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Phenolic acids contribution to antioxidant activities and comparative assessment of phenolic content in mango pulp and peel

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ABSTRACT

A high performance thin layer chromatography (HPTLC) method to analyse antioxidant activity as free-radical scavenging activity and metal-chelation in selected mango varieties was developed and validated. The contributions of common phenolic acids to antioxidant activity in pulp and peel samples was assessed. Polyphenolic content in pulp and peel was correlated with chlorogenic and gallic acid concentration ($R > 0.90$). Free radical scavenging activity in the peel was related to gallic and chlorogenic acids ($R = 0.83$), but wasn't dependent on caffeic acid concentration. Free radical scavenging activity in the pulp could not be predicted from the gallic or chlorogenic acid ($R = 0.25$ and 0.45) indicating that pulp contains other, more powerful free radical scavengers.

1. Introduction

Mango fruit is widely consumed in both fresh and processed form (Jahurul et al., 2015). However, processing of mangos generates large amounts of by-products (mainly peel and seeds), which are usually discarded as waste (Dorta et al., 2012). However, the presence of bioactive compounds such as polyphenolic compounds, carotenoids, enzymes, vitamins E and C, fibre, cellulose, hemicellulose, lipids, protein, enzymes, pectin and fats in mango peels, means that they may be useful as supplements in food products (Jahurul et al., 2015). The aim was to develop a simple, rapid and reproducible high performance thin layer chromatographic (HPTLC) method to analyse phenolic acid content and to quantify and compare antioxidants as free-radical scavengers or metal-chelating agents in the pulp and peel of different mango varieties. We also wanted to correlate antioxidant activity to the major phenolic acids present in mango pulp and peel extracts. Antioxidants, such as phenolic acids are capable of preventing the harmful effects of oxidative stress and act as free-radical scavengers or metal-chelating agents (Agatonovic-Kustrin and Morton, 2016). They are a large group of phenolic compounds belonging to the non-flavonoid family. Their antioxidant properties result from the presence of an aromatic ring, a carboxyl group, and one or more hydroxyl and/or methoxyl groups in the molecule (Agatonovic-Kustrin et al., 2017). They are classified as either hydroxybenzoic acids (e.g. gallic acid, vanillic acid, hydroxybenzoic, and syringic acids) or hydroxycinnamic acid derivatives (e.g. caffeic, chlorogenic, ferulic, p-coumaric, and sinapic acids), and may occur in their acid or conjugated forms (esters) (Laghari et al., 2011; Haminiuk et al., 2014). They are excellent antioxidants, and are more effective than vitamins C, E, and carotenoids (Dai and Mumper, 2010). Unfortunately, there is no previous work identifying/quantifying phenolic acids in mango pulp and peel and relating this to the total observed antioxidant activity. Many *in vitro* methods are available to determine antioxidant and free radical scavenging capacity (Arnao et al., 2001). One popular method is the 2,2-diphenyl-1-picrylhydrazyl (DPPH•) free radical scavenging method, where antioxidants in a sample react with purple DPPH• reducing it to its yellow form (Huang et al., 2005; Wozniak et al., 2010). The disadvantage of these methods is that they only measure antioxidant activity of the whole extract (Kedare and Singh, 2011). This limitation can be overcome if an assay method is combined with chromatographic separation of the sample. Unfortunately, when liquid chromatography is used, slow reaction kinetics result in inaccurate measurements (Zhao et al., 2010). However, thin layer chromatography (TLC) combined with DPPH• assay is not affected by slow reaction kinetics, and has been successfully used to measure both total sample

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antioxidant activity, and antioxidant activity of individual sample components (Agatonovic-Kustrin and Morton, 2016).

2. Materials and methods

2.1. Sample collection and preparation

Samples from 9 different mango varieties (*Mangifera Indica* Linn. Cv. Golden Water Lily (sample 1), *Mangifera Indica* Linn. Cv. Rainbow (sample 2), *Mangifera Indica* Linn. Cv. Chokanan (sample 3), *Mangifera Indica* Linn. Cv. Golden Phoenix (sample 4), *Mangifera Indica* Linn. Cv. Susu (sample 5), *Mangifera Indica* Linn. Cv. Farlan (sample 6), *Mangifera Indica* Linn. Cv. Aust R2E2 (sample 7), *Mangifera Indica* Linn. Cv. Telur (sample 8), *Mangifera Indica* Linn. Cv. Harumanis (sample 9)) were randomly collected from different regions of Selangor, Malaysia and overseas (Fig. 1). *Mangifera Indica* Linn. Cv. Farlan was from Thailand and *Mangifera Indica* Linn. Cv. Aust R2E2 was from Australia. The other 7 varieties were from Selangor, Malaysia. All fruits were harvested between January and February 2015. Mango peel was carefully separated from mango pulp, and both peel and pulp were then frozen at −80 °C. The frozen samples were lyophilised in a freeze-drier (Labconco, Missouri). Approximately 1 g of dried sample was finely ground and then extracted with 50 mL of ethanol using a FOSS Soxtec™ 2050 extractor (Sweden). Ethanolic extracts of mango pulp and peel were then evaporated to a smaller volume of around 9 mL, filtered and transferred into 10.00 mL volumetric flasks, and made up to volume with absolute ethanol. All extracts were stored in amber glass containers at 4 °C to minimize degradation of extract components.

