



**Bioactive compounds from mango peel (*Mangifera indica* L. var. Tommy Atkins) obtained by supercritical fluids and pressurized liquids extraction**

**Compuestos bioactivos de cáscara de mango (*Mangifera indica* L. var. Tommy Atkins) obtenidos de fluidos supercríticos y extracción presurizada de líquidos**

N. Sánchez-Mesa<sup>1\*</sup>, J.U. Sepúlveda-Valencia<sup>1</sup>, H.J. Ciro-Velásquez<sup>1</sup>, M.A. Meireles<sup>2</sup>

<sup>1</sup>Universidad Nacional de Colombia, Faculty of Agricultural Sciences, Department of Food and Agricultural Engineering, Road 65 # 59A - 110, 050034, Colombia..

<sup>2</sup>University of Campinas, Faculty of Food Engineering, Department of Food Engineering, Street Monteiro Lobato, 80, 13083-862, SP-Brazil.

Received: June 21, 2019; Accepted: October 11, 2019

**Abstract**

Currently, large quantities of by-products of mango are generated due to the high consumption of this fruit worldwide. In order to give it an added value, two "clean" technologies: supercritical fluid extraction (SFE) and pressurized liquid extraction (PLE) were evaluated for obtaining their analytes. Results indicated that although higher yields were obtained by PLE, in general a higher concentration of compounds (gallotannins, flavonoids, xanthones, gallic acid, etc.) was obtained by SFE, except for gallic acid due to its high solubility in pressurized water. The best results for SFE were obtained at 50 °C, 20 MPa and co-solvent flow rate corresponding to 20% of the CO<sub>2</sub> flow; while for PLE the best condition was at 6.67 g min<sup>-1</sup> Milli-Q water, 40 °C and 10 MPa. This work provides additional information on the phytochemical composition of Brazilian Tommy Atkins mango peel and its possible use as a functional ingredient.

*Keywords:* Analytes, by-product, extraction, mango, Tommy Atkins.

**Resumen**

En la actualidad se generan grandes cantidades de cáscara de mango debido al alto consumo de este fruto. Con el fin de darle un valor agregado se evaluaron dos tecnologías "limpias", extracción con fluidos supercríticos (EFS) y con líquidos presurizados (ELP) para el aprovechamiento de sus analitos. Los resultados indicaron que aunque por ELP se obtuvieron rendimientos más altos, en general se obtuvo una mayor concentración de compuestos por EFS (galotaninos, flavonoides, xantonas, etc.), excepto para el ácido gálico debido a su alta solubilidad en el agua presurizada. Los mejores resultados para EFS se obtuvieron a 50 °C, 20 MPa y flujo de co-solvente correspondiente al 20% del flujo de CO<sub>2</sub>, mientras que para ELP la mejor condición fue a 6.67 g min<sup>-1</sup> agua Milli-Q, 40 °C y 10 MPa. Este trabajo proporciona información adicional sobre la composición fitoquímica del extracto de la cáscara de mango Tommy Atkins brasileño y su posible uso como ingrediente funcional.

*Palabras clave:* Analitos, sub-producto, extracción, mango, Tommy Atkins.

**1 Introduction**

Mango (*Mangifera indica* L.) is one of the most important tropical fruits in the world in terms of production and acceptance by consumers. It is grown in 90 countries around the world, ranking fifth in world production, among other important fruit crops including bananas, citrus fruits, grapes and

apples (Ribeiro *et al.*, 2008; Ibarra *et al.*, 2015; Santhirasegaram *et al.*, 2015; Morales-de la Peña *et al.*, 2018). Mango processing industry generates high amounts of by-products and their elimination represents both a cost to the food processor and a negative impact on the environment like another by-products (Dávila-Hernández *et al.*, 2019). The main by-products produced in mango fruits processing are peels and seeds that represent between 35% and 60% of the whole fruit (Meneses *et al.*, 2015).

\* Corresponding author. E-mail: nysanche@unal.edu.co

Tel. 574 430-91-70

<https://doi.org/10.24275/rmiq/Alim657>

issn-e: 2395-8472

Research over the past 20 years has revealed that many of these by-products could serve as a potentially valuable source of bioactive compounds (Ruiz *et al.*, 2014); other studies report the use of these residues as a source of pectin, dietary fiber and biogas production (Berardini *et al.*, 2005). Peel is one of the most important by-products during the process, constituting approximately 15-20 g 100 g<sup>-1</sup> of the fresh fruit weight.

The phytochemical profile of mango peel contains polyphenols, carotenoids and vitamins with different properties and health benefits, mainly due to antioxidant activity of these compounds (Robles-Sánchez *et al.*, 2009; Dos Santos *et al.*, 2013). Different polyphenolic compounds such as gallates, gallotannins, flavonoids, xanthenes, benzophenones, gallic acid and derivatives have been identified in extracts of mango peel (Schieber *et al.*, 2003; Berardini *et al.*, 2004, 2005b; Ajila *et al.*, 2010; Dorta *et al.*, 2014; Mercado-Mercado *et al.*, 2019). Berardini *et al.* (2005b) showed phenolic composition and main compounds of mango peel, such as mangiferin, mangiferin derivatives, quercetin and quercetin derivatives (Meneses *et al.*, 2015). Mangiferin is a xanthone-C-glucoside and the main phenolic compound of mango. It is an interesting active compound due to its pharmacological properties such as antioxidants, antiallergic, anti-inflammatory, antimicrobial, antitumor, antidiabetic and radioprotective, immunomodulatory effects, among others (Wauthoz *et al.*, 2007; Rajendran *et al.*, 2008, Acosta *et al.*, 2009; Ling *et al.*, 2009; Yang *et al.*, 2016).

Various extraction techniques can be applied to obtain bioactive compounds. Traditional methods include Soxhlet extraction process, maceration and solid-liquid extraction used in industrial processes, however, they often consume a lot of time, require relatively large amounts of organic solvents, as well as prolonged operation periods (Prado *et al.*, 2013; Ruiz-Montanez *et al.*, 2014), causing a possible negative effect on the activity as thermal and chemical degradation of labile compounds, and harmful residue of the solvent that affects the quality and safety of the extract. In fact, the direct use of mango extracts in food or pharmaceutical industry is not allowed without quality guarantees of low toxic solvent residues (Dorta *et al.*, 2012; Wijngaard *et al.*, 2012). According to Gao and Liu (2005), emerging extraction methods are based on improving the efficiency of traditional methods employing physical action on the material.

