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Optimization of total polyphenol extraction and flavonoid screening by mass spectrometry in mango (*Mangifera indica L.*) waste from Peru

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Abstract

This research aims to optimize the extraction yield of total phenolic compounds (TPC) and quantify flavonoids by mass spectrometry in peel and kernel of mango (Mangifera indica L.), varieties: Edward, Kent, Haden, and Criollo from the department of Lambayeque, Peru, which resulted in eight samples. Mango peels and kernels were manually separated, frozen at -20 °C, freeze-dried, and ground (300 µm). For the extraction, the Central Composite Design was applied with the factors of ethanolic solution, time, and sample/volume ratio. The extracts determined TPCs by Folin-Ciocalteu and UV-Vis spectrophotometry expressed as gallic acid equivalent. Optimization was performed by the desirability function; Quercetin was also quantified by liquid chromatography-mass spectrometry (m/z). The highest yield of TPC content for Criollo mango kernel was obtained with 67.99% ethanolic solution, 89.94 min, and 0.343 g sample/10 mL ethanolic solution with R2 of 0.8966, and for Edward mango peel with 73.996% ethanolic solution, 58.5 min, and 0.432 g sample/10 mL ethanolic solution with R2 of 0.8020. For peel, the methanolic extract from Criollo mango peel had the highest Quercetin value at (23.28 ± 2.35 mg QE/100 g) ($p < 0.05$), and for kernels, in both extractions (ethanolic and methanolic), the four varieties did not present differences ($p > 0.05$).

Keywords: kernel; peel; desirability; polyphenols; Quercetin; spectrometry; untargeted metabolomic.

Practical Application: Considering the importance of mango by-products, focused on their phenolic properties and the mechanism antioxidant, these compounds can exhibit bioactive properties which can be further exploited as natural pigments and antioxidants for use as functional food ingredients and nutraceuticals.

1 Introduction

Currently, fruit consumption has increased due to its potential direct and indirect antioxidant activity, preventing the negative health effects of free radicals (Farrés-Cebrián et al., 2016), as reflected in the increase of agro-industrial companies engaged in the processing of fruits and vegetables, which generate large amounts of waste and inedible by-products that could be used as raw material to recycling active phytochemicals (Gil-Martín et al., 2022; Martins et al., 2022; Pérez-Chabela et al., 2022), such as in the case of mangoes, being peels and seeds the main by-products that are generally discarded as waste, becoming a source of environmental pollution (Peng et al., 2019). Mango (*Mangifera indica L.*) is one of the most popular and important tropical fruits in the world (Castro-Vargas et al., 2019; Sánchez-Mesa et al., 2020), with a world production of 52.08 million tons in 2018 (Food and Agriculture Organization, 2019). In 2020, Peru exported 242,879,787 kg of fresh mango of improved varieties such as Kent, Edward, Haden, and Tomy Atkins. There are ungrafted varieties such as the Criollo from different parts of Peru (Tuisima Coral & Escobar-Garcia, 2021). Its industrial processing generates between 35% to 60% of waste (Braga et al., 2016; Sánchez-Mesa et al., 2020). The peel represents 15-20% and the seed, including the kernel, 20-45% of the fresh weight of the whole fruit depending on the genotype (Serna-Cock et al., 2016).

Mango peels and kernels provide energy, dietary fiber, carbohydrates, protein, and fat (Correa et al., 2019; Iuit-González et al., 2019; Marcillo-Parra et al., 2021) and are rich in phytochemicals such as phenolic compounds (Sauthier et al., 2019; Gómez-Caravaca et al., 2016; Lenucci et al., 2022; López-Cobo et al., 2017; Marcillo-Parra et al., 2021) and flavonoids (Ballesteros-Vivas et al., 2019; Peng et al., 2019). These bioactive compounds are interesting due to their high antioxidant capacity (Braga et al., 2016; Lenucci et al., 2022), therapeutic properties (Asif et al., 2016; Castro-Vargas et al., 2019; Serna-Cock et al., 2016), and as ingredients for the food, nutraceutical, and pharmaceutical industries (Lenucci et al., 2022; Monribot-Villanueva et al., 2019; Peng et al., 2019). It is also worth noting that mango peel is an important by-product rich in polyphenols and could have a high economic value if used effectively.

Polyphenols can be extracted using organic solvents while their antioxidant potential may vary depending on the type of

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extraction, conditions, and choice of solvents (Farrés-Cebrián et al., 2016), aglycone flavonoids are soluble in methanol and ethanol, and glycoside flavonoids are soluble in water. Bioactive compounds are chemically unstable when exposed to high temperatures, light, and humidity (Koop et al., 2022).

There are different studies to identify phenolic compounds in mango by-products. However, in Peru, there are few studies related to this, in mango by-products of Edward, Kent, Haden, and Criollo varieties, typical of the northern region of Peru, and no studies were found using the HPLC/MS detection technique. Therefore, this research was aimed at optimizing the extraction of total polyphenols from the peel and kernel of four varieties of mango (*Mangifera indica L.*) from Peru, using the desirability function and identification of flavonoids by mass spectrometry.

2 Materials and methods

2.1 Reagents

The reagents used in the extraction were of analytical grade and purchased from Sigma-Aldrich Chemie (Steinheim, Germany), gallic acid monohydrate (PubChem CID: 24721416), and phenol reagent of Folin Ciocalteu. Analytical or higher-grade ethanol, from Supelco, chemical grade methanol, from Merk and Quercetin standard (PubChem CID: 5280343).

2.2 Samples

Ripe mango fruits of the Edward, Kent, Haden, and Criollo varieties were collected in the department of Lambayeque, Peru. The fruits were selected without mechanical damage, washed, and disinfected; the peels and kernels were manually removed, and, then, they were frozen at -20 °C, as shown in Figure 1.

2.3 Analysis of fruit components

Mango fruits of each variety were weighed in 3 kg, and the peel and seed were manually separated. The kernel is expressed as a percentage (%) of the latter.

