further use of the seed and waste sediment from the first extraction.

**Keywords:** Agro-industrial waste, Polyphenols, Trolox, Yield.

**Resumen**
El mango (*Mangifera indica* L.) es una fruta importante a nivel mundial, con una elevada producción, que permite su uso para la obtención de productos como jugos y mermeladas. Su procesamiento genera residuos como las semillas, que han sido exploradas por su perfil fitoquímico y bioactividad. Sin embargo, los procesos de extracción de semillas de mango generan sedimentos residuales que no han sido analizados para determinar si aún se encuentran presentes polifenoles o antioxidantes. Por lo tanto, el objetivo de este trabajo fue realizar una extracción exhaustiva de semillas de mango y evaluar su contenido polifenólico (CP) y actividad antioxidante (AA). Los resultados mostraron una alta relación entre el contenido del material extraído con el CP y la AA. La extracción 1 mostró un CP de 24.79 ± 1.41 mg GAE/g (hidrolizables) y 12.38 ± 1.70 mg CE/g (condensados) y la AA fue DPPH: 334.65 ± 18.71 mg TE/g, ABTS: 576.50 ± 16.91 mg TE/g y FRAP: 325.20 ± 14.09 mg TE/g, estos resultados fueron significativos en relación a las extracciones 2, 3, 4 y 5. Sin embargo, la extracción 2 fue significativa en taninos hidrolizables, ABTS y FRAP frente a las extracciones 3, 4 y 5. Las extracciones 4 y 5 obtuvieron los resultados más bajos, sin diferencias significativas entre ellas en todos los ensayos. Por lo tanto, es posible hacer al menos dos extracciones de la semilla de mango y obtener valores significativos de polifenoles y antioxidantes en la segunda extracción, lo que permite un mayor aprovechamiento de la semilla y del sedimento residual de una primera extracción.

**Palabras clave:** Polifenoles, Rendimiento, Residuo agroindustrial, Trolox.

## INTRODUCTION

Mango (*Mangifera indica* L.) is one of the most important tropical fruits worldwide, in 2019 world mango production was 55.9 million tons (Medina-Rendon et al., 2021) and it is expected to be 84.0 million tons by 2030 (OECD/FAO, 2021). The main producers include India, China, Thailand, Indonesia, Pakistan, Mexico, and Brazil (Altendorf, 2019). In Mexico, several varieties of mango are produced, such as Manila, Kent, Tommy Atkins, Haden, Criollo, and Ataulfo, with the last being the most important variety, which even has an Appellation of Origin. According to data from the "Secretaria de Agricultura y Desarrollo Rural (SADER)", mango production in Mexico in 2021 was 2 million 156 thousand tons, 30% of which corresponded to Ataulfo mango (SADER, 2023).

Mangoes are consumed fresh or processed into products, such as juices, jams, dehydrated pulp, or pulp in syrup. However, mango processing generates a large amount of waste, approximately 14 million tons (Torres-León et al., 2021a). Although the Ataulfo variety is exported more for fresh consumption, several companies in Mexico use it for processing into various products (Espinosa-Palomeque et al., 2023). It has been estimated that a medium-sized plant that can process 200 tons of mangoes per day can generate 84 tons of waste, consisting of peel (15-20%) and seed (20-45%) (Torres-León et al., 2021b).

Mango processing wastes have attracted attention for their chemical composition, as they have been shown to be rich in bioactive compounds such as polyphenols, which have potential benefits for human health. In addition, the mango seed has been described as the part of the fruit with the highest concentration of bioactive compounds (Nicolás García et al., 2023). Torres-León et al. (2021c) conducted extensive research on Ataulfo mango seed, showing its potential as an antioxidant and antimicrobial agent.