Pressurized liquids extraction (PLE) and supercritical fluids extraction (SFE) present advantages in comparison with traditional extraction techniques, since degradation and decomposition of active compounds at reduced temperatures are avoided in the absence of light and oxygen. In the case of the SFE, it is possible to modulate the solvent power of supercritical carbon dioxide to perform a selective extraction. However, carbon dioxide (CO<sub>2</sub>) has a very limited ability to dissolve polar and high molecular weight compounds; this limitation can be overcome with the use of co-solvents (Meneses *et al.*, 2015). The use of CO<sub>2</sub> as a supercritical solvent is safe (GRAS = Generally Recognized as Safe), non-flammable, non-toxic and inexpensive, highly compressible with a surface tension that promotes better penetration in the matrix compared to conventional processes (Raventós *et al.*, 2002). On the other hand, PLE uses liquid solvents at elevated temperature and pressure, which produces a reduction in the surface tension of the solvent and facilitates its penetration into the pores of the matrix, the process causes its disruption, therefore, it improves mass transfer of the analyte from the sample to the solvent (Mustafa *et al.*, 2012).

Because the characterization of compounds present in mango by-products is also important for the integral use of natural resources, and that peels are not used currently for commercial purposes, their use could be an important and sustainable opportunity not only for reducing pollution but also for the extraction of bioactive compounds that promote health and development of enriched foods. Therefore, the aim of this work was to obtain extracts from mango peels by SFE and PLE to study the influence of operation parameters of the process on the overall yield and metabolites extraction such as gallic acid, ellagic acid, quercetin and mangiferin.

## 2 Materials and methods

### 2.1 Raw Material

Mango fruits (*Mangifera indica* L. var. Tommy Atkins) were obtained in 'Casa da Uva' market from 'Frutale importação e exportação Ltda.' supplier located in Itaberaba, Juazeiro (Brazil). Peels were removed with a sharp knife and the underlying pulp was removed by scraping gently with the blunt edge of the knife. To obtain the mature peel (complete maturity or consumption phase), fruits were allowed

to mature at room temperature (25 °C) and were considered optimal for their use when reaching a total soluble solids (TSS) of 12-14 °Brix.

## 2.2 Preparation of the sample

Peels obtained were dried at room temperature (25 °C) in trays for 30 hours, until reaching a moisture content of 25% w.b. (wet basis). Then a cryogenic grinding process (Marconi model MA340) was performed at -25 °C and sieved through four sieves with different sizes (1.00, 1.41, 2.00 and 2.38 mm) to obtain a more uniform particle size; fines were also collected (Catel 452 vibration sieve).

## 2.3 Moisture content

The water content of peels was determined by gravimetric method according to AOAC 931.04, in a vacuum oven (Tecnal, model TE-3951) at 60 °C until constant weight was obtained. The determination was made in triplicate.

## 2.4 Supercritical fluid extraction (SFE)

Extraction experiments were carried out using a two pumps unit (CO<sub>2</sub> and modifier). The extractor was pressurized with fluids (CO<sub>2</sub> + ethanol as modifier) and in up flow. The system was equipped with temperature controllers, pressure valves and CO<sub>2</sub> flow meter. Finally, extracted compounds were transported to a separation vessel. For the extractions, 20 g of mango peel was used in the extraction vessel. The flow of CO<sub>2</sub> was set at 2,000 mL h<sup>-1</sup>, static time 20 min and dynamic time 90 min for all experiments.

## 2.5 Pressurized liquid extraction (PLE)

Extraction experiments were carried out with the same SFE system, using only the modifier pump. In this case, the extractor was pressurized with Milli-Q® water. The extraction process was the same used for SFE. For all experiments, a sample size of 20 g of peel was used, static time 20 min and dynamic time 90 min.

## 2.6 Determination of global yield (X<sub>0</sub>)

In the case of SFE, the extracts were collected in bottles and then evaporated (WB Laborota 4001, Heidolph and CH 9230, Buchi, Flawil, Switzerland, with vacuum pump control Rotavac, Heidolph, Instruments, GmbH, Viertrieb, Germany) at a temperature of 50 °C, to completely eliminate

the ethyl alcohol used as co-solvent. Extracts obtained by PLE were lyophilized (Liotop L101) at -44 °C to eliminate the water content. The mass of extracts contained in the bottles was measured using an analytical balance (Sartorius Analytic A200S, ± 0.0001 Sartorius GmbH Göttingen, Germany). The overall yield (X<sub>0</sub>) was determined by relating total mass (M<sub>extract</sub>) of the extract and feed mass of the raw material on a dry basis (M<sub>sample</sub>) according to the Eq. (1):

$$X_0 = \left( \frac{M_{extract}}{M_{sample}} \right) \times 100 \quad (1)$$

## 2.7 Quantification by HPLC

Analyzes were carried out in an HPLC system (Finningan Surveyor Plus system Thermo Electron Corporation) equipped with a diode detector (PDA). Separation was performed on a C18 Kinetex Phenomenex column (2.6 μm, 100 x 4.6 mm). The temperature of the column oven was set at 40 °C, an injection volume of 5 μL, and a flow rate of 1 mL min<sup>-1</sup> were used along the gradient. The mobile phase used was A: water Milli-Q acidified 1% acetic acid and B: acetonitrile acidified 1% acetic acid. The following gradient was applied: 0 min, 10% B; 14 min, 22% B; 15 min, 38% B; 20 min, 90% B; 23 min, 10% B. Initial conditions were maintained for 3 min and spectral measurements were made in the range of 220-450 nm. The content of gallic acid (GA), ellagic acid (EA), mangiferin (M) and quercetin (Q) was quantified; using calibration curves obtained from commercial standards in different concentration levels from 6 to 200 μg mL<sup>-1</sup>. For each concentration, three repetitions were prepared and injected.

## 2.8 Statistical design

In order to evaluate the effect of extraction process variables on the performance and concentration of bioactive compounds, a complete factorial design was carried out, in the case of SFE: temperature (40 and 50 °C), pressure (10, 15 and 20 MPa) and volume of co-solvent (ethanol 5, 10 and 15%) were evaluated, and in PLE: temperature (40 and 50 °C), pressure (10, 15 and 20 MPa) and solvent flow rate of extraction (100, 200 and 400 g h<sup>-1</sup> of water).

