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Research Article

Total Phenolic Content of Mango Pulp (*Mangifera indica* L.) Extracted using Three Different Solvents

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Abstract

Mango is probably the most variegated fruit of India. Mango fruit is not only rich in macro and micro nutrients, but also rich in natural antioxidants. Phytochemical and nutrient content appears to vary across mango cultivars. Seven varieties of mango available were purchased from the local market by following a sampling plan. The pulp was recovered, blended and used for further extraction and analysis. Three solvents namely ethanol, methanol and water were used for extraction. Total phenolic content was determined by the Folin-Ciocalteu method. The TPC of mango varieties were from 111.73 ± 3.56 to 889.72 ± 15.73 µg of GAE/g FW. The results were superior in water extracts, medium in ethanol and least in methanol in all the mango varieties except Alphonsa mango. Quantification of total phenolic components would be used to evaluate antioxidant activities

Keywords: Mango Varieties; Solvent Extraction; Total Phenols; Folin-Ciocalteu; Mango Pulp

Introduction

Mangifera indica L. (mango), "the king of fruits" belonging to the family *Anacardiaceae*, is one of the most popular fruits in tropical regions. Mango is probably the most variegated fruit of India. There are almost 500-1000 cultivars of different mangoes in the country. This huge variety not only differs in its shape, size and colour but also in its scrumptious taste. They are seasonal, available from April to July. However it is not possible to obtain all varieties in one particular place.

Databases on mango varieties list the most number of varieties from India. To name them viz. Alphonso, 'Banganapalli, 'Bombay, 'Bombay Green, 'Chausa, 'Dashehari, Fazli, Fernandian, Himsagar, Kesar, Kishen Bhog, Langra, Mallika, Mankurad, Mulgoa, Neelum, Pairi, Samar Behisht Chausa, 'Suvarnarekha, 'Totapuri, Vanraj, Zardalu, Amrapali, Bangalora, Gulabkhas in all more than 25 varieties [1].

Mango is one of the most important fruits in terms of production, marketing, and consumption. Among tropical fruits, it ranks second, next to banana in international trade. Mango fruit is not only rich in macro and micro nutrients, but also rich in natural antioxidants. Mango had a higher content of ascorbic acid, which was nine and four times that of the apple and banana, respectively [2]. Similarly, its carotenoid content also held a significant superiority, with nearly 10 times that of the apple or orange. α-tocopherol was another preponderant composition, which was more than 18 times that of the apple and outdistances that of the grape, orange, tangerine, and banana. Moreover, many previous studies have showed that those major antioxidants (i.e., vitamins, flavonoids, carotenoids, polyphenols, mangiferin, etc.) contribute directly to the total antioxidant capacity of mango [3-5].

In one study five varieties of mangoes from four countries were evaluated with multiple harvests over one year to compare the beta-carotene, ascorbic acid, and total phenolic contents and antioxidant capacities of the fruit pulp and to compare the phenolic profiles of the individual varieties. Phytochemical and nutrient content appeared to vary across mango cultivars. Up to 25 different carotenoids were isolated from the mango pulp, the densest of which was beta-carotene, which accounted for the yellow-orange pigmentation of most mango cultivars. Total phenolic content ranged from 19.5 to 166.7 mg of gallic acid equivalents (GAE)/100 g of puree. The varieties Tommy Atkins, Kent, Keitt, and Haden had similar total phenolic contents, averaging 31.2+/-7.8 mg GAE/100 g of puree, whereas the variety Ataulfo contained substantially higher values. Similar trends were observed in the DPPH radical scavenging activities among the five varieties. In contrast, the country of origin and harvest dates had far less influence on these parameters. Ataulfo mangoes contained significantly higher amounts of mangiferin and ellagic acid than the other four varieties. Large fruit-to-fruit variations in the concentrations of these compounds occurred within sets of mangoes of the same cultivar with the same harvest location and date [3].