2.4 Color analysis

The color of the freeze-dried peel and kernel of the four mango varieties was determined using an NS800 3NH digital colorimeter (Shenzhen, China). Thus, the parameters of lightness (L*) were measured: 0 = black, 100 = white, red (a*) and green (-a), yellow (b*) and blue (-b) or Chroma (C*) or saturation and hue (h*) or hue angle, and the color difference between a and b (ΔE) was also determined using the Equation 1:

$$\Delta E = \sqrt{\Delta a^2 + \Delta b^2 + \Delta L^2} \quad (1)$$

2.5 Sample preparation

Peels (CM) and kernels (AM) of the four varieties (Edward - E, Kent - K, Haden - H, and Criollo - C). Eight samples were obtained: Edward mango peel (CME), Edward mango kernel (AME), Kent mango peel (CMK), Kent mango kernel (AMK), Haden mango peel (CMH), Haden mango kernel (AMH), Criollo mango peel (CMC), and Criollo mango kernel (AMC).

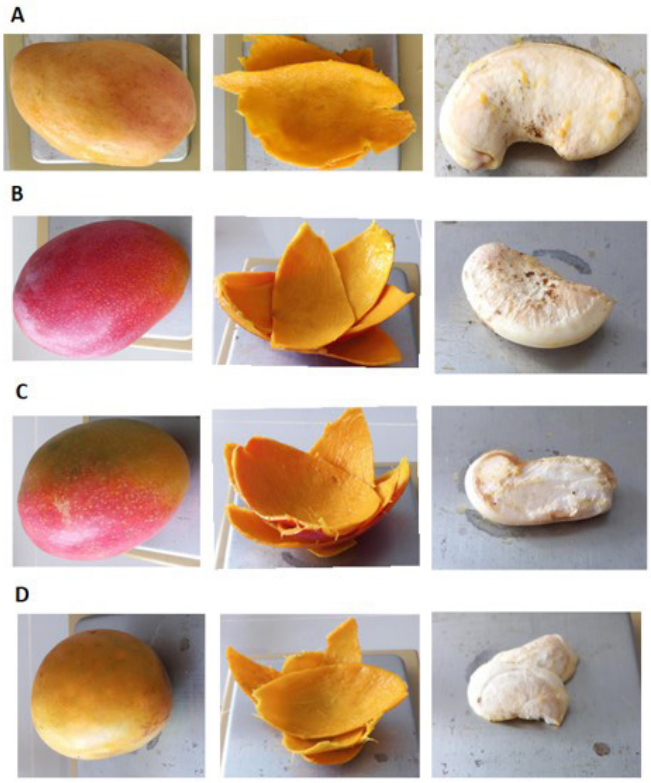

Figure 1. Mango varieties (left), peel (meddle) and kernel (right) wastes (A) E (Edward), (B) K (Kent), (C) H (Haden), (D) C (Criollo).

Samples were frozen at -20 °C, and dried in a BioBase BK-FD10PT freeze dryer to temperatures from -45 to -50 °C for 2 h, until the cold trap temperature reached ≤ -56 °C, going on to sublimation at 5 to 7Pa pressure for 18 to 24 h depending on the type of sample (kernel or peel). Drying was completed when the temperature reached 28.5 °C (room T°) and humidity below 6%. It was milled in an IKA M20 UNIVERSAL MILL with a stainless-steel star-shaped blade; then, it was sent to a Tyler Ro Tap RX 29-16 sieve shaker with a mesh size between 8 and 200. Fractions between 300 to 150 µm (retained at 100 mesh) were separated and packed in hermetic, self-sealing polyethylene films and 5 mL cryovials wrapped with aluminum foil and stored in a Velp Scientifica FOC 2151 cooled incubator at 20 °C until characterization and extraction of total polyphenols and flavonoid content.

2.6 Extraction optimization

The samples (CME, CMK, CMH, CMC, AME, AMK, AMH, AMC) were extracted with ethanol solution according to the conditions of the Central Composite Design (CCD), with 18 treatments and 4 central points (Table 1). With independent variables (VIs) of ethanol/water ratio, time, and ratio g sample/10 vol solution. A stirring procedure was performed by multirotor, at 90 rpm (orbital), 45 deg (Reciprocal), and 5º (Vibro/pause), for 30 to 89.9 min, according to the design. The extracts obtained were centrifuged (5000 xg at 4 °C for 15 min) and the supernatants were separated and transferred to a 15 mL beaker. They were covered with aluminum foil and kept at -20 °C until

Table 1. Central Composite Design (CCD) and results of total phenolic compounds for the samples (mango peel and kernel).

Ethanol/ water ratio (%)	Time (min)	sample/ solution ratio (g/10 mL)	CME	CMK	CMH	CMC	AME	AMK	AMH	AMC
			Total phenolic compounds (mg GAE/100 g db.)							
68.1	42.2	0.44	2536.0	1829.9	2020.0	1884.2	3975.4	3024.9	3323.6	3676.7
68.1	42.2	0.86	1797.7	1311.4	1269.7	1519.8	2325.7	1964.5	2020.0	2464.7
68.1	77.8	0.44	2373.1	1857.1	1721.3	2400.2	4056.9	3215.0	3269.3	4192.7
68.1	77.8	0.86	1769.9	1603.2	1367.0	1339.2	2589.7	2131.2	2228.5	2561.9
91.9	42.2	0.44	1992.9	1422.5	1504.0	1857.1	3405.1	2943.4	2943.4	3052.0
91.9	42.2	0.86	1353.1	1353.1	1075.2	1061.3	2020.0	1589.3	1742.1	2117.3
91.9	77.8	0.44	1884.2	1965.7	1585.5	1775.6	3106.3	2780.5	2590.3	2671.8
91.9	77.8	0.86	1575.4	1367.0	1047.4	1241.9	1895.0	1505.9	1644.9	1950.6
60.0	60	0.65	1918.9	1992.5	1569.6	1974.1	2801.4	3205.8	2709.4	2801.4
100.0	60	0.65	1091.6	484.2	1091.6	289.3	304.0	399.6	359.2	576.1
80	30.1	0.65	1937.3	1955.7	1349.0	2010.8	2488.8	3095.5	2066.0	2838.1
80	89.9	0.65	1790.2	1753.5	1551.2	1790.2	2525.6	3022.0	2341.8	2709.4
80	60	0.29	2652.8	2158.3	2694.0	2405.6	4053.8	4136.2	4548.2	5001.5
80	60	1	1605.8	1103.9	1283.2	1366.8	1976.2	1952.3	2036.0	2155.5
80	60	0.65	2231.4	1532.8	1496.1	1349.0	2783.0	2709.4	2801.4	2838.1
80	60	0.65	2599.1	1551.2	1459.3	1514.5	2764.6	2599.1	2194.7	2948.4
80	60	0.65	2157.9	1440.9	1992.5	1679.9	2819.7	2893.3	2525.6	3132.3
80	60	0.65	2305.0	1367.4	1606.4	1863.8	2801.4	2580.7	2415.3	2838.1

further spectrophotometric and chromatographic analysis. For HPLC- MS analysis, peel and kernel extracts were filtered through a 0.45 µm syringe filter.