Statgraphics Centurion XVII software (USA) was used to analyze the results. The following model of second order was used to fit the data:

$$Y = \beta_0 + \sum_{i=1}^n \beta_i X_i + \sum_{i=1}^n \beta_{ii} X_i^2 + \sum_{i < j=2}^n \sum_{i=1}^n \beta_{ij} X_i X_j \quad (2)$$

where Y is the answer as a function of the independent factors or variables ( $X_i$ ),  $\beta_0$  is the coefficient for the intercept,  $\beta_i$  are the linear coefficients,  $\beta_{ii}$  are coefficients of the double interaction of each factor and  $\beta_{ij}$  is the coefficient of the product of the interactions of the facts. The adequacy of the model was predicted through regression analysis ( $R^2$ ) and ANOVA ( $p < 0.05$ ).

### 3 Results and discussion

#### 3.1 Yield

Fig. 1 shown the global yields of the extractions studied by both technologies. Higher yield was evident by using a larger amount of co-solvent (20% ethanol for SFE and 6.67 g min<sup>-1</sup> water for PLE). The increases in ethanol percentage promotes a higher SFE yield due to increased solubility of the compounds in the solvent. CO<sub>2</sub> being a nonpolar molecule restricts low polarity substance extractions (Paula *et al.*, 2014; García-Mendoza *et al.*, 2015). The addition of a co-solvent of polar characteristic together with supercritical extraction, makes polar compounds not extracted by pure carbon dioxide to be solubilized by the mixture of CO<sub>2</sub> and ethanol (co-solvent) (Souza, 2015). Likewise, the use of organic solvents at high pressures can favor mass transfer of the solutes to the solvent, due to an increase in the interaction between the solvent and the matrix, as well as solvent power, thus improving extraction yields (Mustafa and Turner, 2011; Luthria, 2012).

According to the statistical model and the ANOVA (table 1 and 2), the results show that with supercritical fluids, the extract with the highest yield (8.2%) was obtained using 20 MPa, 50 °C and 20% ethanol, the following higher values were obtained working at pressures of 15 and 20 MPa at 40 °C and 20% ethanol (8.1% and 8.1% respectively).

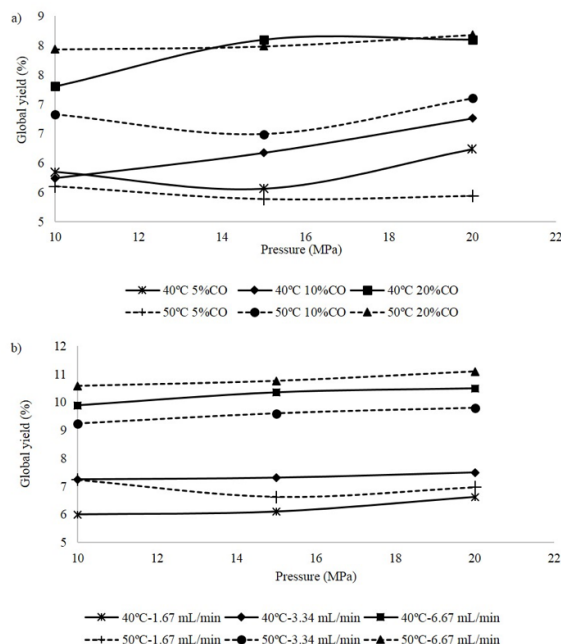


Fig. 1. Extraction curves for supercritical extraction with CO<sub>2</sub> and co-solvent (CO) (a) and pressurized water (PE) (b).

The lowest yield obtained was in the condition of 15 MPa, 50 °C and 5% ethanol. As for PLE, the best yields (11.1, 10.8 and 10.6%) were obtained using higher flow rate (6.67 g min<sup>-1</sup> water), temperature (50 °C) and pressure (20, 15 and 10 MPa) and the lowest yield (6.00%) was obtained in the lowest conditions (1.67 g min<sup>-1</sup> water, 40 °C and 10 MPa). The extraction yield was slightly lower than those reported using conventional methods by different authors who have found yields of mango peel from 4.6 to 18.5 g 100 g<sup>-1</sup> with solvents such as HCl, ethanol, water and mixtures of both (Koubala *et al.*, 2008; Dorta *et al.*, 2012; Garcia-Mendoza *et al.*, 2015). This behavior was reported by Barreto *et al.* (2008), however, this conventional overall performance does not differentiate between extraction of functional and non-functional compounds.

In general, results show a positive effect of the flow on the yield of the extracts, since their increase decreases mass transfer resistance (Yin *et al.*, 2005). Likewise, several studies have shown that there is an optimum flow with which solvent is saturated just before leaving the extractor.

Table 1. p-value and R<sup>2</sup> of each variable in SFE.

| Variable           | Main effects |        |         | Interaction effects |        |         |        |         | R <sup>2</sup> (adj) |
|--------------------|--------------|--------|---------|---------------------|--------|---------|--------|---------|----------------------|
|                    | A            | B      | C       | AA                  | AB     | AC      | BC     | CC      |                      |
| Yield (%)          | 0.0000*      | 0.3772 | 0.0621* | 0.6359              | 0.2832 | 0.5576* | 0.1742 | 0.4660* | 0.93                 |
| Mangiferin (μg/g)  | 0.0627*      | 0.2506 | 0.5089* | 0.4914              | 0.6076 | 0.5479* | 0.3316 | 0.1619* | 0.85                 |
| Gallic ac. (μg/g)  | 0.0083*      | 0.4226 | 0.0479* | 0.0692              | 0.5116 | 0.0299* | 0.7992 | 0.0193* | 0.8                  |
| Ellagic ac. (μg/g) | 0.0001*      | 0.5529 | 0.4231* | 0.97                | 0.7673 | 0.5916* | 0.7715 | 0.3613* | 0.91                 |
| Quercetin (μg/g)   | 0.0000*      | 0.051  | 0.5105* | 0.1196              | 0.2541 | 0.8202* | 0.1533 | 0.8670* | 0.87                 |

A: co-solvent flow (ethanol); B: temperature; C: pressure

\* Significant at  $p < 0.05$ .

Table 2. p-value and R<sup>2</sup> of each variable in PLE.

| Variable           | Main effects |         |        | Interaction effects |        |        |        |        | R <sup>2</sup> (adj) |
|--------------------|--------------|---------|--------|---------------------|--------|--------|--------|--------|----------------------|
|                    | A            | B       | C      | AA                  | AB     | AC     | BC     | CC     |                      |
| Yield (%)          | 0.0000*      | 0.0017* | 0.2344 | 0.0813              | 0.465  | 0.6385 | 0.7211 | 0.7215 | 0.91                 |
| Mangiferin (μg/g)  | 0.0000*      | 0.0028* | 0.1798 | 0.34                | 0.7286 | 0.2396 | 0.0801 | 0.6202 | 0.9                  |
| Gallic ac. (μg/g)  | 0.0008*      | 0.3234* | 0.3111 | 0.721               | 0.2789 | 0.4469 | 0.0916 | 0.6276 | 0.89                 |
| Ellagic ac. (μg/g) | 0.0830*      | 0.0522* | 0.1377 | 0.1242              | 0.7246 | 0.3821 | 0.0878 | 0.3078 | 0.88                 |

A: flow; B: temperature; C: pressure

\* Significant at  $p < 0.05$ .