Extraction processes that have been used for Ataulfo mango seeds include conventional methods such as maceration and the use of emerging technologies such as microwaves and ultrasound (Cárdenas-Hernández et al., 2021). Additionally, the potential of fermentation to recover bioactive compounds has been explored (Torres-León et al., 2019). All the techniques used have been optimized to obtain the highest amount of bioactive compounds possible, considering zero waste and circular bioeconomy strategies; however, in most cases, at the end of the extraction processes, sediment remains as a final waste that is usually discarded. Therefore, it is necessary to evaluate the residual material to determine the possibility of performing another extraction process to obtain a greater amount of bioactive compounds, considering that many of these compounds are bound to the plant matrix. Therefore, the objective of this study was to perform an exhaustive extraction process on mango seeds and evaluate the polyphenol content and antioxidant activity to determine the number of times mango seeds could be extracted to obtain bioactive compounds with antioxidant potential.

## MATERIALS AND METHODS

### Material and reagents

Mango seed was obtained from processing mango (*Mangifera caesia* Jack ex Wall) purchased in Saltillo, Coahuila. The seeds were dried in an oven at 40 °C for 48 h, reaching a constant moisture content of 5.37%. The dried seeds were ground in a blender and passed through a 40-mesh sieve. Mango seed powder was stored in plastic bags at room temperature (25 °C).

### Chemicals

2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), Folin-Ciocalteu's reagent, sodium carbonate, and gallic acid (3,4,5-trihydroxybenzoic acid) were purchased from Sigma Aldrich.

### Exhaustive extraction methodology

Mango seed powder was extracted using 67 % ethanol (solid/liquid ratio: 1 g/23 mL) in flasks. The flasks were then mixed and placed in a heat bath at 60 °C for 30 min. The extract was then transferred to conical tubes and centrifuged at 2000 rpm. Finally, the extracts were stored in light-protected flasks. The sediments in the conical tubes were dried at 40 °C for 24-48 h. Then, the dried sediment was weighed, the volume of ethanol (67 %) was adjusted to maintain the solid/liquid ratio (1 g/23 mL), and the extraction was performed in the same way as the first extraction. The sediment was recovered and extracted again 3 more times. The main extract was obtained at the end of the experiment and four extracts were obtained from sediment extraction.

The percentage of the extracted material was calculated using the following equation:

% extracted material= 100-((dry weight of sediment obtained*100)/(dry weight of the material to be extracted))

### Determination of polyphenol content

Hydrolysable Tannins (HT)

HT was determined according to the methodology described by Wong-Paz et al. (2014) using Folin-Ciocalteu reagent. In a microplate well, 20 µL of sample was added. Subsequently, 20 µL of Folin-Ciocalteu's reagent was added and mixed for 5 min. Subsequently, 20 µL of sodium carbonate (10 mM) was added to the mixture and allowed to react for another 5 min. Finally, 125 µL of distilled water was added and the absorbance was read at 790 nm using microplate reader. The results were expressed as milligrams of gallic acid equivalents (GAE) per gram of seed (mg GAE/g seed) according to a gallic acid standard curve (0-1,000 mg/L; $R^2 = 0.994$).

Condensed Tannins (CT)

CT was performed according to the methodology described by Sepulveda et al. (2020), using HCl-butanol and ferric reagents. Each sample (250 µL) was placed in a test tube. Then, 1,500 µL of HCl-butanol (1:9) was added, followed by 50 µL of the ferric reagent. The test tubes were capped, covered with aluminum foil, and placed in a boiling water bath for 1 h. Next, the tubes were allowed to cool to room temperature (25 °C) and 200 µL were placed in a microplate well. Finally, the absorbance was read at 550 nm using a microplate reader. The results are expressed as milligrams of catechin equivalents per gram of seed (mg CE/g seed) according to a catechin standard curve (0-1,000 mg/L; $R^2 = 0.991$).

### Antioxidant activity assays

DPPH antioxidant activity assay

DPPH assay was performed as described by Torres-León et al. (2017). A total of 193 µL of 60 µM DPPH solution was mixed with 7 µL of sample in each microplate well. After 30 min of reaction in the dark, the absorbance was measured at 517 nm using a microplate reader. The results were expressed in milligrams of Trolox equivalents per gram of seed (mg TE/g seed) according to a Trolox standard curve (0-250 mg/L; $R^2 = 0.997$).