Methanolic vs. ethanolic extraction

0.5 g of powdered peels and kernels were dissolved in 10 mL methanol/water 80:20% (v/v) solution, in a stirrer, for 30 min, by the modified method of Gómez-Caravaca et al. (2016). The extracts obtained were centrifuged at 5000 xg at 4 °C for 15 min and the supernatants were separated and transferred to a 25 mL beaker. The extraction and centrifugation steps were repeated 3 times and, then, the supernatants were combined in the beaker. The methanolic extracts were compared with the best ethanolic extraction condition from the previous item. Both extractions (methanolic and ethanolic-optimal) were applied to the 8 samples (CME, CMK, CMH, CMC, AME, AMK, AMH, AMC).

2.7 Total polyphenols by the Folin-Ciocalteu method

The Methodology (Magalhães et al., 2010; Singleton et al., 1999) was applied with some modifications, using the Folin-Ciocalteu reagent and absorbance reading in a UV-Vis spectrophotometer at 765 nm. gallic acid was used as a standard. Total polyphenol content was expressed as milligrams gallic acid equivalent (mg GAE/100 g db.).

2.8 Determination of Quercetin content by HPLC-MS

Quercetin quantification in the extracts was performed using the modified method of Irakli et al. (2021), by Shimadzu LCMS 2020 high-performance liquid chromatography (HPLC), using a C18 column (150 mm x 4.6 nm, 5 µm), with electrospray ionization (ESI), negative SIM, column temperature of 40 °C. As mobile phase, formic acid-water (0.1% v/v, solvent A) and acetonitrile (solvent B) were used with gradients: 15% B (0 min), 25% B (0-5.5 min), 35% (5.5-11 min), 60% B (11-31 min), 15% B (31-31.01 min), 15% B (31.01-35 min). From each extract, 10 uL was injected, previously filtered with 0.22 µm PTFE membrane, at a flow of 0.5mL/min. As standard, Quercetin was used and the results were expressed as mg Quercetin per 100 g sample (mg QE/100 g db.).

2.9 Screening of secondary metabolites

It was performed using an LC-MS system with a mass spectrometer as a detector. The separation was performed on a column C18 150 mm x 4.6 nm, 5 µm at a flow of 0.5 mL/min and an injection volume of 10 uL. Detection was performed in the range of 100 to 1100 m/z with electrospray ionization (ESI) in Scan mode. Formic acid-water (0.1% v/v, solvent A) and acetonitrile (solvent B) were used as mobile phase, with gradients: 15% B (0 min), 25% B (0 -5.5 min), 35% B (5.5-11 min), 60% B (11-31 min), 15% B (31-31.01 min), 15% B (31.01-35 min). The extracts were filtered with a 0.45um syringe filter into a 2 mL vial and placed in the autosampler for analysis of their metabolic profiles. The data were processed using LabSolutions software, and the scans were used for the detection of possible biomarkers of the samples by multivariate techniques.

2.10 Statistical data analysis

A one-way ANOVA was used to compare the proportions of mango fruit components of the four varieties, followed by Tukey's test of multiple comparison of means for the cases where significant differences were detected ($p < 0.05$). The optimization of the extraction process based on the total polyphenol yield from

the peel and kernel of Edward, Kent, Haden, and Criollo mango varieties was carried out using the CCD, and the results were adjusted to the second order polynomial model (Equation 2).

$$Y_i = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 + \varepsilon \quad (2)$$

Where: Yi is the total polyphenol yield; X^1, X^2, and X^3 are the independent variables (VIs) of ethanol/water ratio, time, and ratio g sample/10 vol solution; βo, βi, βij are the coefficients of the model. The best models were considered as those with the highest R^2adj and no Lack of fit. The desirability function was used to maximize the obtainment of the best VIs conditions to improve yield. All bivariate statistics were performed with a significance level of 5%.

Finally, in the screening of secondary metabolites, an untargeted metabolomic approach was used, performing multivariate statistics on the scans (chromatographic peaks), which were expressed as a percentage of the area within each sample. Scans that were present in at least 60% of the samples were used to perform the Principal Component Analysis (PCA), PCA was performed after data standardization, the Hierarquical Clustering of Principal Components (HCPC) was applied to confirm the suggested groups of PCA, HCPC was performed using Euclidian distances and Ward method to group, HeatMAP was also corriet out to evaluate visually the amount of pick through the samples.

Statistical analyses were performed using Statsoft STATISTICA V. 10. software and R program.

3 Results

The Edward variety was the heaviest ($p < 0.05$) and Criollo the lightest ($p < 0.05$) (Table 2). The percentage of peel ranged from 11.5% to 15.3%, being the Haden variety the one with the highest percentage of peel ($p < 0.05$). The Criollo variety had the highest seed percentages at a value of 14.0% and kernel at 7.4% ($p < 0.05$). Close values were reported by Tuisima Coral & Escobar-Garcia (2021) for mango from Piura, in Peru: Edward variety (peel 15.30%, seed 6.30%); Kent (peel 10.80%, seed 7.00%), and Haden (peel 17.5%, seed 10.90%). These values are in contrast to those reported by Correa et al. (2019) for the Criollo variety (peel 19.01% and kernel 8.57%).

There is a statistical difference ($p < 0.05$) in the color parameters between some mango varieties, both for peel and kernel (Table 3). Regarding the values of L*, which indicates brightness, for the peel, it was obtained by the Haden and Kent varieties (with no differences between these two ($p > 0.05$). The Haden variety presented the most reddish peel with higher values ($p < 0.05$) of the parameters a*, b*, and c* of 14.32, 48.07, and 50.16, respectively. The parameter h* that defines the mean hue presented a difference ($p < 0.05$) among all varieties. These same trends were observed for the parameters corrected as delta L*(ΔL). Regarding a* red and b* yellow, in the peels of the four varieties, the yellow hue predominated in comparison with the kernels. These values compared with those obtained by Silva et al. (2022), who evaluated the color in mango pulp of the "Maria" variety, fresh 25.30 and industrialized, presented a lower hue of yellow because the pigment predominates more in the peels.