With larger flows there is not enough contact time to saturate the solvent and, for smaller flows, the axial dispersion and the interfacial mass coefficient decrease the concentration of the solute (Rosa and Meireles, 2009). Although results show that the increase in pressure and temperature generally favor performance, in the case of SFE only co-solvent percentage affected it significantly ( $p < 0.05$ ) while in PLE both co-solvent flow rate and temperature had a significant effect ( $p < 0.05$ ).

The increases of pressure can improve the yield due to the tendency of solubility to increase with pressure (Sovová and Aleksovski, 2007). The yields increased with temperature, except for the 5% ethanol flow in the SFE, where the yields of extracts 1, 2 and 3 were higher than those of extracts 4, 5 and 6. This may be due to the fact that the effect of solvent density was significantly greater than that caused by the increase in vapor pressure of the solute with temperature (Meireles *et al.*, 2005). This behavior is known as retrograde phenomenon and has been reported by various authors (Michielin *et al.*, 2005; Almeida and Ferreira, 2007; Michielin, 2009; Prado *et al.*, 2013). Likewise, a possible inversion region is observed at the point corresponding to the pressure of 15 MPa and 20% ethanol, where yield increases with the reduction of temperature, predominantly the effect of solvent density. After this region, up to 20 MPa the effect of vapor pressure is dominant.

### 3.2 Characterization of analytes

Table 3 shows the content of mangiferin (M), gallic acid (GA), ellagic acid (EA) and quercetin (Q) of extracts obtained by SFE and PLE. Data are expressed on a dry basis.

#### 3.2.1 Mangiferin

HPLC analyzes showed that mango peel extracts obtained by SFE have a much higher mangiferin content than those obtained by PLE. For the first method, results are in the range of 964.26-2,131.73 μg g<sup>-1</sup> DM (DM = dry matter), while for PLE it was between 59.33-108.57 μg g<sup>-1</sup> DM. These values are within or above the range reported by Berardini *et al.* (2005), who reported values of 11.2-1,297 μg g<sup>-1</sup> DM as characteristic of the compound, given that mangiferin is the main polyphenolic compound present in mango peel; and it is lower in the case of PLE than the found by Meneses *et al.* (2015) who reported a value of 218.24 μg g<sup>-1</sup> DM by solid-liquid extraction for 6 hours, followed by a subsequent adsorption/desorption stage to purify polyphenolic compounds fraction. It is important to note that in SFE, although the ethanol percentage had the greatest influence on extraction, no factor had a significant effect; contrary to PLE in which both water flow and temperature had a significant effect ( $p < 0.05$ ) in mangiferin extractive process.



Table 3. Concentrations of biocompounds by the extraction processes studied.

| Run | SFE                                     | PLE    | SFE                                     | PLE      | SFE                                      | PLE      | SFE                                    |
|-----|---|--------|---|----------|--|----------|--|
|     | <b>Mangiferin</b><br>$\mu\text{g/g DM}$ |        | <b>Gallic ac.</b><br>$\mu\text{g/g DM}$ |          | <b>Ellagic ac.</b><br>$\mu\text{g/g DM}$ |          | <b>Quercetin</b><br>$\mu\text{g/g DM}$ |
| 1   | 1,719.67                                | 61.54  | 718.72                                  | 4,872.85 | 2,053.51                                 | 3,207.66 | 1,978.74                               |
| 2   | 1,813.96                                | 94.48  | 543.38                                  | 5,757.28 | 2,405.64                                 | 3,204.21 | 1,816.68                               |
| 3   | 2,131.73                                | 93.74  | 566.18                                  | 6,411.58 | 3,099.90                                 | 3,134.76 | 2,050.86                               |
| 4   | 1,911.34                                | 70.74  | 588.85                                  | 5,705.03 | 2,912.26                                 | 3,684.08 | 2,145.94                               |
| 5   | 1,330.75                                | 77.11  | 621.93                                  | 5,778.19 | 2,963.31                                 | 2,937.45 | 1,992.02                               |
| 6   | 1,609.92                                | 71.47  | 728.84                                  | 6,052.22 | 3,047.03                                 | 3,736.91 | 2,090.39                               |
| 7   | 1,571.30                                | 104.09 | 662.18                                  | 5,848.58 | 3,315.30                                 | 2,316.04 | 1,738.90                               |
| 8   | 1,861.61                                | 102.61 | 601.74                                  | 6,054.68 | 3,499.28                                 | 2,545.12 | 1,822.16                               |
| 9   | 2,008.62                                | 106.48 | 759.92                                  | 8,678.50 | 3,643.91                                 | 2,908.39 | 1,276.27                               |
| 10  | 2,016.84                                | 101.29 | 712.47                                  | 6,675.91 | 3,452.37                                 | 3,693.76 | 1,661.09                               |
| 11  | 1,460.49                                | 78.08  | 565.74                                  | 7,680.74 | 3,237.73                                 | 3,334.97 | 1,620.58                               |
| 12  | 1,527.27                                | 59.33  | 641.57                                  | 5,392.45 | 3,367.57                                 | 2,196.63 | 1,674.44                               |
| 13  | 1,452.46                                | 108.57 | 337.55                                  | 9,106.22 | 5,050.38                                 | 2,997.95 | 1,307.09                               |
| 14  | 1,553.71                                | 100.22 | 402.4                                   | 7,542.44 | 4,844.40                                 | 2,178.07 | 1,067.17                               |
| 15  | 1,380.88                                | 98.3   | 606.95                                  | 9,261.19 | 4,237.63                                 | 2,744.21 | 1,050.34                               |
| 16  | 1,277.34                                | 95.62  | 483.25                                  | 7,751.56 | 4,807.97                                 | 3,589.32 | 1,294.77                               |
| 17  | 964.26                                  | 94.18  | 394.99                                  | 7,618.98 | 3,967.96                                 | 3,001.99 | 1,518.32                               |
| 18  | 1,870.90                                | 90.24  | 694.8                                   | 7,669.64 | 5,861.52                                 | 2,721.10 | 1,574.29                               |

Quercetin was not detected by PLE.