Some of the varieties available in the present study area are described below

- **Alphonso:** Also known as Hapoo, this is the most expensive variety of mangoes in India. Maharashtra, Gujarat and Karnataka are its prime producers. It has a fibreless pulp and douses into the mouth as a smooth-creamy mango. This variety of mango is popular all over the world.
- **Banganapalli:** These mangoes have a very beautiful yellow colour and are sweet and fibreless. It is the most popular variety of mangoes which comes straight from Andhra Pradesh.
- **Neelam:** This is one variety that grows throughout the country. With its specialty in Hyderabad, it is a large-yielding variety of mangoes. Though it arrives early in the season, the best variety is available only in June.
- **Sindoora:** Its reddish color at the top has entitled this variety with the name of Sindoora. Extremely juicy and pulpy, this mango is one of the tastiest mangoes one can ever have.
- **Malgova:** Mammoth in size, the Malgova is legendary to Hyder Ali's Mysuru orchards, where each fruit weighed between 1-1.5 kg a piece! When compared to the waif-like Alphonso, the Malgova almost looks unsightly. The flavour though, is a welcoming sweetness with the slightest undercurrent of tartness. The pink blush at the tip is the only indicator of its ripeness.
- **Imam Pasand:** This variety originated Andhra Pradesh. This variety is also called Imam Pasand. Himayat is popular among the Nawabs. Each weighs around 450g-500g and hence each fruit is large. Ripened fruit has negligible amounts of dietary fibre and has a silky flesh with deep sweet flavour and distinctive citrus overtones. The skin is green and when it ripens, it attains yellow colour.

Materials and Methods

Sampling and extraction

The mango fruit samples were purchased from retail outlets in the Coimbatore area and prepared for analysis during the harvest time. The retail outlets included supermarkets, independent retailers and catering suppliers. One kilogram each was purchased at least from three outlets and was combined into composite samples for analysis. Each composite was made up of three sub-samples, combined on an equal weight basis. Sub-samples included were based on the need to take into account factors including cultivar and region. From this the laboratory sample and test sample were derived for the determination of TP.

The fresh fruits were sampled in seasons (summer) where the cultivars and geographic origin were known to change between seasons. This process allows a single, robust set of nutrient values to be derived for each fruit. A voucher specimen was identified by at least three persons viz., the investigator, the vender or farmer and botanist deposited at the Department's Nutrition laboratory for analyses and future reference. The samples were stored in the refrigerator and extracted the same day after removing the skin, stem and seeds.

The seven types of cultivars of ripe mangoes (Alphonso, Senthuran, Malgova, Nadusalai, Emmabasanth, Banganapalli and Kudhadath) were purchased from local market in Coimbatore. The mangoes were washed with warm water and peels were removed by using sharp stainless steel knife and underlying pulp was recovered by scraping with the blunt edge of the knife. The pulp was ground using mixer and grinder (Preethi Chef Pro-750W) individually up to the formation of a coarse texture.

The blended sample (0.3 g) was weighed using an analytical balance (2007 TX/TXB Series, Shimadzu). The sample was then transferred into a Borosil test tube. For each test tube, 6 ml of extracting solvent was added to the sample (solvent ratio of 1:20). The test tube was then placed on a water bath shaker (Precision) of 130 rpm at a room temperature of 30ºC for 60 min for the extraction. The extracts were collected and filtered using a syringe filter before the determination of total phenolic content. All the extractions were carried out in three replicates. Extracts were stored in brown bottles in the freezer until further analyses.

Polyphenols are isolated by the three steps of extraction, incubation and filtration of the supernatant. It is widely accepted that the extraction step is one of the most important stages in isolation of polyphenols, but based on literature, there is no consensus about one single and effective standard extraction method. On the contrary, there are several reported methods with very accurate results, and according to the literature in some cases, the solid-liquid extraction with different types of solvents is more adequate [6].

In order to extract different phenolic compounds from plants with a high degree of accuracy, three solvents of differing polarities were used. Solvents used for the extraction of bio molecules from plants are chosen based on the polarity of the solute of interest. A solvent of similar polarity to the solute will properly dissolve the solute. Multiple solvents can be used sequentially in order to limit the amount of analogous compounds in the desired yield. However, in our study we used three different solvents for extraction viz., ethanol, methanol and deionised water. Variations in the yields and phenolic contents of various extracts are attributed to polarities of different compounds present in the sample. A higher content of polyphenols can be obtained with an increase in the polarity of the solvent used. Several authors have reported a good correlation between total phenolic contents and reducing power ($r^2 = 0.86$) and radical scavenging activity ($r^2 = 0.82$) assays. The polarity of solvents in the increasing order is as follows: Hexane < Chloroform < Ethylacetate < Acetone < Methanol < Water [7].