As expected, the kernel presented very different values ($p < 0.05$) in all parameters when compared with the peel, but statistical differences were also detected in these parameters

Table 2. The average weight of four mango varieties and the percentage of wastes.

Variety	Entire (g)	peel (%)	seed (%)	kernel (%)
Edward	561.7 ± 63.5 a	11.8 ± 1.8 bc	5.8 ± 1.6 b	2.2 ± 0.2 c
Kent	495.6 ± 31.9 b	11.5 ± 1.7 c	7.7 ± 2.4 b	3.5 ± 0.5 b
Haden	457.5 ± 32.9 b	15.3 ± 2.2 a	7.5 ± 1.3 b	2.9 ± 0.3 bc
Criollo	289.1 ± 26.8 c	14.4 ± 2.0 ab	14.0 ± 2.8 a	7.4 ± 0.7 a

The values represent the mean ± SD (n = 3). Different letters in the same column indicate statistical differences ($p < 0.05$).

Table 3. Peel and kernel color parameters of four mango varieties.

Variety	Peel					
	L*	a*	b*	C*	h*	ΔE*
Criollo	76.76 ± 0.04 B;b	10.09 ± 0.08 A;c	45.66 ± 0.33 A;b	46.76 ± 0.33 A;b	77.54 ± 0.08 B;b	75.41 ± 0.19 A;b
Edward	80.36 ± 0.23 B;a	8.39 ± 0.32 A;d	44.96 ± 0.58 A;b	45.74 ± 0.62 A;b	79.43 ± 0.27 A;a	77.61 ± 0.2 A;a
Haden	68.72 ± 0.21 B;c	14.32 ± 0.14 A;a	48.07 ± 0.16 A;a	50.16 ± 0.19 A;a	73.41 ± 0.11 B;c	71.74 ± 0.19 A;c
Kent	69.11 ± 0.43 B;c	13.38 ± 0.23 A;b	41.03 ± 0.31 A;c	43.16 ± 0.36 A;c	71.93 ± 0.18 B;d	67.23 ± 0.13 A;d
	Kernel					
Criollo	88.82 ± 0.09 A;a	1.73 ± 0.01 B;d	11.12 ± 0.06 B;c	11.25 ± 0.05 B;c	81.16 ± 0.09 A;a	34.05 ± 0.08 B;a
Edward	87.86 ± 0.19 A;b	2.25 ± 0.06 B;c	11.52 ± 0.15 B;b	11.74 ± 0.16 B;b	78.95 ± 0.16 A;b	33.12 ± 0.16 B;b
Haden	86.04 ± 0.04 A;d	2.71 ± 0.03 B;a	12.17 ± 0.06 B;a	12.46 ± 0.06 B;a	77.43 ± 0.06 A;c	31.42 ± 0.03 B;d
Kent	87.45 ± 0.05 A;c	2.4 ± 0.05 B;b	10.61 ± 0.04 B;d	10.87 ± 0.05 B;d	77.24 ± 0.23 A;c	32.54 ± 0.04 B;c

for the kernels of the four varieties. Thus, it was observed that the Criollo variety had the highest value of L* (88.82) and h* (81.16), and the lowest value of a* (1.73), while the kernels of the Haden variety had the highest value of parameter a* with 2.71 and the lowest value in L* (86.04).

Color parameter values. Mean ± SD (n = 3). Lowercase letters in the same column indicate a significant difference ($p < 0.05$) between mango varieties within each part of the fruit, by Tukey's test. Capital letters in the same column indicate a significant difference ($p < 0.05$) between peel and kernel when comparing within each variety, by t-test for paired samples.

PCA of the color parameters for the peel and kernel samples of the four mango varieties (Figure 2a) shows that there was a clear differentiation between peel and kernel, which was confirmed by HCPC (Figure 2b), which clearly shows two large clusters (one for each part of the fruit). The mango peel had the highest b*, c*, and a* values, while the kernel of the same variety had the highest L* and h* values.

The Haden and Kent varieties were classified very closely (Figure 2b), resulting in the same subgroup for both cases (peel and kernel). In the case of kernels, the most different variety was the Criollo, and, in the case of peel, it was the Edward variety.

3.1 Optimization of total polyphenol extraction (TPC)

Optimization in total polyphenols from mango peel and kernel of Edward, Kent, Haden, and Criollo varieties was achieved by CCD to obtain the maximum extraction yield (Table 1).

For extraction from mango peel, the mathematical models showed no lack of fit (Table 4), and the one for the Edward variety had an adjusted coefficient of determination of 80%, indicating an adequate model fit.

When extraction from the kernel was performed, mathematical models with R^2adj greater than 78% and no lack of fit were obtained for most of the samples except for the Edward variety (Table 4). The three independent variables (ethanol percentage, time, and sample/solution ratio) had a linear effect ($p < 0.05$) for all eight samples (four of peel and four of kernel). The ethanol percentage, in addition to the linear effect, also had a quadratic effect for the Edward and Kent peels, as well as for the peels of the four varieties. On the other hand, the time variable presented a quadratic effect for the peels of the Kent and Edward varieties. Finally, the sample/solution ratio had a quadratic effect for almost all the kernels, except for the Kent variety.

Optimization, using the desirability function (Table 4) applied to the peel extracts, indicated higher extraction for all varieties under the following conditions: ethanol between 70% and 74%; the recommended time in minutes was 58.5, 89.9, 54.1, and 89.9 for the varieties Haden, Kent, Edward, and Criollo; and the sample/solvent ratio was between 0.3 and 0.56, depending on the variety, similar to reported for Safdar et al. (2022), in polyphenols extraction with ethanol in peels mango, obtaining a higher yield in the extraction at 80% ethanol. In the case of extracts from kernels, the desirability function indicated ranges (depending on variety) of ~68% - ~76% ethanol, ~76 min - ~90 min, and 0.29 to 0.43 g sample/10 mL.