SFE process was more effective in this case, mainly due to the polarity of this compound. During dissolution, the cohesive energy of bonds holding solute together and the energy cost of the solvent-solvent bond breaking must be overcome by the cohesive energy released by the formation of solute-solvent bonds. If these energies are approximately equal, what happens when solute and solvent are structurally similar, then the solute will dissolve in the solvent. The molecular structure of mangiferin shows that it is a molecule with intermediate polarity; therefore, it can dissolve in ethanol more easily than in water, as has been reported by several authors (Acosta *et al.*, 2009; Acosta *et al.*, 2016). From the results obtained it can also be observed that mango peel is a good source of mangiferin, according as reported by Luo *et al.* (2012).

### 3.2.2 Gallic acid

Contrary to the results obtained for M, the content of GA was significantly higher for the PLE technology. The high polarity of this compound and its affinity for water improved its recovery by using this pressurized solvent. In this case, the extracts obtained varied in the range of 4,872.85-9,261.19  $\mu\text{g g}^{-1}$  DM, where the flow rate of co-solvent (ethanol) has a statistically

significant effect ( $p < 0.05$ ). The values of this study are higher than those reported by Palafox *et al.* (2012) in mango pulp (946 to 987  $\mu\text{g g}^{-1}$  DM).

For SFE, results were between 337.6-759.9  $\mu\text{g g}^{-1}$  DM and were affected ( $p < 0.05$ ) by flow and pressure. Results obtained indicate that this phenolic acid was not sensitive to temperature under the conditions studied and, on the contrary, pressurized water favored its extraction due to its high-water solubility (Li *et al.*, 2016). Miron *et al.* (2011) extracted phenolic compounds from oregano leaves (*Origanum vulgare*) by PLE at different temperatures and proportions of ethanol/water solvents and obtained the highest amount of phenols and the highest antioxidant activity, expressed as GAE (gallic acid equivalents) when using 100% water, suggesting that, among others, the solvent influenced both the yield of the extract and its quality. Other authors have also reported the potential of this technique to extract polyphenols from fruit and vegetable by-products (King and Grabiell, 2007; Moraes *et al.*, 2013; Ibarra *et al.*, 2015).

### 3.2.3 Ellagic acid

It is a common tannin in mangoes and has been identified in different species (Arogba, 2000; Sun and Chen, 2012; Pierson *et al.*, 2014). Among the results

obtained by both technologies, it is observed that there was not a big difference compared to previous compounds: 2,178.1-3,736.9  $\mu\text{g g}^{-1}$  DM for PLE and 2,053.5-5,861.5  $\mu\text{g g}^{-1}$  DM for SFE. Only the flow rate had a significant effect ( $p < 0.05$ ) during SFE, showing a directly proportional relationship. In this case, CO<sub>2</sub> and ethanol as a co-solvent demonstrated its effectiveness in separating a larger fraction of EA from mango peels. Soong and Barlow (2004; 2006) reported values between 31-1,180  $\mu\text{g EA g}^{-1}$  DM seed, for mango seed extracts; likewise, they suggested that ethanol is a good solvent for its extraction and indicated that a high yield of this compound could contribute to a greater antioxidant activity of ethanolic extracts in comparison with methanolic extracts. López-Cobo *et al.* (2017) quantified by HPLC-DAD-QTOF-M several free polar compounds, including the EA in pulp, peel, seed and pod in three different mango crops and did not detect this phenolic acid in the peel of any of the three crops.

### 3.2.4 Quercetin

This compound was only found in extracts obtained by SFE as shown in Table 3. Due to its polar nature, ethanol is more efficient solvent compared with water. Values between 1,050.3 and 2,145.9  $\mu\text{g g}^{-1}$  DM were reached, the highest concentration was obtained at 50 °C, 10 MPa and 5% ethanol. This last variable was the only one with a significant effect ( $p < 0.05$ ) in the extractive process, showing that Q presents a behavior inversely proportional to the percentage of ethanol. SFE process could promote changes in the interactions between solutes and the matrix, allowing to better solubilize polar phenolic compounds, to facilitate its extraction with the help of co-solvent. Results obtained in this study are higher to those found by Rodríguez *et al.* (2017) and Meneses *et al.* (2015) who reported values of  $960.61 \pm 37.72 \mu\text{g g}^{-1}$  DM and 480.8-612  $\mu\text{g g}^{-1}$  DM respectively, in mango by-products consisting of peel and pulp remnant. The identification of flavonoids in mango peel and to a lesser extent in its pulp is reported; both show important differences in the content of flavonoids, phenolic acids and other antioxidants according to Masibo and He (2008). On the other hand, Ajila *et al.* (2010) reported that ellagic acid, gallic acid and quercetin are the main polyphenols in green and mature mango peel extracts.

## Conclusions

For SFE the co-solvent flow and CO<sub>2</sub> pressure were significant ( $p < 0.05$ ) while in PLE the flow and temperature of water had a significant effect ( $p < 0.05$ ). The regression equations developed had high R<sup>2</sup> ( $\geq 0.8$ ) indicating a good model fit. The results showed that the optimum conditions extraction of the phytochemicals evaluated for SFE was obtained at 50 °C, 20 MPa and 20% ethanol and for PLE at 50 °C, 10 MPa and 6.67 g min<sup>-1</sup> Milli-Q water. Likewise, greater concentration was reached by SFE for almost all compounds while by PLE a higher yield was achieved, as well as a higher concentration of gallic acid due to its water solubility, but no quercetin was detected. The yield and the amounts of mangiferin, gallic acid, ellagic acid and quercetin could be enhanced by controlling appropriately the extraction variables evaluated. Therefore, both techniques could be combined successfully to extract higher concentrations of bioactive compounds from mango peel.

## Acknowledgements

This work was supported financially by the CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico), Brazil. Likewise, we would like to thank COLCIENCIAS, Colombia (Call 647 - 2014) for the financial support provided during the internship.