ABTS antioxidant activity assay

ABTS assay was performed as described by Ordoñez-Torres et al. (2020). For ABTS radical formation, 2.45 mM potassium persulfate ($K_2S_2O_8$) was mixed with 7 mM ABTS solution (1:1 v/v). The mixture was then allowed to stand for 24 h in the dark at 25 °C. Subsequently, the absorbance was measured at 734 nm and the solution was diluted in ethanol until an absorbance of 0.700 nm was reached. For this study, 10 µL of the sample was mixed with 1 mL of ABTS solution and the absorbance at 734 nm was measured using a spectrophotometer. The results were expressed in milligrams of Trolox equivalents per gram of seed (mg TE/g seed) according to a Trolox standard curve (0-250 mg/L; $R^2 = 0.994$).

Ferric Reducing Antioxidant Power (FRAP) assay

The FRAP assay was performed as described by Torres-León et al. (2017). 10 µL of the sample was mixed with 290 µL of FRAP reagent in a 96-well microplate. The reaction mixture was then incubated at 37 °C for 15 min. Absorbance was measured at 593 nm using a microplate reader. The results were expressed in milligrams of Trolox equivalents per gram of seed (mg TE/g seed) according to a Trolox standard curve (0-125 mg/L; $R^2 = 0.999$).

### Statistical analysis

All experiments were performed in quadruplicate, and the values are presented as the mean ± standard deviation. The experimental design was completely randomized. The data obtained were analyzed using one-way analysis of variance (ANOVA, $p < 0.05$) and Tukey's mean comparison test ($p < 0.05$). Data analysis was conducted using the Statistica 7.0. Pearson’s correlation was calculated using Microsoft Excel.

## RESULTS AND DISCUSSION

### Extracted material

The results of the extraction process are shown in Figure 1. The first (29.09 ± 0.59 %) and second (18.76 ± 1.13 %) extraction had a high percentage of extracted material above 10 %, which were significantly different from each other and against extractions 3 (5.87 ± 1.63 %), 4 (5.41 ± 1.51 %) and 5 (5.09 ± 0.65 %). Extractions 3, 4, and 5 had yields of less than 6 % with no significant differences between them. The results obtained are in accordance with those reported for other agro-industrial wastes such as chestnut wood (15-20 %) (Aimone et al., 2023), grape pomace (7.92 %), peanut shell (15.17 %), mango bagasse (37.07 %) (Braga et al., 2016), and mango seed Chok-Anan (3.31-11.90 %) (Maisuthisakul, 2009).

Mango seeds seem to have a higher extraction yield than different waste varieties, however, factors such as extraction method, temperature, solvent, time, and nature of the sample may influence these results (AL Ubeed et al., 2022). The extraction yield showed that up to a second extraction of mango seed, it was possible to obtain a high amount of extract, however, from the third extraction, the yield was low and there was no significant difference in extractions 4 and 5. Therefore, two extractions of mango seeds may be adequate to obtain a high percentage of the extract.

This is mainly due to the amount of extractable material available although binary ethanol-water mixtures allow the extraction of both polar and less polar compounds (Lim et al., 2019), there is a limit to the amount of material that can be extracted. In agro-industrial wastes such as mango seed there is a certain amount of free phenols, while another part of the phenols are bound in the plant matrix by covalent bonds (Vilas-Franquesa et al., 2023), for this reason, even if more extractions are carried out, it will be impossible to have a greater amount of extract. The largest amount of extract was found in the first two extractions.