3.2 Flavonoid content (Quercetin)

For the extracts obtained from the peel, the methanol solvent obtained higher and lower Quercetin values ($p < 0.05$) for the Haden and Criollo varieties, respectively (Table 5); while, for the

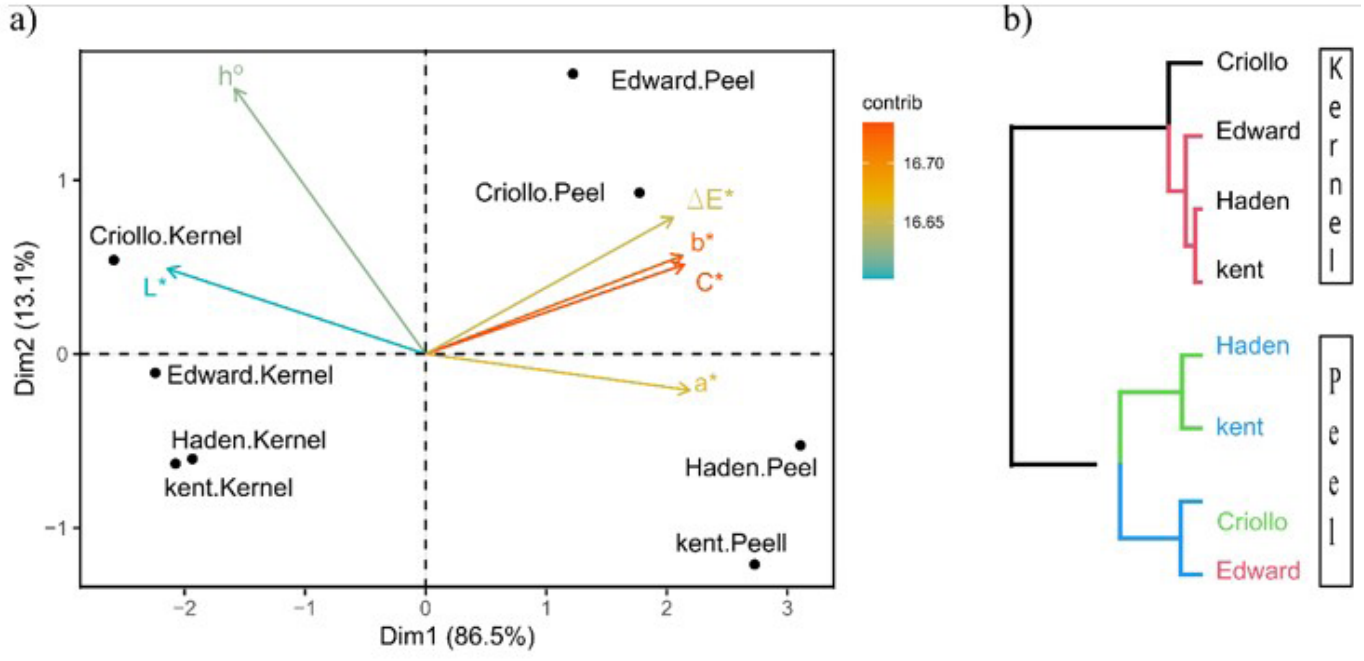

Figure 2. PCA biplot from peel and kernel color data of four mango varieties (a) and their subsequent clustering by HCPC (b).

Table 4. Mathematical models of ethanolic extraction of total polyphenols from mango peels and kernels, and optimization by desirability function.

Variety	Equation	R2_adj	Lack of Fit	Optimization Values		
				Ethanol/ water ratio (X1)	Time (X2)	sample/ solution ratio (g/10 mL) (X3)
Peel						
Edward	$Y = 2152,44 - 224,25X_1 - 233,25\ X_1^2 - 23,76X_2 - 295,91X_3$	0.8020	0.5838	73.996	58.503	0.432
Kent	$Y = 1532,93 - 221,86X_1 - 92,60X_1^2 + 39,24X_2 + 125,28X_2^2 - 235,31X_3$	0.5678	0.0327	69.993	89.936	0.5562
Haden	$Y = 1537,08 - 144,24X_1 + 14,08X_2 - 326,47X_3$	0.5775	0.5135	71.995	54.013	0.29
Criollo	$Y = 1628,20 - 295,89X_1 + 4,65X_2 - 329,76X_3$	0.5612	0.2376	71.995	89.936	0.503
Kernel						
Edward	$Y = 2766,39 - 492,15X_1 - 309,46\ X_1^2 - 1,21X_2 + 28,02X_2^2 - 667,55X_3 + 197,5X_3^2$	0.7831	<0.001	69.993	89.936	0.432
Kent	$Y = 2836,77 - 456,61X_1 - 391,21X_1^2 - 0,96X_2 - 617,83X_3$	0.8025	0.0523	75.997	89.936	0.3326
Haden	$Y = 2402,57 - 430,06X_1 - 290,12X_1^2 - +12,27X_2 - 630,49X_3 + 324,09X_3^2$	0.8593	0.3381	70.994	76.465	0.29
Criollo	$Y = 2906,99 - 501,34X_1 - 398,61X_1^2 - 10,99X_2 - 673,23X_3 + 263,36X_3^2$	0.8966	0.0883	67.992	89.936	0.34325

Table 5. Quercetin content (mg/100 g) in peels and kernels of Edward, Kent, Haden, and Criollo mango varieties, according to the type of solvent by LC-MS.

Variety	Solvent	Quercetin
	Peel	
Edward	ethanol	7.685 ± 1.158 A;c
Edward	methanol	7.66 ± 0.39 A;c
Kent	ethanol	16.629 ± 1.623 A;b
Kent	methanol	14.46 ± 0.104 A;b
Haden	ethanol	15.69 ± 1.99 B;b
Haden	methanol	19.54 ± 1.29 A;a
Criollo	ethanol	23.28 ± 2.345 A;a
Criollo	methanol	18.66 ± 0.312 B;a
	Kernel	
Edward	ethanol	—
Edward	methanol	0.06 + 0.03 b
Kent	ethanol	—
Kent	methanol	0.14 + 0.02 a
Haden	ethanol	—
Haden	methanol	0.11 + 0.05 a
Criollo	ethanol	0.12 + 0.06 A
Criollo	methanol	0.03 + 0.00 B;b

Capital letters in the same column indicate statistical differences ($p < 0.05$) between solvents within each variety and part of the fruit. Lowercase letters in the same columns indicate statistical differences ($p < 0.05$) between mango varieties when compared within each solvent and part of the fruit.

