Comparison of methods of extraction of antioxidant compounds from the peel from Mango (*Manguifera indica* L.)

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1. Introduction

The total content of phenolic compounds in mango peel ranges from 9.0 to 109.0 mg/g dry peel with more extractable phenolic compounds than the flesh (Ajila *et al.,* 2007b; Berardini *et al.,* 2005; Machado and Schieber, 2010). The peel contains carotenoids (tetraterpenoids), mono-, di- and triterpenoids, including ocimene, myrcene or limonene, terpinolene, and carene. β-Carotene, violaxanthin and lutein are also present. These compounds are effective antioxidants *in vitro*.

1.1 Aims and Objectives

To compare the efficiency of different methods for the extraction of polyphenolic compounds from mango peel and the influence of different drying methods on antioxidant capacity and antioxidant composition of different extracts.

2. Materials and Methods

2.1 Preparation of the mango peels

Peels from the mango variety 'Tommy Atkins' were frozen (-20 ºC) before drying. 100 g samples of the peel were either dried in an oven at 70 ºC (O), or freeze dried (F), then made into fine powders in a coffee grinder. All preparations were duplicated.

2.2 Extraction of polyphenols

The polyphenols were extracted as shown in Table 1.

Table 1. Extraction methods; O – oven dried, F – freeze-dried

Acidic methanol-acetone-water (MA)	Methanol-water (MW)	Water (W)
O or F powders, 1g + 40 mL of acidic methanol-water (50:50 v/v, pH 2) Shaken 1 h at 20 – 22 oC Centrifuged (2150 g/20 min) Supernatant removed Residue + 40 mL of acetone/water (70:30, v/v) Repeat shaking/centrifugation Supernatants combined Stored at -10 ºC (Perez et al., 2008)	Fresh peel, 30 g, crushed + 100 mL methanol-water (80:20 v/v) Shaken 5 h at 20 – 22 oC Filtered: Whatman No. 1, Centrifuged (2150g/20 min) Supernatant: (a) used for direct analysis; (b) rotary evaporation (3 h/40 oC) – crude extract This extract was used to compare the effects of the other methods. (Chaira et al., 2010)	O or F powders, 1g + 80 mL of ultrapure distilled water Heated 70 – 80 oC/2 h, with agitation Filtered: Whatman No. 1 Centrifuged (2150g/25min) Supernatants used for analyses

2.3 Determination of phenolic content

Extracts were filtered (0.45 μm). Phenolics determined by reverse phase HPLC-UV analysis: 40 ºC, C18 KNAUER Eurospher column, 100 Å pore size, 5 μm particle size, 250 x 4.6 mm internal diameter (KNAUER, Berlin, Germany). Mobile phase: methanol 99.8% (A) and 0.1% acetic acid in ultrapure water (B). Detector: Dionex MWD-3000 (Thermo Scientific, U.K.), 280 nm. Multi-step gradient analysis: from start to 8 min, 0.3 ml/min and 10% (A); 8.1 min to 25 min, 0.5 ml/min and 15% (A); 25.1 min to 65 min, 0.8 ml/min and 24 (A).

2.4 Determination of antioxidant capacity

Antioxidant capacity was determined in triplicate using the Oxygen Radical Absorbance Capacity (ORAC) assay with Trolox™ standards (Fegredo et al., 2009). The antioxidant capacity was expressed as equivalence to Trolox units.

2.5 Statistical analysis

Results were compared by two-way anova and post-hoc tests using SPSS Predictive Analytics software.

3. Results

Over 10 phenolic compounds were detected (Table 2). The total phenolic contents of the extracts were not significantly different.

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Table 3. Phenolic compounds identified in dried mango peel extracts (mg/g), mean values (SD). MA – acidic methanol-acetone; MW – methanol-water; W – water. -O, -F – oven dried and freeze-dried, respectively

Phenolic compound	MA-O	MA-F	W-F	W-O	MW
Gallic acid	64.86 (0.50)	64.28 (0.33)	3.26 (1.36)	12.37 (3.74)	1.25 (0.16)
Catechin hydrate	1.962 (0.27)	0.333 (0.01)	0.601 (0.07)	7.84 (0.58)	3.26 (0.38)
Chlorogenic acid	0.697 (0.05)	0.012 (0.001)	0.384 (0.02)	2.52 (1.26)	1.37 (0.09)
(-)-Epicatechin	-	-	-	0.199 (0.06)	0.23 (0.22)
Caffeic acid	0.0089 (-)	0.063 (0.01)	0.032 (0.001)	0.050 (-)	0.029 (0.005)
Vanillic acid	-	-	-	-	0.130 (0.020)
Ethyl gallate	-	0.043 (0.04)	0.332 (-)	0.365 (-)	0.029 (-)
P-coumaric acid	-	-	-	-	0.006 (-)
Sinapic acid	0.326 (0.01)	0.338 (-)	-	0.117 (0.07)	2.28 (0.41)
Penta-O-galloyl-β-D-glucose hydrate	3.503 (2.27)	7.59 (5.01)	35.72 (29.21)	0.566 (0.24)	38.47 (2.66)
(a) Total Phenolic Content	134.58 (1.40)	139.02 (10.56)	152.01 (19.24)	132.51 (14.80)	137.16 (6.83)

(-) No value identified. (a) The total phenolic content was expressed as the sum of the phenolic compounds listed and other un-identified compounds detected but not included here.

There were no significant differences in the antioxidant capacity of the MA and MW extracts (Table 3) but the water extracts gave significantly higher mean values ($F(1, 5) = 11.203; P = 0.020$).

Table 3. Total Antioxidant Capacity (TAC) of mango peel extracts, mean (SD) μM Trolox equivalence/g dried peel. MA – acidic methanol-acetone, W – water, MW – methanol-water. -O, -F – oven dried and freeze-dried, respectively.

Extract	*Mean TAC*
MA-O	8340 (1693)
MA-F	7409 (1605)
W-O	10797 (1443)
W-F	11938 (1542)
MW	4540 (995)

4. Conclusions

The predominant phenolic compound was gallic acid. The aqueous method (W) developed empirically in this investigation was shown to be the significantly better method for obtaining extracts with higher antioxidant capacity than the other methods used. The simplicity of this method and its efficiency suggests it is a potential alternative for the extraction of useful hydrophilic compounds with antioxidant properties.

5. References

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