Chemicals

Gallic acid was purchased from Sigma-Aldrich USA. Folin- Ciocalteu reagent was obtained from Merck, Germany. All other chemicals used in the study were of analytical grade. These were supplied by the local agent in the study area.

Determination of total phenolic content

The total phenolic content of the mango extract was determined by using Folin-Ciocalteu reagent following a slightly modified method [11]. Gallic acid was used as a reference standard for plotting calibration curve (Figure 1). Ten mg of gallic acid was dissolved in 100 ml of 50% methanol (100 µg/ml) and then further diluted to 6.25, 12.5, 25 or 50 µg/ml [8]. 1 ml of aliquots and standard gallic acid (10, 20, 30, 40 and 50 µg/ml) was positioned into the test tubes and 5 ml of distilled water and 0.5 ml of Folin Ciocalteu's reagent was mixed and shaken. After 5 minutes, 1.5 ml of 20% sodium carbonate was added and volume made up to 10 ml with distilled water. The reaction mixture was incubated at room temperature for 30 min with intermittent shaking for colour development. The absorbance of the resulting blue colour was measured at 765 nm using UV-VIS Double Beam Spectrophotometer (Systronics, India). The linear equation of a standard curve prepared with Gallic acid was used to determine the total phenolic contents of the extracts. The content of total phenolic (TP) compounds was expressed as µg/g Gallic acid equivalent (GAE) of fresh weight.

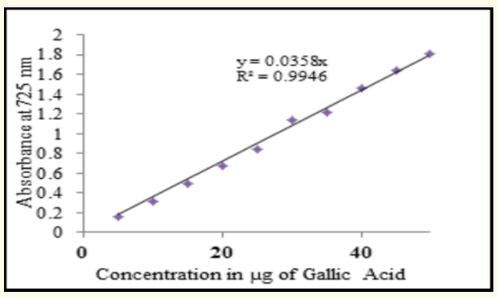

Figure 1: Standard Curve of Gallic Acid –Total Phenol Assay.

The total phenolics content of the extracts were determined colorimetrically, using the Folin-Ciocalteu method, as described by Singleton and Rossi [9], using gallic acid as a standard. Different aliquots of sample solution were made up to 1ml each with distilled water and added 1.5 ml of Folin– Ciocalteu reagent (previously diluted 10 folds with distilled water), kept at room temperature for 5 min and added 4 ml of 20% sodium carbonate (Na2CO3), and kept at room temperature for 30 min. The absorbance was read against the blank at 750 nm. Here, blank as except test sample (extracts, standard). The total phenolics content was represented as milligrams of Gallic acid equivalent per gram of dry mass (mg GAE/ g) [10].

Results

The preservation of vegetables and fruits phenolic content has a great impact on the contribution of phenols not only in enzymatic browning reaction but also on nutrient content of the product as antioxidants capacity. The results of this study indicate that all the fruits have phenolics that vary widely in different extracts and agricultural patterns. Further research is needed to find out the various factors influencing phenolic content in plants.

The TPC of mango varieties were from 111.73 ± 3.56 to 889.72 ± 15.73 µg of GAE/g FW. The results were superior in water extracts, medium in ethanol and least in methanol in all the mango varieties analysed except in the alphonsa mango. The order of TPC in mango variants is given in following decreasing order: alphonsa mango> nadusalai> senthuram> malgova> emmabasanth> kudhadath (Table 1). TP extraction in water was significantly higher when compared to the ethanolic and methanolic extracts ($p \leq 0.05$).