Edward and Kent varieties, no differences ($p > 0.05$) were detected between the solvents. On the other hand, when comparing the varieties within each type of solvent, it can be observed that, for the methanol solvent, the highest Quercetin value was found in the extract from the Haden variety, and, for ethanol, in the Criollo variety. Finally, for kernels, the methanol solvent had a better extraction value than ethanol. Likewise, there was very little (or no) Quercetin compared to the peel.

3.3 Secondary metabolite profile by LC-MS

Through the PCA from the scans of chromatograms of all the samples, it is possible to observe that the extracts from peels were very different from those of kernels (Figures 3a and 3b) since the extracts from the peels had higher scan values of 273, 463, and 191. The extracts from the kernels had higher scan values of 421 and 453 (Figure 4a). As chromatograms of the extracts from

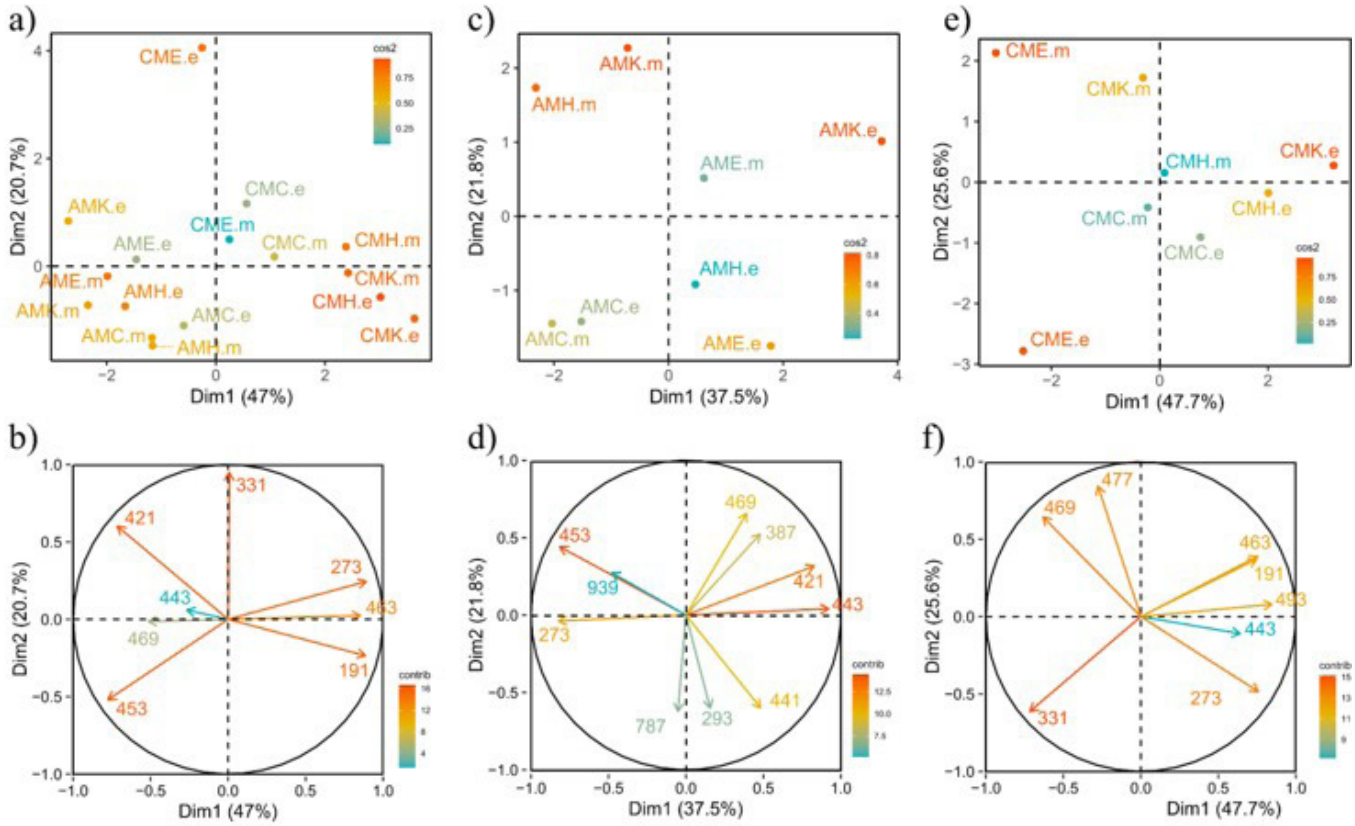

Figure 3. PCAs from scans present in at least 65% of the samples. a) PCAs of the total extracts methanolic and ethanolic of Edwar, Kent, Haden and Criollo mango peels and kernels, b) PCAs of the scans of the chromatograms of the total extract, c) PCAs of kernels extract, d) PCAs of the scans of the kernels chromatograms, e) PCAs of peel extracts and f) PCAs of the scans of the peels chromatograms.