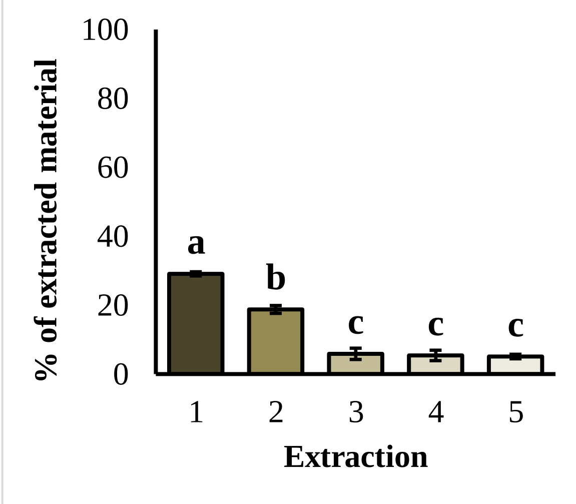


**Figure 1.** Percentage of material extracted from mango seeds. Different letters indicate significant differences ($p < 0.05$).

### Polyphenol content

The polyphenol content results are shown in Figure 2. In general, mango seeds have a higher content of hydrolysable tannins than condensed tannins (Torres-León et al., 2021b). The content of hydrolysable tannins (24.79 ± 1.41 mg GAE/g) was similar to that reported by Cárdenas-Hernández et al. (2021) for Ataulfo mango seed (29 mg GAE/g), and these results are higher than those reported for red grape seeds (20. 69 ± 0.13 mg GAE $g^{-1}$) (Di Stefano et al., 2022), pomegranate seed (1.84-4.67 mg GAE $g^{-1}$) (Falcinelli et al., 2017), and litchi cv. Jizui pericarp (11.79 ± 1.05 mg GAE/g) (Li et al., 2012). Condensed tannins (12.38 ± 1.70 mg CE/g) were higher than those reported in *Anabasis articulata* stems (4.03 ± 0.34 mg CE/g) (Benhammou, N. et al., 2013) and *Passiflora* leaves (0.65 ± 0.04 mg CE $g^{-1}$) (Pineli et al., 2015).

Statistical analysis shows that the first (24.79 ± 1.41 mg GAE/g) and second (14.18 ± 2.34 mg GAE/g) extraction were significantly different among all extractions for hydrolysable tannins, while for condensed tannins only the first extraction (12.38 ± 1.70 mg CE/g) was significantly different from all others. This indicates that by performing an additional extraction it is possible to obtain a significant amount of hydrolysable tannins, however, for the condensed tannin content the amount obtained (4.97 ± 0.77 mg CE/g) is neither high nor significant with respect to the third extraction (3.94 ± 0.62 mg CE/g).

These results are related to those obtained for the yield of the extracted material, where the most influential factors were the nature of the sample and the solvent used. In general, mango seeds contain more hydrolysable tannins than condensed tannins (Torres-León et al., 2021b); thus, they are predicted to have more hydrolysable molecules. In addition, a large part of the polyphenols is bound, which makes their extraction difficult; thus, after a certain number of extractions, it is no longer possible to obtain more polyphenols. A study by Torres-León et al. (2019) showed that a large part of the polyphenols in mango seeds are bound (~1500 mg GAE/100 g) while free polyphenols represent a content of 713.6 ± 70 mg GAE/100 g. In terms of solvents, ethanol has proven to be a suitable solvent for the extraction of mango seed compounds, moreover, ethanol is preferred for food applications. In fact, binary ethanol-water mixtures have proven to be better for the recovery of bioactive compounds, and a study by Lim et al. (2019) showed that mixtures of 25-75 % ethanol-water allowed the recovery of the highest amount of polyphenols in mango seed (76.52 - 101.68 mg GAE/g), because in the mixture, water dissolves the polar compounds, and the organic solvent (ethanol) recovers the less polar constituents.