Name of the Fruits	Total Phenols (µg of GAE/ g of FW)			
	Ethanol	Methanol	Water	Total
Mango (Banganapalli)	539.23 ± 12.85	446.22 ± 6.63	889.72 ± 15.73	1875.17
Mango (Kudhadath)	176.37 ± 8.46	143.92 ± 4.82	193.31 ± 8.70	513.60
Mango (Emmabasanth)	172.55 ± 3.79	147.27 ± 10.59	218.91 ± 7.17	538.73
Mango (Malgova)	273.26 ± 4.70	236.16 ± 0.34	301.67 ± 4.43	811.09
Mango (Nadusalai)	322.21 ± 16.47	310.23 ± 16.46	357.29 ± 14.33	989.73
Mango (Senthuram)	265.77 ± 22.89	224.36 ± 20.00	327.51 ± 12.32	817.64
Mango (Alphonsa)	556.06 ± 4.89	493.94 ± 8.01	383.35 ± 1.94	1433.89

Table 1: Total Phenols in three different extracts of Mango Pulp.

Discussion

Ribeiro., *et al.* [11] investigated the pulp composition of four mango cultivars (Haden, Tommy Atkins and Ubá) at the ripening stage in relation to three components with antioxidant potential (total phenolics, carotenoids and ascorbic acid). Total phenolic compound content was estimated by the Folin-Ciocalteu reagent and total carotenoid content by spectrophotometry at 450 nm. The contents of beta-carotene and total vitamin C (ascorbic acid and dehydroascorbic acid) were quantified by high performance liquid chromatography. Differences were found among the four mango cultivars in all the components analyzed. The content of phenolic compounds ranged from 48.40 (Haden) to 208.70 mg/100 g (Ubá); total carotenoid from 1.91 (Haden) to 2.63 mg/100 g (Palmer); beta-carotene from 661.27 (Palmer) to 2,220 microg/100 g (Ubá) and total ascorbic acid ranged from 9.79 (Tommy Atkins) to 77.71 mg/100 g (Ubá). The results from several studies also show that mangoes are a good source of antioxidants [8,12].

Eight genotypes of the mango fruit were evaluated for antioxidant potential by several biochemical assays (DPPH, ABTS, ORAC, FRAP, SASR and MCC) and tested for their polyphenol composition and vitamin C contents. The significance analysis demonstrated that the antioxidant capacity of Tainong 1 fruits were significantly

higher than that of other genotypes, which was about 2.1–6.3-fold higher than Guifei assayed in ABTS, DPPH, ORAC and FRAP methods. Total polyphenols and flavonoids might be major contributors to the antioxidant activity. Quantification of total phenolic components would be used to evaluate antioxidant activities of mango genotypes. The total polyphenols and flavonoids contents showed a great variety amongst mango genotypes and high positive correlation with the total antioxidant capacity. It is concluded that significant genotypic difference exists in the total antioxidant capacity of mango fruits. Both total polyphenols and flavonoids are major contributors to the total antioxidant capacity in mango fruit. They highlighted that antioxidant activity had significant differences amongst the genotypes. The total polyphenols and flavonoids contents showed a similar variety [5].

Li., *et al*. [12], concluded that (a) there were diversities in antioxidant contents and in vitro antioxidant capacities among cultivars and among groups, e.g., Guire No. 82 had superior polyphenol content, flavonoid level, and in vitro antioxidant capacity, while the highest level of α-tocopherol was observed in Renong No. 1; (b) higher contents of ascorbic acid, total polyphenol, total flavonoid, and total *in vitro* antioxidant capability were observed in green peel mangoes than that in red peel or yellow peel mangoes; (c) correlation analysis and principal component regression analysis demonstrated that positive correlations exist among the measured antioxidants and total in vitro antioxidant capability, except α-tocopherol. Besides, polyphenol chemicals were deemed to be one group of the most critical factors affecting the total in vitro antioxidant capability. Meanwhile, the strong synchronism of total polyphenol and flavonoid contents in pulp were found [12].

Conclusion

Total phenols which are a major contributor of antioxidant activity was estimated in seven varieties of mango. Three solvents were used for the extraction of TP. We obtained a greater extraction of TP in water. The commonly available variety of Alphonsa mango had the highest TP. We suggest that other varieties popular in the Northern parts of India may be analysed for the TP and AO content.

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