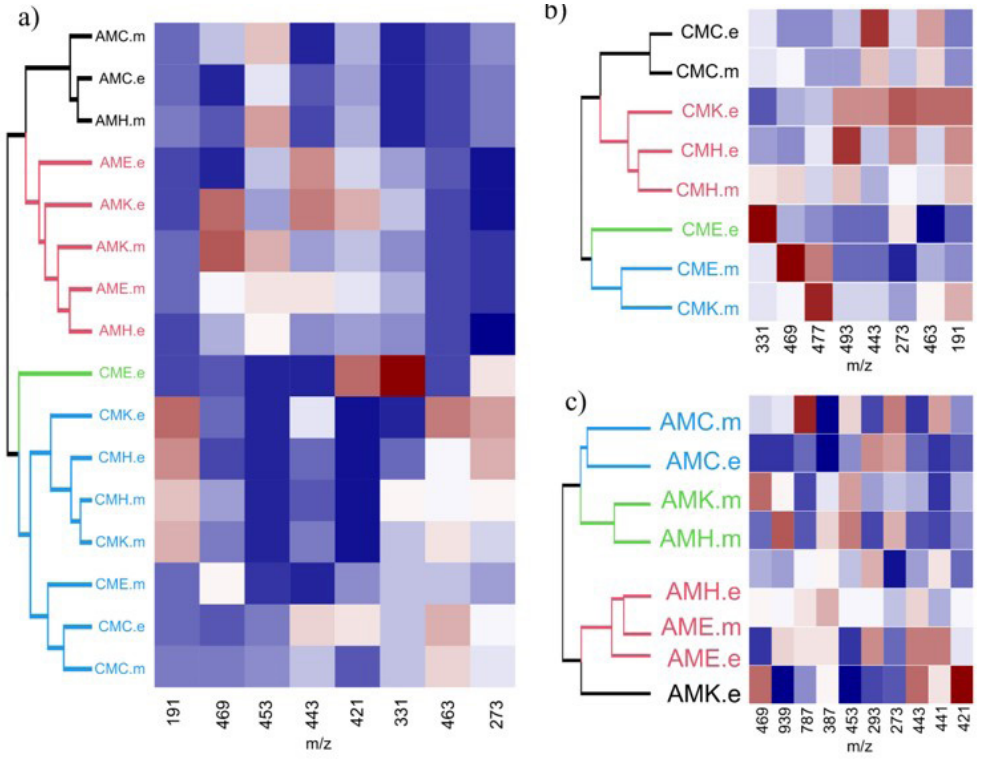

Figure 4. HeatMAP of the scans present in at least 65% of the samples. a) The heatMAP of chromatograms of the extracts from peels and kernels b) The heatMAP of chromatograms of the peels extracts and c) The heatMAP of chromatograms of the kernel extracts.

peels and kernels were very different, it was decided to perform PCAs for kernels (Figures 3c and 3d) and peels (Figures 3e and 3f), separately, to better detect the differences between samples and solvent type. Thus, when looking at the PCA (Figures 4) of the kernels, it is possible to see that the methanolic extracts of the Haden and Kent varieties were similar. The heatMAP of chromatograms of the kernel extracts (Figure 4b) could indicate certain markers, for example, the kernel of the Kent variety in the ethanolic extract had higher scan values of 421, 469, and 443, while for the methanolic Criollo variety, a higher scan value of 787 was obtained.

Finally, for the extracts from peels (Figure 4c), it is possible to observe that the Criollo variety had higher scan values of 443 and 463 for the case of the two solvents. It can also be observed that the ethanolic extracts of the Kent and Haden varieties were represented by scans 493, 273, and 191.

4 Conclusions

This study optimized the extraction yield of total phenolic compounds (TPC) and quantified flavonoids by mass spectrometry in mango (*Mangifera indica L.*) peel (CM) and kernel (AM) of Edward (E), Kent (K), Haden (H) and Criollo (C) varieties from the department of Lambayeque, Peru.

The desirability function applied to the extracts of peels indicated higher extraction for all varieties. In the Quercetin content, the peel had the highest content, the kernel had almost nothing.

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References

Asif, A., Farooq, U., Akram, K., Hayat, Z., Shafi, A., Sarfraz, F., Sidhu, M. A. I., Rehman, H. U., & Aftab, S. (2016). Therapeutic potentials of bioactive compounds from mango fruit wastes. *Trends in Food Science & Technology*, 53, 102-112. http://dx.doi.org/10.1016/j.tifs.2016.05.004.

Ballesteros-Vivas, D., Álvarez-Rivera, G., Morantes, S. J., Sánchez-Camargo, A. del P., Ibáñez, E., Parada-Alfonso, F., & Cifuentes, A. (2019). An integrated approach for the valorization of mango seed kernel: Efficient extraction solvent selection, phytochemical profiling and antiproliferative activity assessment. *Food research International*, 126, 108616. http://dx.doi.org/10.1016/j.foodres.2019.108616. PMid:31732074.

Braga, G. C., Melo, P. S., Bergamaschi, K. B., Tiveron, A. P., Massarioli, A. P., & Alencar, S. M. (2016). Rendimento de extração, atividade antioxidante e compostos fenólicos dos subprodutos agro industriais de uva, manga e amendoim. *Ciência Rural*, 46(8), 1498-1504. http://dx.doi.org/10.1590/0103-8478cr20150531.

Castro-Vargas, H. I., Ballesteros Vivas, D., Ortega Barbosa, J., Morantes Medina, S., Aristizabal Gutiérrez, F., & Parada-Alfonso, F. (2019). Bioactive phenolic compounds from the agroindustrial waste of Colombian mango cultivars 'sugar mango' and 'tommy atkins': an alternative for their use and valorization. *Antioxidants*, 8(2), 1-19. http://dx.doi.org/10.3390/antiox8020041. PMid:30781395.

Correa, D., Romero, B., & León, N. (2019). Extraction of tannins from creole mango seed (Mangifera indica L.) and its application as tanning. *Journal of Agro-Industry Sciences*, 1(2), 51-55. http://dx.doi.org/10.17268/JAIS.2019.007.

Farrés-Cebrián, M., Seró, R., Saurina, J., & Núñez, O. (2016). HPLC-UV polyphenolic profiles in the classification of olive oils and other vegetable oils via principal component analysis. *Separations*, 3(33), 1-13. http://dx.doi.org/10.3390/separations3040033.

Food and Agriculture Organization – FAO. (2019). *Major tropical fruits: statistical compendium 2018*. Rome: FAO. https://doi.org/10.4060/ca5688en

Gil-Martín, E., Forbes-Hernández, T., Romero, A., Cianciosi, D., Giampieri, F., & Battino, M. (2022). Influence of the extraction method on the recovery of bioactive phenolic compounds from food industry by-products. *Food Chemistry*, 378, 131918. http://dx.doi.org/10.1016/j.foodchem.2021.131918. PMid:35085901.

Gómez-Caravaca, A. M., López-Cobo, A., Verardo, V., Segura-Carretero, A., & Fernández-Gutiérrez, A. (2016). HPLC-DAD-q-TOF-MS as a powerful platform for the determination of phenolic and other polar compounds in the edible part of mango and its by-products (peel, seed, and seed husk). *Electrophoresis*, 37(7–8), 1072-1084. http://dx.doi.org/10.1002/elps.201500439. PMid:26703086.

Irakli, M., Skendi, A., Bouloumpasi, E., Chatzopoulou, P., & Biliaderis, C. G. (2021). Lc-ms identification and quantification of phenolic compounds in solid residues from the essential oil industry. *Antioxidants*, 10(12), 2016. http://dx.doi.org/10.3390/antiox10122016. PMid:34943119.