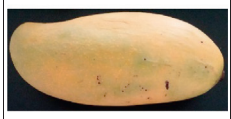
No	code	Name	Image
1	1A – pulp 1B – peel	Mango golden Waterlily Scientific name : *Mangifera Indica* Linn. Cv. Golden Waterlily	
2	2A – pulp 2B – peel	Name : Mango Rainbow Scientific name : *Mangifera Indica* Linn. Cv. Rainbow	
3	3A – pulp 3B – peel	Mango Chokanan Scientific name : *Mangifera Indica* Linn. Cv. Chokanan	
4	4A – pulp 4B – peel	Mango Golden Phoenix Scientific name : *Mangifera Indica* Linn. Cv. Golden Phoenix	
5	5A – pulp 5B – peel	Mango Susu Scientific name : *Mangifera Indica* Linn. Cv. Susu	
6	6A – pulp 6B – peel	Mango Farlan Scientific name : *Mangifera Indica* Linn. Cv. Farlan	
7	7A – pulp 7B – peel	Mango Aust R2E2 Scientific name : *Mangifera Indica* Linn. Cv. Aust R2E2	
8	8A – pulp 8B – peel	Mango Telur (egg-sized shape) Scientific name : *Mangifera Indica* Linn. Cv. Telur	
9	9A – pulp 9B – peel	Mango Harumanis Scientific name : *Mangifera Indica* Linn. Cv. Harumanis	

Fig. 1. Images of mango samples used.

2.2. Chemicals, solvents and solutions

All standards and solvents were of analytical grade. Caffeic acid, chlorogenic acid, DPPH•, ethanol, and gallic acid were from Sigma-Aldrich (Germany) while $FeCl_3$ was from Merck (Germany). 1.00 mg mL^{-1} standard solutions of chlorogenic, caffeic and gallic acid and a 0.4% *w/v* DPPH• solution were prepared using absolute ethanol. Freshly prepared 2% w/v ethanolic $FeCl_3$ was neutralized by adding a few drops of diluted sodium hydroxide solution. The solution was then filtered and the clear solution was used for derivatization (Sethi, 2006). All extracts were stored in amber glass containers at 4 °C to minimize degradation.

2.3. High performance thin layer chromatography

HPTLC plates were pre-washed with a blank run of ethanol, then dried and activated, by heating in an oven at 105 °C for 15 min. Samples were sprayed as 8 mm wide bands using a 25 μL HPTLC syringe (Hamilton, Switzerland) with an Automatic TLC sampler 4 (CAMAG, Switzerland), 8 mm from the lower edge, with 14 mm distance from each side, and a minimum distance of 2 mm between each track. Post chromatographic derivatization involved spaying a plate using either 2% *v/w* $FeCl_3$ or 0.4% *w/v* DPPH•. TLC plates were developed in an Automated Multiple Development Chamber (AMD2, CAMAG, Switzerland) with a n-hexane:ethyl acetate:acetic acid (20:10:1) mobile phase, and then photographed both before and after derivatization. Before being photographed, plates derivatized with DPPH• were stored in dark for 30 min, while plates derivatized with $FeCl_3$ were heated at 110 °C for 10 min. Plate images were recorded using a TLC-visualizer (CAMAG, Switzerland) equipped with a 12-bit charged couple device (CCD) digital camera and win CATS software (CAMAG, Switzerland). Quantification of chlorogenic acid and gallic acid in samples was achieved by UV densitometry at 320 nm using a TLC scanner III (CAMAG, Switzerland) controlled by winCATS software (CAMAG, Switzerland). Image analysis software, Sorbfil TLC Videodensitometer (Sorbpolymer, Russia), was used for quantitative evaluation of plate images.

2.4. Method validation

The method was validated according to the current International Conference on Harmonization (ICH) guidelines for validation of analytical procedures (International Conference on Harmonization, November, 2005). The working concentration ranges for chlorogenic and gallic acids at 320 nm and chlorogenic acid at 360 nm, after derivatization with DPPH• and $FeCl_3$ were determined by plotting chromatographic peak areas versus applied amounts of standards using the least squared method. Specificity was assessed by the ability of the optimized mobile phase to separate sample components. Method precision was determined by repeatability, by applying three replicates of each standard at three concentrations (low, medium and high) within