## Nomenclature

|                 |   |
|-----------------|---|
| SFE             | supercritical fluids extraction                       |
| PLE             | pressurized liquids extraction                        |
| DM              | dry matter  |
| ABTS            | 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonicacid) |
| DPPH            | 2,2-diphenyl-1-picrylhydrazyl                         |
| TEAC            | Trolox Equivalent Antioxidant Capacity                |
| CO <sub>2</sub> | carbon dioxide (MPa)                                  |
| Q               | quercetina ( $\mu\text{g g}^{-1}$ DM)                 |
| EA              | ellagic acid ( $\mu\text{g g}^{-1}$ DM)               |
| GA              | gallic acid ( $\mu\text{g g}^{-1}$ DM)                |
| M               | mangiferina ( $\mu\text{g g}^{-1}$ DM)                |

## References

- Acosta, J., Nuevas, L., Amaro, D. and Álvarez, J. (2009). Estudio del proceso de lixiviación de la corteza vegetal de *Mangifera indica* L. *Latin American Journal of Pharmacy* 28, 27-31.
- Acosta, J., Sevilla, I., Salomón, S., Nuevas, L., Romero, A. and Amaro, D. (2016). Determination of mangiferin solubility in solvents used in the biopharmaceutical industry. *Journal of Pharmacy & Pharmacognosy Research* 4, 49-53.
- Ajila, C.M., Jaganmohan Rao, L., and Prasada Rao, U.J.S. (2010). Characterization of bioactive compounds from raw and ripe *Mangifera indica* L. peel extracts. *Food and Chemical Toxicology* 48, 3406-3411. <https://doi.org/10.1016/j.fct.2010.09.012>
- Almeida, P.P. De. and Ferreira, S.R.S. (2007). Crossover pressure for supercritical fluid extraction of spearmint (*Mentha spicata* L.) essential oil with pure CO<sub>2</sub> and CO<sub>2</sub> plus ethanol. In: I Iberoamerican Conference on Supercritical Fluids (PROSCIBA). Foz do Iguaçu, Caderno de Resumos do PROSCIBA.
- Arogba, S.S. (2000). Mango (*Mangifera indica*) kernel: chromatographic analysis of the tannin, and stability study of the associated polyphenol oxidase activity. *Journal of Food Composition and Analysis* 13, 149-156. <https://doi.org/10.1006/jfca.1999.0838>
- Barreto, J.C., Trevisan, M.T., Hull, W.E., Erben, G., De Brito, E.S., Pfundstein, B., Würtele, G., Spiegelhalter, B. and Owen R.W. (2008). Characterization and quantitation of polyphenolic compounds in bark, kernel, leaves, and peel of mango (*Mangifera indica* L.). *Journal of Agricultural and Food Chemistry* 56, 5599-5610. <https://doi.org/10.1021/jf800738r>
- Berardini, N., Carle, R., and Schieber, A. (2004). Characterization of gallotannins and benzophenone derivatives from mango (*Mangifera indica* L. cv. 'Tommy Atkins') peels, pulp and kernels by high performance liquid chromatography / electrospray ionization mass spectrometry. *Rapid Communications in Mass Spectrometry* 18, 2208-2216. <https://doi.org/10.1002/rcm.1611>
- Berardini, N., Fezer, R., Conrad, J., Beifuss, U., Carle, R. and Schieber, A. (2005). Screening of mango (*Mangifera indica* L.) cultivars for their contents of flavonol O- and xanthone C glycosides, anthocyanins and pectin. *Journal of Agricultural and Food Chemistry* 53, 1563-1570. <https://doi.org/10.1021/jf0484069>
- Berardini, N., Knödler, M., Schieber, A., and Carle, R. (2005b). Utilization of mango peels as a source of pectin and polyphenolics. *Innovative Food Science and Emerging Technologies* 6, 442-452. <https://doi.org/10.1016/j.ifset.2005.06.004>
- Dávila-Hernández, G., Sánchez-Pardo, M.E., Gutiérrez-López, G.F., Necochea-Mondragon, H., Ortiz-Moreno, A. (2019). Effect of microwave pretreatment on bioactive compounds extraction from xoconostle (*Opuntia joconostle*) by-products. *Revista Mexicana de Ingeniería Química* 1, 191-204. <https://doi.org/10.24275/uam/izt/dcbi/revmexingquim/2019v18n1/Davila>
- Dorta, E., González, M., Lobo, M. G., Sanchez-Moreno, C., and De Ancos, B. (2014). Screening of phenolic compounds in by-product extracts from mangoes (*Mangifera indica* L.) by HPLC-ESI-QTOF-MS and multivariate analysis for use as a food ingredient. *Food Research International* 57, 51-60. <https://doi.org/10.1016/j.foodres.2014.01.012>
- Dorta, E., Lobo, M.G. and González, M. (2012). Using drying treatments to stabilise mango peel and seed: Effect on antioxidant activity. *LWT - Food Science and Technology* 45, 261-268. <https://doi.org/10.1016/j.lwt.2011.08.016>
- Dos Santos, W.J., Silva, E.A., and Taranto, O.P. (2013). Supercritical fluid extraction from mango (*Mangifera indica* L.) leaves: experiments and modeling. *Chemical Engineering Transactions* 32, 2005-2010. <https://doi.org/10.3303/CET1332335>
- Gao, M., and Liu, C.Z. (2005). Comparison of techniques for the extraction of flavonoids