Iuit-González, M., Betancur-Ancona, D., Santos-Flores, J., & G. Cantón-Castillo, C. ((2019). Mermelada enriquecida con fibra dietética de cáscara de Mango (Mangifera indica L.). *Revista Tecnología En Marcha*, 32, 193-201. http://dx.doi.org/10.18845/tm.v32i1.4128.

Koop, B. L., Silva, M. N., Silva, F. D., Lima, K. T. S., Soares, L. S., Andrade, C. J., Valencia, G. A., & Monteiro, A. R. (2022). Flavonoids, anthocyanins, betalains, curcumin, and carotenoids: sources, classification and enhanced stabilization by encapsulation and adsorption. *Food Research International*, 153, 110929. http://dx.doi.org/10.1016/j.foodres.2021.110929. PMid:35227467.

Lenucci, M. S., Tornese, R., Mita, G., & Durante, M. (2022). Bioactive compounds and antioxidant activities in different fractions of mango fruits (Mangifera indica L., Cultivar Tommy Atkins and Keitt). *Antioxidants*, 11(3), 1-21. http://dx.doi.org/10.3390/antiox11030484. PMid:35326134.

López-Cobo, A., Verardo, V., Diaz-de-Cerio, E., Segura-Carretero, A., Fernández-Gutiérrez, A., & Gómez-Caravaca, A. M. (2017). Use of HPLC- and GC-QTOF to determine hydrophilic and lipophilic phenols in mango fruit (Mangifera indica L.) and its by-products. *Food Research International*, 100(Pt 3), 423-434. http://dx.doi.org/10.1016/j.foodres.2017.02.008. PMid:28964365.

Magalhães, L. M., Santos, F., Segundo, M. A., Reis, S., & Lima, J. L. F. C. (2010). Rapid microplate high-throughput methodology for assessment of Folin-Ciocalteu reducing capacity. *Talanta*, 83(2), 441-447. http://dx.doi.org/10.1016/j.talanta.2010.09.042. PMid:21111158.

Marcillo-Parra, V., Anaguano, M., Molina, M., Tupuna-Yerovi, D. S., & Ruales, J. (2021). Characterization and quantification of bioactive compounds and antioxidant activity in three different varieties of mango (Mangifera indica L.) peel from the Ecuadorian region using

HPLC-UV/VIS and UPLC-PDA. *NFS Journal*, 23, 1-7. http://dx.doi.org/10.1016/j.nfs.2021.02.001.

Martins, S. H. F., Pontes, K. V., Fialho, R. L., & Fakhouri, F. M. (2022). Extraction and characterization of the starch present in the avocado seed (Persea americana mill) for future applications. *Journal of Agriculture and Food Research*, 8(January), 100303. http://dx.doi.org/10.1016/j.jafr.2022.100303.

Monribot-Villanueva, J. L., Elizalde-Contreras, J. M., Aluja, M., Segura-Cabrera, A., Birke, A., Guerrero-Analco, J. A., & Ruiz-May, E. (2019). Endorsing and extending the repertory of nutraceutical and antioxidant sources in mangoes during postharvest shelf life. *Food Chemistry*, 285(January), 119-129. http://dx.doi.org/10.1016/j.foodchem.2019.01.136. PMid:30797326.

Peng, D., Zahid, H. F., Ajlouni, S., Dunshea, F. R., & Suleria, H. A. R. (2019). LC-ESI-QTOF/MS Profiling of australian mango peel by-product polyphenols and their potential antioxidant activities. *Processes (Basel, Switzerland)*, 7(10), 764. http://dx.doi.org/10.3390/pr7100764.

Pérez-Chabela, M. de L., Cebollón-Juárez, A., Bosquez-Molina, E., & Totosaus, A. (2022). Mango peel flour and potato peel flour as bioactive ingredients in the formulation of functional yogurt. *Food Science and Technology (Brazil)*, 42, e38220. http://dx.doi.org/10.1590/fst.38220.

Safdar, M. N., Kausar, T., Nadeem, M., Murtaza, M., Sohail, S., Mumtaz, A., Siddiqui, N., Jabbar, S., & Afzal, S. (2022). Extraction of phenolic compounds from (Mangifera indica L.) and kinnow (Citrus reticulate L.) peels for the development of functional fruit bars. *Food Science and Technology (Brazil)*, 42, 1-8. http://dx.doi.org/10.1590/fst.09321.

Sánchez-Mesa, N., Sepúlveda-Valencia, J. U., Ciro-Velásquez, H. J., & Meireles, M. A. (2020). Bioactive compounds from mango peel (Mangifera indica L. var. tommy atkins) obtained by supercritical fluids and pressurized liquids extraction. *Revista Mexicana de Ingeniería Química*, 19(2), 755-766. http://dx.doi.org/10.24275/rmiq/Alim657.

Sauthier, M. C. S., Silva, E. G. P., Santos, B. R. S., Silva, E. F. R., Caldas, J. C., Minho, L. A. C., Santos, A. M. P., & Santos, W. N. L. (2019). Screening of Mangifera indica L. functional content using PCA and neural networks (ANN). *Food Chemistry*, 273, 115-123. http://dx.doi.org/10.1016/j.foodchem.2018.01.129. PMid:30292356.

Serna-Cock, L., García-Gonzales, E., & Torres-León, C. (2016). Agro-industrial potential of the mango peel based on its nutritional and functional properties. *Food Reviews International*, 32(4), 364-376. http://dx.doi.org/10.1080/87559129.2015.1094815.

Silva, E. S., Santos, H. B. Jr., Guedes, T. J. F. L., Sandes, R. D. D., Rajan, M., Leite, M. T. S. Na., & Narain, N. (2022). Comparative analysis of fresh and processed mango (Mangifera indica L, cv. "Maria") pulps: influence of processing on the volatiles, bioactive compounds and antioxidant activity. *Food Science and Technology (Brazil)*, 42, 1-10. http://dx.doi.org/10.1590/fst.54020.

Singleton, V., Orthofer, R., & Lamuela-Raventós, R. M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. *Methods in Enzymology*, 299, 152-178. http://dx.doi.org/10.1016/S0076-6879(99)99017-1.

Tuisima Coral, L. L., & Escobar-Garcia, H. A. (2021). Characterization of fruits of varieties of mango (Mangifera indica) conserved in Peru. *Revista Brasileira de Fruticultura*, 43(2), 1-8. http://dx.doi.org/10.1590/0100-29452021710.