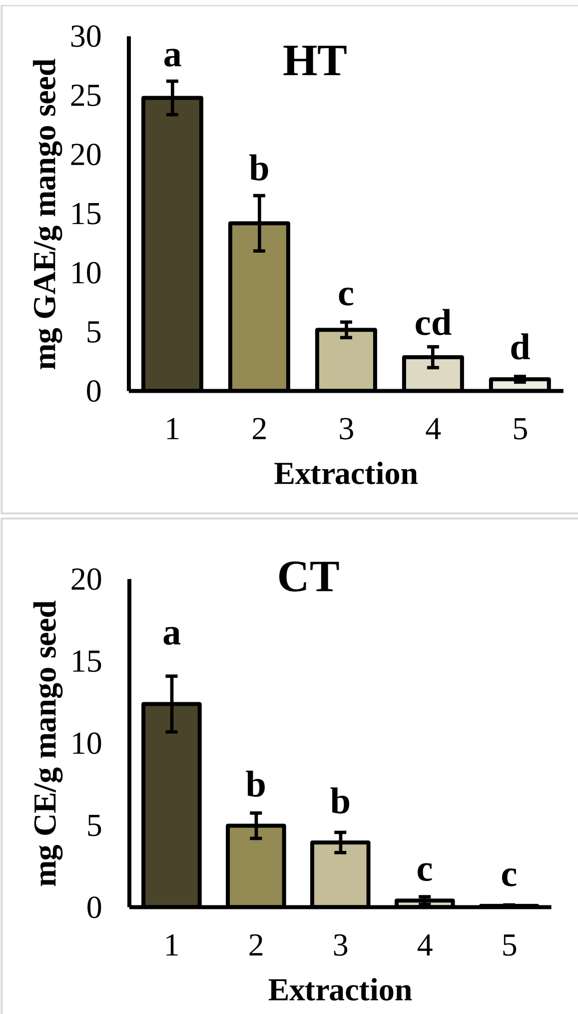


**Figure 2.** Polyphenol content of mango seed extracts. Different letters indicate significant differences at $p < 0.05$. HT: Hydrolysable Tannins, CT: Condensed Tannins, GAE: Gallic Acid Equivalent, CE: Catequin Equivalent.

### Antioxidant activity

The antioxidant activity results are shown in Figure 3. The ABTS assay showed the highest antioxidant activity (576.50 ± 16.91 mg TE/g), followed by DPPH (334.65 ± 18.71 mg TE/g) and FRAP (325.20 ± 14.09 mg TE/g) (Yap et al., 2023). These results are similar to those reported by Torres-León et al. (2017) for Ataulfo mango seed for DPPH (431.1 ± 114.90 mg TE/g), ABTS (499.2 ± 38.43 mg TE/g) and FRAP (455.1 ± 76.53 mg TE/g) and higher than those reported for various Indian legumes (DPPH: 42.9-571.1 mg TE/100 g, ABTS: 4.5-194.9 mg TE/100 g, FRAP: 90.6-2,773.5 mg TE/100 g) (Parikh & Patel, 2018).

All three assays showed that the first extraction was significantly different from the others. Extractions 2, 3, 4, and 5 had much lower values, below 70 mg TE/g, and even between extractions 4 and 5, there was no significant difference in any of the assays. A second extraction allows obtaining a considerable amount of hydrolysable tannins; moreover, extractions 2 and 3 still provide considerable antioxidant activity since extractions 4 and 5 show lower activities of DPPH, ABTS, and FRAP between 3.76 - 5.99 mg TE/g. Although a second extraction is optimal for obtaining a higher amount of polyphenols, it is necessary to evaluate its adequacy, considering that the highest antioxidant activity is obtained in the first extract.

Antioxidant activity studies use the DPPH, ABTS and FRAP assays in a complementary way, the ABTS assay has the ability to interact with hydrophilic and lipophilic type molecules, which allows a greater detection range than DPPH (Munteanu & Apetrei, 2021), this can be corroborated as the assay with the highest antioxidant activity was ABTS, the DPPH assay can only interact with lipophilic molecules which is a disadvantage, however, DPPH is stable, reproducible and need not be generated compared to ABTS (Bibi-Sadeer et al., 2020). The FRAP assay, as well as ABTS, can interact with hydrophilic and lipophilic molecules; however, the mechanism of action is different, since ABTS and DPPH are based on the neutralization of a radical, while FRAP is based on the reduction of the ferric ion ($Fe^{3+}$) to ferrous ($Fe^{2+}$) because the results of the assays can be different (Munteanu & Apetrei, 2021). Therefore, a single antioxidant assay does not allow to be broadening the view of antioxidants; therefore, the use of different assays is recommended to distinguish the dominant mechanisms of different antioxidants and their potential.