- from cultured cells of *Saussurea medusa* maxim. *World Journal of Microbiology and Biotechnology* 21, 1461-1463. <https://doi.org/10.1007/s11274-005-6809-1>
- García-Mendoza, M.P., Paula, J.T., Paviani, L.C., Cabral, F. A., and Martínez-Correa, H.A. (2015). Extracts from mango peel by-product obtained by supercritical CO<sub>2</sub> and pressurized solvent processes. *LWT - Food Science and Technology* 62, 131-137. <https://doi.org/10.1016/j.lwt.2015.01.026>
- Ibarra Herrero, M., Sánchez-Camargo, A.P., Cifuentes, A., and Ibáñez, E. (2015). Trends in analytical chemistry plants, seaweeds, microalgae and food by-products as natural sources of functional ingredients obtained using pressurized liquid extraction and supercritical fluid extraction. *Trends in Analytical Chemistry* 71, 26-38. <https://doi.org/10.1016/j.trac.2015.01.018>
- King, J.W. and Grabel, R.D. (2007). *Isolation of Polyphenolic Compounds from Fruits or Vegetable Utilizing Sub-Critical Water Extraction*, US7208181-B1, Washington, DC (USA).
- Koubala, B.B., Kansci, G., Mbome, L.I., Crépeau, M.-J., Thibault, J.-F. and Ralet, M.-C. 2008. Effect of extraction conditions on some physicochemical characteristics of pectins from “Améliorée” and “Mango” mango peels. *Food Hydrocolloids* 22, 1345-1351. <https://doi.org/10.1016/j.foodhyd.2007.07.005>
- Li, S., Chen, F., Jia, J., Liu, Z., Gu, H., Yang, L., and Wang, F. (2016). Ionic liquid-mediated microwave-assisted simultaneous extraction and distillation of gallic acid, ellagic acid and essential oil from the leaves of *Eucalyptus camaldulensis*. *Separation and Purification Technology* 168, 8-18. <https://doi.org/10.1016/j.seppur.2016.05.013>
- Ling, L. T., Yap, S.A., Radhakrishnan, A.K., Subramaniam, T., Cheng, H. M. and Palanisamy, U.D. (2009). Standardised *Mangifera indica* extract is an ideal antioxidant. *Food Chemistry* 113, 1154-1159. <https://doi.org/10.1016/j.foodchem.2008.09.004>
- López-Cobo, A., Verardo, V., Díaz-de-Cerio, E., Segura-Carretero, A., Fernández-Gutiérrez, A., and 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, 423-434. <https://doi.org/10.1016/j.foodres.2017.02.008>
- Luo, F., Lv, Q., Zhao, Y., Hu, G., Huang, G., and Zhang, J. (2012). Quantification and purification of mangiferin from chinese mango (*Mangifera indica* L.) cultivars and its protective effect on human umbilical vein endothelial cells under H<sub>2</sub>O<sub>2</sub>-induced stress. *International Journal of Molecular Sciences* 13, 11260-11274. <https://doi.org/10.3390/ijms130911260>
- Luthria, D.L. (2012). Optimization of extraction of phenolic acids from a vegetable waste product using a pressurized liquid extractor. *Journal of Functional Foods* 4, 842-850. <https://doi.org/10.1016/j.jff.2012.06.001>
- Masibo, M., and He, Q. (2008). Major mango polyphenols and their potential significance to human health. *Comprehensive Reviews in Food Science and Food Safety* 7, 309-319. <https://doi.org/10.1016/j.jff.2012.06.001>
- Meireles, A., Carvalho Jr, R., Moura, L. and Rosa, P. (2005). Supercritical fluid extraction from rosemary (*Rosmarinus officinalis*): Kinetic data, extract's global yield, composition, and antioxidant activity. *The Journal of Supercritical Fluids* 35, 197-204. <https://doi.org/10.1016/j.supflu.2005.01.009>
- Meneses, M.A., Caputo, G., Scognamiglio, M., Reverchon, E. and Adami, R. (2015). Antioxidant phenolic compounds recovery from *Mangifera indica* L. by-products by supercritical antisolvent extraction. *Journal of Food Engineering* 163, 45-53. <https://doi.org/10.1016/j.jfoodeng.2015.04.025>
- Mercado-Mercado, G., Montalvo-González, E., Sánchez-Burgos, J.A., Velázquez-Estrada, R.M., Álvarez-Parrilla, M., González-Aguilar, G.A., Sáyago-Ayerdi S.G. (2019). Optimization of β-carotene from “Ataulfo” mango (*Mangifera*

- indica* L.) By-products using ultrasound-assisted extraction. *Revista Mexicana de Ingeniería Química* 18, 1051-1061. <https://doi.org/10.24275/uam/izt/dcbi/revmexingquim/2019v18n3/Mercado>
- Michielin, E.M.Z. (2009). Obtention of whale grass extract (*Cordia verbenacea* D.C.) by different techniques: measurement of biological activity, mathematical modeling and determination of phase equilibrium. Florianópolis: UFSC. Dissertation (Master in Food Engineering), Department of Chemical Engineering and Food Engineering, Federal University of Santa Catarina. (In Portuguese).
- Michielin, E.M.Z., Bresciani, L.F.V., Danielski, L., Yunes, R. and Ferreira, S.R.S. (2005). Composition profile of horsetail (*Equisetum giganteum* L.) oleoresin: comparing SFE and organic solvents extraction. *Journal of Supercritical Fluids* 33, 131-138. <https://doi.org/10.1016/j.supflu.2004.07.004>
- Miron, T.L., Plaza, M., Bahrim, G., Ibáñez, E. and Herrero, M. (2011). Chemical composition of bioactive pressurized extracts of romanian aromatic plants. *Journal of Chromatography A* 1218, 4918-4927. <https://doi.org/10.1016/j.chroma.2010.11.055>
- Morales-de la Peña, M., Rosas-González, M.C., Martín-Belloso, O., Welti-Chanes, J. (2018). Changes in bioactive compounds concentration and physicochemical properties of mango smoothies treated by ultrasound. *Revista Mexicana de Ingeniería Química* 1, 131-144. <https://doi.org/10.24275/uam/izt/dcbi/revmexingquim/2018v17n1/Morales>
- Mustafa, A., Trevino, L.M. and Turner, C. (2012). Pressurized hot ethanol extraction of carotenoids from carrot by-products. *Molecules* 17, 1809-1818. <https://doi.org/10.3390/molecules17021809>
- Mustafa, A. and Turner, C. (2011). Pressurized liquid extraction as a green approach in food and herbal plants extraction: A Review. *Analytica Chimica Acta* 703, 8-18. <https://doi.org/10.1016/j.aca.2011.07.018>
- Moraes, M.N., Zobot, G. L., Prado, J. M., and Meireles, M.A. (2013). Obtaining antioxidants from botanic matrices applying novel extraction techniques. *Food and Public Health* 3, 195-214. <https://doi.org/10.5923/j.fph.20130304.04>
- Palafox-Carlos, H., Yahia, E.M., and González-Aguilar, G.A. (2012). Identification and quantification of major phenolic compounds from mango (*Mangifera indica*, cv. Ataulfo) fruit by HPLC - DAD - MS / MS-ESI and their individual contribution to the antioxidant activity during ripening. *Food Chemistry* 135, 105-111. <https://doi.org/10.1016/j.foodchem.2012.04.103>
- Paula, J.T., Paviani, L.C., Foglio, M.A., Sousa, I.M.O., Duarte, G.H.B., Jorge, M.P., Eberlinc, M.N., Cabral, F.A. (2014). Extraction of anthocyanins and luteolin from *Arrabidaea chica* by sequential extraction in fixed bed using supercritical CO<sub>2</sub>, ethanol and water as solvents. *The Journal of Supercritical Fluids* 86, 100-107. <https://doi.org/10.1016/j.supflu.2013.12.008>
- Pierson, J.T., Monteith, G.R., Roberts-Thomson, S.J., Dietzgen, R.G., Gidley, M.J., and Shaw, P.N. (2014). Phytochemical extraction, characterisation and comparative distribution across four mango (*Mangifera indica* L.) fruit varieties. *Food Chemistry* 149, 253-263. <https://doi.org/10.1016/j.foodchem.2013.10.108>
- Prado, I.M., Prado, G.H.C., Prado, J. M., and Meireles, M.A.A. (2013). Supercritical CO<sub>2</sub> and low-pressure solvent extraction of mango (*Mangifera indica*) leaves: Global yield, extraction kinetics, chemical composition and cost of manufacturing. *Food and Bioprocess Processing* 91, 656-664. <https://doi.org/10.1016/j.fbp.2013.05.007>
- Rajendran, P., Ekambaram, G., and Sakthisekaran, D. (2008). Effect of mangiferin on benzo (a) pyrene induced lung carcinogenesis in experimental Swiss albino mice. *Natural Product Research* 22, 672-680. <https://doi.org/10.1080/14786410701824973>
- Raventós, M., Duarte, S., and Alarcón, R. (2002). Application and Possibilities of Supercritical CO<sub>2</sub> Extraction in Food Processing Industry: An Overview. *Food Science and Technology*