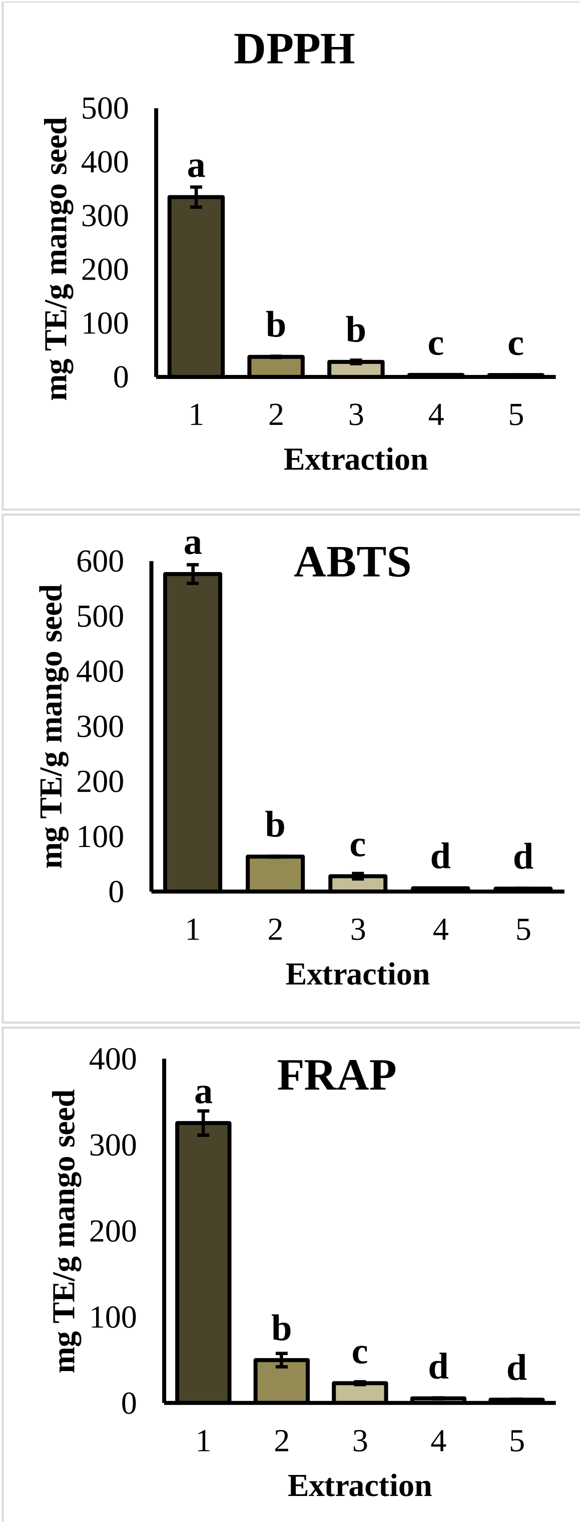


**Figure 3.** Antioxidant activity of mango seed extracts by DPPH, ABTS and FRAP assays. Different letters indicate significant differences at $p < 0.05$. TE: Trolox Equivalent.

**Correlation analysis of Yield with HT, CT, DPPH, ABTS and FRAP**

In addition, Pearson's correlation analysis was performed to determine the extraction yield, polyphenol content, and antioxidant activity (Table 1). The results showed a strong correlation of extraction yield with HT and CT, as well as DPPH, ABTS, and FRAP. These results are predictable since, as the amount of extractable material available decreases, the amount of polyphenols that can be obtained is reduced; at the same time, as there are fewer polyphenols, the antioxidant activity is decreased, as shown by strong correlation between HT and CT with DPPH, ABTS, and FRAP. Moreover, the antioxidant assays are strongly and positively related to each other, in fact, DPPH and ABTS have the highest correlation between antioxidants, but FRAP had the best correlation with HT and CT, all these results are similar to those reported by Ribeiro et al. (2013) for exotic and native fruits from Brazil, Muñoz-Bernal et al. (2020) for wine samples, Xiong et al. (2020) for dried yellow bean samples, Tymczewska et al. (2023) for spice extracts and Rumpf et al. (2023) for lignins, therefore although the type of plant material is different the correlations between polyphenols and antioxidants show similar trends.