- International* 8, 269-284. <https://doi.org/10.1106/108201302029451>
- Ribeiro, S.M.R., Barbosa, L.C.A., Queiroz, J.H. and Knödler, M. (2008). Phenolic compounds and antioxidant capacity of Brazilian mango (*Mangifera indica* L.) varieties. *Food Chemistry* 110, 620-626. <https://doi.org/10.1016/j.foodchem.2008.02.067>
- Robles-Sanchez, R.M., Rojas-Graü, M.A., Odrizola-Serrano, I., Gonzalez-Aguilar, G.A., and Martín-Belloso, O. (2009). Effect of minimal processing on bioactive compounds and antioxidant activity of fresh-cut 'Kent' mango (*Mangifera indica* L.). *Postharvest Biology and Technology* 51, 384-390. <https://doi.org/10.1016/j.postharvbio.2008.09.003>
- Rodríguez-González, S., Gutiérrez-Ruíz, I.M., Pérez-Ramírez, I.F., Mora, O., Ramos-Gomez, M., and Reynoso-Camacho, R. (2017). Mechanisms related to the anti-diabetic properties of mango (*Mangifera indica* L.) juice by-product. *Journal of Functional Foods* 37, 190-199. <https://doi.org/10.1016/j.jff.2017.07.058>
- Rosa, Paulo T.V. and Meireles, M.A.A. (2009). Supercritical and pressurized fluid extraction applied to the food industry. In. *Extracting Bioactive compounds for Food Products: Theory and Applications*, MAA Meireles, CRC Press, Boca Raton, FL. 269-401.
- Ruiz-Montanez, G., Ragazzo-Sanchez, J. A., Calderon-Santoyo, M., Velazquez-de la Cruz, G., Ramírez de Leon, J. A., and Navarro-Ocaña, A. (2014). Evaluation of extraction methods for preparative scale obtention of mangiferin and lupeol from mango peels (*Mangifera indica* L.). *Food Chemistry* 159, 267-272. <https://doi.org/10.1016/j.foodchem.2014.03.009>
- Santhirasegaram, V., Razali, Z., George, D. S., and Somasundram, C. (2015). Comparison of UV-C treatment and thermal pasteurization on quality of Chokanan mango (*Mangifera indica* L.) juice. *Food and Bioprocess Technology* 94, 313-321. <https://doi.org/10.1016/j.fbp.2014.03.011>
- Schieber, A., Berardini, N., and Carle, R. (2003). Identification of flavonol and xanthone glycosides from mango (*Mangifera indica* L. Cv. "Tommy Atkins") peels by high-performance chromatography-electrospray ionization mass spectrometry. *Journal of Agricultural and Food Chemistry* 51, 5006-5011. <https://doi.org/10.1021/jf030218f>
- Soong, Y. and Barlow, P.J. (2006). Quantification of gallic acid and ellagic acid from longan (*Dimocarpus longan* Lour.) seed and mango (*Mangifera indica* L.) kernel and their effects on antioxidant activity. *Food Chemistry* 97, 524-530. <https://doi.org/10.1016/j.foodchem.2005.05.033>
- Soong, Y.Y. and Barlow, P.J. (2004). Antioxidant activity and phenolic content of selected fruit seeds. *Food Chemistry* 88, 411-417. <https://doi.org/10.1016/j.foodchem.2004.02.003>
- Souza, M.E.A.O. (2015). Antioxidant potential of mango peel extracts (*Mangifera indica* L.) from the Tommy Atkins variety obtained by low and high pressure methods and sizing of a column for supercritical extraction. Thesis (doctorate) - Federal University of Santa Catarina, Technological Center, Post-Graduate Program in Food Engineering, Florianópolis. <https://repositorio.ufsc.br/xmlui/handle/123456789/135287>. (In Portuguese).
- Sovová, H. and Aleksovsky, S. (2007). Supercritical CO<sub>2</sub> extraction of *Salvia officinalis* L. *The Journal of Supercritical Fluids* 40, 23-245. <https://doi.org/10.1016/j.supflu.2006.07.006>
- Sun, J., and Chen, P. (2012). Ultra high-performance liquid chromatography with high resolution mass spectrometry analysis of African mango (*Irvingia gabonensis*) seeds, extract, and related dietary supplements. *Journal of Agricultural and Food Chemistry* 60, 8703-8709. <https://doi.org/10.1021/jf302703u>
- Wauthoz, N., Balde, A., Balde, E.S., Damme, M.V. and Duez, P. (2007). Ethnopharmacology of *Mangifera indica* L bark and pharmacological studies of its main glucosylxanthone, mangiferin. *International Journal of Biomedical and Pharmaceutical Sciences* 1, 112-119.

- Wijngaard, H., Hossain, M., Rai, D., and Brunton, N. (2012). Techniques to extract bioactive compounds from food by-products of plant origin. *Food Research International* 46, 505-513. <https://doi.org/10.1016/j.foodres.2011.09.027>
- Yang, Z., Weian, C., Susu, H., and Hanmin, W. (2016). Protective effects of mangiferin on cerebral ischemia-reperfusion injury and its mechanisms. *European Journal of Pharmacology* 771, 145-151. <https://doi.org/10.1016/j.ejphar.2015.12.003>
- Yin, J. Wang., Wei, W., Liu, Y. and Shi, W. (2005). Analysis of de operation conditions for supercritical fluid extraction of seed oil. *Separation and Purification Technology* 43, 163-167. <https://doi.org/10.1016/j.seppur.2004.10.016>