**Table 1.** Pearson's correlation of yield against HT, CT, DPPH, ABTS, and FRAP assays.

| Test | Yield | HT | CT | DPPH | ABTS | FRAP |
|---|---|---|---|---|---|---|
| **Yield** | 1 | | | | | |
| **HT** | 0.9912 | 1 | | | | |
| **CT** | 0.9322 | 0.9665 | 1 | | | |
| **DPPH** | 0.8829 | 0.9018 | 0.9425 | 1 | | |
| **ABTS** | 0.8884 | 0.9032 | 0.9364 | 0.9994 | 1 | |
| **FRAP** | 0.9037 | 0.9190 | 0.9485 | 0.9988 | 0.9992 | 1 |

Due to the previously mentioned, studies of yield, polyphenol content and antioxidant activity of previously extracted plant matrices can help to determine the viability of exhaustive extraction of the material to obtain high amounts of polyphenols or antioxidants by conventional techniques, because once the content of free phenols is exhausted it is no longer possible to recover more compounds, this can be corroborated by the fact that at least two extractions of the mango seed seem to be adequate to obtain significant values, but more than two extractions is no longer suitable, therefore emerging technologies such as ultrasound, microwaves, pulsed electric fields and supercritical fluids could be more appropriate, as they have been shown to have the ability to cause more damage to the plant cell wall than conventional techniques, but they have a higher acquisition cost in general (Cristianini & Guillén Sánchez, 2020). In addition, it is necessary to evaluate in these technologies an exhaustive extraction to determine their potential to use the greatest amount of material possible, because some extraction methodologies using emerging technologies perform two or three extractions of the material without previously evaluating whether there is a greater recovery of polyphenols or antioxidants in those two or three extractions or all is recovered in a single extraction (Alañón et al., 2021), which could imply unnecessary cost and energy expenditure.

In addition to emerging technologies, green technologies such as the use of enzymes and fermentations, can help to produce a greater amount of free polyphenols, since the enzymatic processes involved break the plant walls and release the bound phenols, in addition to favoring the hydrolysis of complex molecules (Vilas-Franquesa et al., 2023). However, it is important to consider molecules of interest. For example, pentagalloylglucose (PGG), which consists of central glucose bound to five galloyl groups, and is the major compound in mango seeds, has attracted attention for its biological activities as an antioxidant, antidiabetic and anticancer agent (Mahmoud et al., 2022). Many studies have focused on obtaining mango seed extracts rich in certain compounds, such as PGG; therefore, fermentation processes might not be suitable because PGG is easily hydrolyzed by fungal enzymes into its main components, gallic acid and glucose (Aharwar & Parihar, 2018; Torres-León et al., 2019). As a result, conventional extraction is still a viable, cheap, and safe option for obtaining bioactive compounds from plant matrices such as mango seeds.

## CONCLUSIONS

This study determined the polyphenol content and antioxidant activity of mango seeds and waste obtained from extraction using an exhaustive extraction methodology. The results indicated a positive correlation between the amount of extracted material, the polyphenol content, and the antioxidant activity. The first extraction yielded the highest content in all parameters evaluated, while the second extraction yielded a considerable amount of hydrolysable tannins, but not condensed tannins, while the antioxidant activity was still significant for ABTS and FRAP, but not for DPPH. Although the highest antioxidant activity was obtained in the first extraction, the second and third extractions allowed for the recovery of a considerable amount of antioxidants. Therefore, it can be concluded that mango seeds can be extracted at least twice using conventional techniques to obtain a significant amount of polyphenols and antioxidant activity.

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